IntechOpen

Soybean and Nutrition

Edited by Hany El-Shemy

SOYBEAN AND NUTRITION

Edited by **Hany A. El-Shemy**

Soybean and Nutrition
http://dx.doi.org/10.5772/1008
Edited by Hany El-Shemy

Notice

Statements and opinions expressed in the chapters are these of the individual contributors and not necessarily those of the editors or publisher. No responsibility is accepted for the accuracy of information contained in the published chapters. The publisher assumes no responsibility for any damage or injury to persons or property arising out of the use of any materials, instructions, methods or ideas contained in the book.

We are IntechOpen,
the world's leading publisher of Open Access books
Built by scientists, for scientists

4,000+
Open access books available

116,000+
International authors and editors

120M+
Downloads

151
Countries delivered to

Our authors are among the
Top 1%
most cited scientists

12.2%
Contributors from top 500 universities

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Meet the editor

Professor Hany A. El-Shemy received his two Ph.D. degrees in biochemistry and genetic engineering from the University of Cairo, Egypt and Hiroshima University, Japan. He became an assistant professor with Biochemistry Department of Cairo University, Egypt from Sept, 1996 and advanced to associate professor in Sept, 2002, as well as full professor in March 2007. His research interests are in the fields of plant biotechnology and medicinal plants. He received 2 patents, wrote 2 book chapters, published more than 60 SCI Journal papers and 25 conference presentation, and served as the technique committee member as well as chair in many international conferences and the editors including PLoS ONE, BMC Genomics, CIMB journals, also reviewer for more than 10 SCI cited journals. He received several awards, including State prize Award from Academy of Science, Egypt (2004), Young Arab Researcher prize Awarded from Shuman Foundation, Jordan (2005).

Contents

Preface

Worldwide, soybean seed proteins represent a major source of amino acids for human and animal nutrition. Soybean seeds are an important and economical source of protein in the diet of many developed and developing countries. Soy is a complete protein and soy-foods are rich in vitamins and minerals.

Soybean protein provides all the essential amino acids in the amounts needed for human health.

Recent research suggests that soy may also lower risk of prostate, colon and breast cancers as well as osteoporosis and other bone health problems and alleviate hot flashes associated with menopause. This volume is expected to be useful for student, researchers and public who are interested in soybean.

Hany A. El-Shemy, Ph.D.
Professor, Director of Biotechnology Labs, FARP,
Biochemistry and Molecular Biology,
Biochemistry Department,
Faculty of Agriculture,
Cairo University, Giza
Egypt

Nutritional Value of Soybean Meal

Teresa Banaszkiewicz
Siedlce University, Natural Faculty,
Poland

1. Introduction

Protein feeds in the European Union (EU) cover only 25% demand for protein, what oblige the particular members to import feeds with a high protein content, where the main place have soybean meal. The share of this feed in total quantity of feeds utilization in EU is about 9%. Yearly consumption of soybean meal in EU is about 32mln ton, from what 5.6% it falls on Poland (Rynek Pasz, 2010). Poland, as another countries, is importer of protein feeds, mainly soybean meal. The utilization of high protein feeds in Poland in the season 2010/2011 is estimated on about 3.5mln ton (Rynek Pasz, 2010), from what in country is produced about 1.6mln ton, in this about 1.2mln ton of rapeseed meal,180thou. ton legume seeds and 18thou. ton of fish meal (Rynek Pasz, 2010). Remaining part of feeds must be imported. Import of high protein feeds estimated on about 2.5mln ton, from this about 2mln ton this is soybean meal, which in the most (1.4mln ton) is imported from the Argentine (Rynek Pasz, 2010).To 2003 year, import of animal meals to Poland was evaluated on about 300thou. ton, which were covered about 30-40% demand of protein need to production mixtures for poultry and pigs. In results of prohibition applying the animal meals import decreased to 70thou. ton (Rynek Pasz, 2010).

Feed	2006/2007	2007/2008	2008/2009	2009/2010 (estimated)	2010/2011 (prognosis)
Soybean meal	1852	1969	1679	1810	1970
Rapeseed meal	540	512	624	861	840
Sunflower meal	207	140	310	502	400
Legume seeds	168	224	195	227	240
Animal meals	23	26	29	30	30
Percentage of soybean meal,%	66.8	69.7	60.5	52.6	55.5

Table 1. Utilization of high protein feeds in Poland, thou. ton (Rynek Pasz, 2010)

In Poland does not write down deficit of protein for ruminant generally, however in feeding of non ruminant animals care, similarly in another countries of UE, this deficit is defined on about 1mln ton (Prusiński, 2008). This deficit of protein is covered by soybean meal (Judziński, 2007). In results of prohibition applying the animal meals, the consumption of soybean meal increased. Till to 2000 year, Poland imported about 900thou. ton of soybean

meal annually, but after prohibition of import and applying the animal meals in animal nutrition import of soybean meal increased to 1.3mln ton, and after 2006 year to about 2mln ton (Rynek Pasz, 2010). Lately in Poland also waste of another of vegetable high protein fodders increased: rapeseed meal to 840thou. ton, legume seeds to 240thou. ton ,whereas the quantity of animal meals decreased to 31thou. ton. Soybean meal is the basic component of fodder mixtures for poultry, in which make up about 70% high protein fodders. Participate of soybean meal in mixtures for particular of animal species shape as follow: the laying hens -20-25%; broiler chickens-25-35%; pigs-10-20% and cows efficiency above 7thou. liters -15% (Brzóska, 2009; Brzóska et al., 2009).This broadly utilization of soybean meal and soybean products be connected mainly with high content of proteins and its amino acid composition. In this description the information relating nutritional value of soybean and soybean meals for animals were introduced. Also the attention   on possibility replacement the part of soybean meal by rape products (rape meal and rape cakes) was turned.

2. Production and nutritional value of soybean

The soybean (*Glycine max*) is grown as a commercial crop in over 35 countries as the major oilseed (Smith & Huyser, 1987). The fruit of soybean is simple or take the shape of crescent pod, length about 3-7cm, including 1 or 2seeds which mass of 1000 seeds take out 115-280g. On the fodder designed the seeds in mass about 180-200g.Unripe seeds are green , and mature have from light-yellow by green to brown colour. In practice are used seeds of different cultivars, what influence on colour and form of seeds. The soybean seeds of modern cultivars have spherical shape, and the yellow and green colour is the most desirable (Sikorski, 2007).The soybean products are use in food industry on whole world. The soybean seeds contain high quantity of protein and its amino acid composition is approximate to composition of animal proteins, therefore is often used as replacement component of meat protein. Soybean seeds are used in oil industry. About 90% of soybean seeds make up cotyledons and 8% there are hulls. In the cotyledons are accumulated proteins and fats , the main components of seeds. In the cotyledons also are accumulated carbohydrates and anti-nutritional factors. In result of separation of this components or their extraction were obtained different soybean products used in people and animals feeding.

2.1 Production of soybean seeds and meal

The world production of the soybean seeds in 2009/2010 season carried out about 260.6mln ton (Rynek rzepaku, 2010) and the same importance producers of seeds and soybean meal are USA, Brazil , Argentine as well as China, which produced about 87% total quantity of soybean seeds. The main exporters of the soybean seeds are USA (about 44%), Brazil (about 33%) and Argentine (about 11%) and main importer are China (about 38%).

On direct consumption is appropriate about 10% of harvest and about 90% of soybean seeds is use as feeds for animals. The production of soybean meal on world is amount over 160mln ton (Rynek Rzepaku,2010) and main exporters are Argentine -about 37%, Brazil-about 29% and USA- about 8%.

The soybean seeds are subjected of different processing (Berk, 1992), so the oil industry supplies many kinds of by-products (cakes, expellers, oilseed meal), which are used in animal nutrition. After preliminary processes of type the cleaning ,smashing, dehulling , conditioning , flaking, boiling or toasting of soybean seeds, the oil is extracted from seeds by mechanical method or by solvent extraction. Most of all wide spread is solvent extraction.

In results of this process the raw oil and defatted flakes are obtained. In order to elimination of anti-nutritional substances the flakes subjected on high temperature (toasted) and the soybean meal is obtained. To the obtained flakes, sometimes the hulls are added back and obtained soybean meals contained different quantity of protein and fiber. When the hulls does not add, the   high protein products are obtained ,which used mainly in poultry nutrition. The other meals containing more or less of hulls application are used in nutrition of another animals (Van Eys et al.2004).

Country/Region	Production, mln ton
United States of America	94.8
Brazil	68,0
Argentina	54.5
China	14.5
India	9.1
Paraguay	6.7
Other	13.00
Total	260.6

Table 2. Production of soybean seeds (Rynek rzepaku, 2010)

Raw oil obtained in results of fat separation is the most important product, but quantitative most of all, the soybean meal from raw or dehulled and extracted solvent   is obtained. Considerably less the soybean meal is obtained by the mechanical press. Before processing of raw soybean seeds are dehulled and obtained by-products contained protein which composition of amino acids are similar to animal protein. About 46% of soybean by-products are appropriate   to poultry, 32% to swine, about 9% to dairy and beef cattle respectively. Remaining part is use in nutrition of pets and aquaculture.

2.2 Chemical composition and nutritional value of seeds and soybean meal

Nutrients content in soybean products are the basic element to optimization diets and estimation of total quantity nutrients give to animals. Knowledge   about composition of feeds let to forecast animal performance results. Soybean meal is the best vegetable protein source considering on quantity as well its quality. From among legume seeds, the soybean seeds content the most of crude protein and the best of amino acid composition. Content of crude fiber (about 6%) is lower in comparison to another vegetable high protein feeds.

2.2.1 Basal nutrients

Soybean seeds contain to 40% of crude protein and about 20% of fat, and soybean meal characterized higher content of crude protein- about 40-49%. Soybean meal standardized on 44 and 49% of protein there is on the feed market . The protein of soybean contains the considerable quantity of lysine (6.2g/16gN), but value of protein is limited by methionine and cystine content (2.9g/16gN). With regard on high protein content, the soybean meal is mainly use in poultry and pigs nutrition. In mixtures for poultry content of soybean meal can approximate to 40%.

Generally soybean seeds content 5,6-11.5% of water, ranges   for crude protein is from 32 to 43,6%, for fat from 15.5 to 24.7%, for crude ash from 4.5 to 6.4%, for neutral detergent fiber

(NDF) from 10 to14,9%, acid detergent fiber (ADF) from 9 to 11,1%, carbohydrates content from 31.7 to 31.85% on a dry matter basis (Ensminger et al., 1990; NRC, 1998; Poultry Feeding Standards,2005).

Nutrient	Soybean seeds % of DM	Soybean meal		SBM(Banaszkiewicz, 2000)%
		44% CP, % of DM	49% CP, % of DM	
Crude protein	37.08	43.8-49.9	52.8-56.3	44.40
Crude ash	4.86	5.6-7.2	5.2-9.1	6.65
Crude fat	18.38	0.55-3.0	1.0-3.3	2.18
Crude fiber	5.12	4.3-7.2	3.1-4.1	6.75
NDF	12.98	12.3-18.9	7.4-12.2	15.51
ADF	7.22	8.9-11.9	5.2-6.7	9.5
N-free-extractive	24.00	34.3	33.2	31.82
Starch	4.66	5.51	5.46	6.3

Table 3. Basic nutrients in soybean seeds and products (Van Eys et al.2004; ENV/JM/MONO (2001)15)

The soybean contain very little of starch (4.66-7%) and quite a lot of hemicellulose and pectins. Protein of soybean products characterized much quantity of lysine, tryptophane, isoleucine, valine and threonine however sulphuric amino acids are less than in protein of rape products (Ensminger et al. 1990; NRC, 1998; Poultry Feeding Standards,2005). Content of essential amino acids of soybean products shown in table 4.

Amino acids	Soybean seeds, % of DM	Soybean meal 44%CP, % of DM	SBM, g/16g N (Banaszkiewicz, 2000)
Arginine	2.45-3.1	3.49-3.78	6.79
Cystine	0.45-0.67	0.66-0.75	1.57
Histidine	1.0-1.22	1.21-1.32	2.58
Isoleucine	1.76-1.98	2.15-2.78	4.24
Leucine	2.2-4.0	3.66-3.92	8.21
Lysine	2.5-2.66	2.99-3.22	6.49
Methionine	0.5-0.67	0.6-0.69	1.50
Phenylalanine	1.6-2.08	2.35-3.0	4.93
Threonine	1.4-1.89	1.89-2.03	3.99
Tryptophan	0.51-2.44	0.66-0.75	1.05
Valine	1.5-2.44	2.24-2.67	5.22

Table 4. Content of essential amino acids of soybean products, [ENV/JM/MONO (2001)15]

Nutritive value of soybean protein is limited by sulphur amino acids and tryptophane. Soybean is characterized the highest digestibility of protein, lysine and methionine. The amino acids content in soybean protein are good supplemented of grain protein and covered requirement of animals.

Amino acids	Soybean meal	Canola meal	Sunflower meal
Crude protein	82	79	79
Arginine	88	89	90
Lysine	85	81	80
Methionine	83	86	90
Threonine	76	80	73
Cysteine	75	77	92

Table 5. Apparent ileal digestibility coefficients of amino acids,% (Ravindran et al. 2005)

According Banaszkiewicz (2000) the nutritive value of soybean protein obtained by chemical methods was lower than rape cakes.

Specification	SBM
Chemical Score(CS) - related to total egg protein	53
CS- related to egg albumin	42
Essential Amino Acids Index (EAAI) - related to total egg protein	81
EAAI- related to egg protein	80
Limiting amino acids- related to total egg protein	Met, Cys, Trp
Limiting amino acids- related to egg albumin	Met, Cys, Trp

Table 6. Nutritive value of soybean meal protein (Banaszkiewicz, 2000)

The mean value of protein of rape cakes obtained by CS index was adequately 60 and 57 and by EAAI- 81 and 82.

Lipid fraction of the soybean seeds contain about 99%of triglycerides, in which content of polyunsaturated fatty acids (linoleic and linolenic) and unsaturated – oleic acid is high. In the lipid fraction of soybean seeds the fatty acids content about 80%, from what about 50% it is the linoleic acid. The range in fatty acid composition in soybean seeds and oil shown in table 6.

Fatty acids	Soybean seeds% of DM	Soybean oil%
Palmitic	1.44-2.31	7-12
Stearic	0.54-0.91	2-5
Oleic	3.15-8.82	19-34
Linoleic	6.48-11.6	48-60
Linolenic	0.72-2.16	2-10
Arachidic	0.04-0.7	< 1.0

Table 7. Fatty acid composition of soybean seeds and oil (ENV/JM/MONO (2001)15)

The concentration of mineral components in the soybean seeds depend on different factors and the most of all on origin, conditions of tillage, variety and technological process. The soybean products contain the considerable quantities of phosphorus. In the region of

intensive animal production the phosphorus content in the fecal excretion is limiting. The phosphorus content in the feeds influence on the its excretion.

| Mineral components | Soybean seeds | Soybean meal | | SBM Banaszkiewicz(2000) |
		Mechanical extracted (cakes)	Solvent extracted 44% CP	
Ca	2.62	2.96	3.12	2.71
P	5.70	6.64	6.37	5.14
Mg	2.80	2.84	2.72	2.27
K	15.93	20.28	19.85	6.66
Na	0.29	0.33	0.18	0.30

Table 8. Content of minerals in seeds and soybean products, g/kg (Van Eys et al.2004)

The content of vitamins in soybean seeds and products shown in table 9. Soybean full fat contain 31 mg/kg of vitamin E ,soybean meals only about 3mg/kg and soybean expeller 6.6mg/kg. The big differences between soybean full fat and another soybean products occurs.

Vitamins	Soybean meal solvent	Soybean meal dehulled solvent	Soybean expeller	Soybean full-fat
E, (mg/kg)	3.0	3.3	6.6	31.0
Thiamin, (mg/kg)	1.7	1.7	1.7	6.6
Riboflavin, (mg/kg)	3.0	2.6	4.4	2.64
Pantothenic acid, (mg/kg)	13.3	13.2	13.8	15.6
Biotin, (µg/kg)	320	320	320	286
Folic acid, (µg/kg)	450	700	450	3542
Niacin, (mg/kg)	59.8	20.9	36.7	22.0

Table 9. Content of vitamin in seeds and soybean products
(http://www.soymeal.org/sbmcomposition.html)

Content of metabolizable energy in soybean seeds for poultry is about 15 MJ/kg DM and for pigs about 17MJ/kg DM. The metabolizable energy content for poultry in the soybean meal is about 9MJ/kg DM and for pigs about 13MJ/kgDM. Jiang (2003) collected soybean meal samples and appreciated the ME value of these soybean meals no relationship were found between the ME and crude protein or crude fat, but ME content was lower for higher crude fiber.

2.2.2 Anti-nutritional factors in soybean

Protease inhibitors

The nutritive value of soybean is limiting mainly by trypsin and chymotrypsin inhibitors, pectins and the protein about immunology activity. The most important there are the trypsin inhibitors - the Kunitz inhibitors and the Bowman-Birk inhibitors (Winiarska – Mieczan,2007). In animal cause they lowering the nitrogen retention, decreasing of performance results and increasing of metabolic nitrogen excretion.

Protease inhibitors (the Kunitz inhibitor and Bowman –Birk inhibitor) are active against trypsin and chymotrypsin (Liener, 1994). These inhibitors interfere with the digestion of proteins resulting in decreased animal growth. Activity of trypsin inhibitor range from 100 to 184 TUI/ mg of protein (Kakade et al.1972). The limit of activity for soy products is to 0.4 urease units. Thacker & Kirkwood (1990) report a range for trypsin inhibitors of 21.1 to 31.1 mg/g. The activity of these inhibitors in soybean products may be decrease by toasted or heated processes. The right warming up of soybean and its products eliminate above 90% of antitrypsin activity. The animals of several species differently react on trypsin inhibitors in feeds. Goslings and chickens are more sensitive on the present trypsin inhibitors than piglets and calves. There are a new cultivars of soybean in which the level of trypsin inhibitors were reduced to 10mg/kg of seeds (Kulasek et al. 1995). Chickens fed diets containing soybean seeds, where TIA level was low, characterized of better growth, and heating these seeds increased its growth yet, what was probably resulting of further decreasing TIA and inactivation of lectins and immunogenic proteins.

Lectins

Lectins (hemaglutinins) are proteins that bind to carbohydrates. In raw soybean can decrease growth and cause increase mortality rate in animals. The level of the lectins in soybean can vary from 37 to 323 HU /mg of protein (Kakade et al.1972). In soybean meal content of lectins joining carbohydrates carried out since 0.2 to 3.1g/kg and there are mainly agglutinating lectins (Fasina et al 2003; Maenz et al.1999). This strong influence of lectins practically disappearance after autoclaving.

Phytoestrogenes

Soybean contain a isoflavones. This compounds have got biochemical activity, including estrogenic, anti-estrogenic and hypocholesterolemic effects. Total isoflavones content ranged from 160.8 to 284.2 mg/100g (Hoeck et al.2000). The isoflavones in soybean and soy products have three types: daidzein, genistein and glycitein in three isomers and three forms. Totally, there are 12 isomers of isoflavones in soybean. The concentrations of total daidzein , genistein and glicitein carried out of 20.2-206 mg, 31.5-268 mg and 10.9-107 mg per 100g raw seed respectively (Douglas, 1996;Wang & Murphy, 1994). These compounds have been implicated in reproduction in animals fed diets containing large amounts of soybean meal (Schutt, 1976). On negative influence of izoflavones on broilers show results obtained by Payne et al.(2001), but a little of quantity of this components in feed for chickens have beneficial anti-carcinogenic effects (Messina & Barnes, 1991). The isoflavones content is greatly influenced by many factors. The soybean products are mainly source of isoflavones which executing important consideration in prevention of neoplasmic diseases and reduced the risk diseases of circulation tract (Radzikowski,2004). The interest of role and utilization of isoflavones increase in animal production, because was proved the influence on immunological tract and improvement of performance results and quality of trait (Payne et al.2001; Kerley & Allee,2003).Lee et al. (2003) show the total content of isoflavones in Korean soybean cultivars range from 110 to 330mg/100g of feeds.

Stachyose and raffinose

The stachyose and raffinose are low molecular weight carbohydrates. These compounds are present in toasted soybean meal, as well as in raw soybean seeds (Padgette et al.1996).The raffinose content of soybean seeds ranges from 0.1 to 0.9g/100g on fresh weight basis and stachiose is from 1.4 to 4.1g/100g (Douglas, 1996; Hymowitz et al.1972).

Phytates

Phytic acid chelates calcium, magnesium, potassium ,iron and zinc rendering them unavailable to non ruminant animals. A lot of phytates in diets decrease availability of this minerals, mainly calcium, phosphorus and zinc. The phytates decrease also activity of enzymes (pepsin, trypsin and amylase) as well as availability of protein , amino acids , starch and energy (Sebastian et al.1998;Ravindran et al.2000). Phytates influence on decrease of feed consumption by chickens as well as their growth (Shan& Davis,1994). Liener (2000) estimated that two-thirds of the phosphorus in soybean is bound as phytate and unless freed is mostly unavailable to animals. Phytic acid is present in soybean and most soybean products at level 1-1.5g/100g of the dry matter.

Allergens

The allergenic effect is attributed to the globulin fraction of soybean proteins. In the soybean seeds the globulins comprise about 85% (80-90%) of total protein (Shinbasaki et al.1980).The most important allergens of soybean are GLY 1 and GLY1B - glicynine and beta-conglicynine (Świderska- Kiełbik et al. 2005). Soybeans contain several antigenic proteins which can stimulate the immune system sensitive of calves, pigs and human (Pedersen,1988). These proteins are not sensitive on temperature. The denaturation of beta-konglicinine needs of temperature about 75oC. Allergens were ascertained also in lecitine of soybean, which is described as occupational allergens at the bakers. The allergic activity can also show tryptase inhibitor present in soybeans. According Moroz & Yong (1980) the heating increase allergenic proprieties of soybean. Now appeared reports on small quantities of soy protein in meat of chickens fed diets contained 25% of soybean seeds (Świderska- Kiełbik et al. 2005).

Pectins

They are very important anti-nutritional substances (Pusztai,1991). They components belong to sensitive substances on high temperature. They answer for agglutination in alimentary tract and mitosis. The thermal processing is little effective to this soy antigens.

Micotoxins

Most mikotoxins in soybean products there is the ochratoxins (mushrum‚s products of Aspergillus ochraceous or Penicyllium varrucosum) and zearalenon as product of Fusarium graminearum. They come into being during storage in bad conditions (high moisture and temperature >20oC). These products show estrogenic activity which can cause disturbance in reproduction. The particular sensibility on zearalenon is observed at pigs. The level of these substances over 1 ppm caused problems in reproduction. The afla-micotoxins in soybean products occur rather seldom.

Oligosaccharides

Oligosaccharides are substances can cause of flatulent problems, decrease of digestibility of nutrients and hypertrophy of intestines (Salgado et al.2002). They can also influence on quantity of microorganisms in intestines (Rubio et al. 1998). The reaction of animals on anti-nutritional substances in soybean depend on animal species and age. The adult ruminants are not sensitive on these substances , whereas the decrease of growth of chickens , pigs, calves and rats were observed, when raw soybean was given. In the feeding of this animals should been used of heated and toasted products. The level of TIA in this products should not exceed 10 mg/kg. Reaction of hens and adult of pigs on raw soybean seeds is less,

therefore in mixtures for this animals can use a little quantity raw soybean products without fear decrease of this performance.

Specification	Soybean seeds (Hanssen,2003; Peisker,2001)	Soybean meal 44%CP (Hanssen,2003; Peisker,2001)	Soybean meal
Oligosaccharides,%	14	15	50-60g/kg[Coon et al.,1990; Kocher et al. ,2002]
Stachiose,% Rafinose,%	4-4.5 0.8-1	4.5-5 1-1.2	
Trypsin inhibitor,mg/g CP Chymotrypsin inhibitor	45-60	4-8	1-8mg/g [Peisker,2001] 10.7mg/g[Douglas et al.,1999] 30.3mg/g[Douglas et al.,1999]
Lectins, ppm	50-200	50-200	0.22-3.1g/kg chelated of sugar, in it0.01-0.87aglutinin[Maenz et al.,1999;Fasina et al.,2003]
Saponins,%	0.5	0.6	600mg/kg[Peisker,2001]
Glicynina mg/g Beta konglicynina,mg/g	150-200 50-100	40-70 10-40	- -
Phytic phosphorus,%	0.6	0.6	8.9g/kg[Glencross&Carter,2007]11.2mg/kg[Refstieetal.,1999] 19.9mg/kg[Glencross,2004]

Table 10. Content of anti-nutritional substances in soybean

2.3 Technological processes improved nutritional value of seeds and soybean products

Raw soybean seeds as well as the soybean products contain many anti-nutrient factors, which decrease their nutritional value and conduct to depression of animals performance and worsening of this health (Liener,1994).Adequate heat processing inactivate these factors. The soybean requires the processing in aim to elimination of anti-nutrient factors particularly in non ruminants feeding. Before of purchase the soybean meal should to be testing , if it was toasted (Van Eys et al.2004) . During to heat of soybean seeds (100-105oC) follow the changes in protein structure and decomposition of trypsin inhibitors. In result of this process the availability of lysine, methionine and cystine increase. Under the heat the trypsin inhibitors are inactivating. The effectiveness of these process depend on temperature and time of working , moisture of seeds as well as the degree of their crumbling. The use of too high temperature cause decrease of protein and some amino acids availability, especially lysine. The measured heating of seeds prevent of decomposition protein in rumen of ruminants, while too strong heating decrease of this utilization also at ruminants. Use of optimal temperature and time of its working can increase of digestibility and availability of protein and amino acids.

The results in table 11 shows that the digestibility of amino acids (lysine, methionine and threonine) increased as the result of autoclaving at 121oC for 0-18 minutes. This improvement in digestibility was the result of the destruction of anti-nutritional factors by the heat treatment.

Autoclave time (minutes)	Lysine	Methionine	Threonine
0	73	65	64
9	78	70	68
18	87	86	82

Table 11. Effect of autoclaved of raw soybean seeds on digestibility of amino acids in chickens,% (Anderson-Haferman et al.,1992)

The excessive hot processing can decrease content digestibility and availability of amino acids. It regards mainly lysine, which can bind in non available products with carbohydrates.

Autoclave time at 121oC(minutes)	Lysine	Cystine	Methionine	Threonine
Digestibility coefficients,%				
0	91	82	86	84
20	78	69	86	86
40	69	62	83	84
Concentration of amino acids ,%				
0	3.27	0.70	0.71	1.89
20	2.95	0.66	0.71	1.92
40	2.76	0.63	0.71	1.87
Concentration of available amino acids,%				
0	2.98	0.57	0.61	1.59
20	2.30	0.46	0.61	1.65
40	1.90	0.39	0.59	1.50

Table 12. Effect of autoclaved of soybean meal on digestibility, concentration and available of amino acids ,% (Dudley-Cash n.d.; Parsons et al., 1992)

The data show on reduction in concentration of lysine and cystine as a results of autoclaved for up to 40 minutes. There was little or no effect this process on methionine and threonine. Under heated soybeans have reduced amino acid digestibility, which reduces growth performance. In the result of processes with high temperature the reduction in protein quality was observed.
The anti-nutrient substances such protease inhibitors, lectins, pectins, urease, lipooxygenases and anti-vitamin substances are decomposed in high temperature or in fermentation processes (Liener,2000). In soybean the anti-nutrients sensitive substances there are trypsin inhibitors, pectins and goitrogenes substances(Liener, 2000).Some substances no destruction in use of temperature: faintly digested of carbohydrates, saponins, estrogens, cyjanogens and phytates (Liener,2000). The utilization of soybean products by animals depend at remainder of anti-nutritive substances. Protein dissolvent is correlated with gain of poultry and pigs (Araba & Dale, 1990).
Protein dissolvent in raw of soybean seeds and soybean products subjected of strong heating should to be about 90%. With protein dissolvent below 72% the decrease of animal performance was observed. These meals are necessary thermal prepared. The urease index does not change much in the first minutes of heating and next suddenly decrease. The

soybean protein quality for ruminants depend from quickly of destruction in rumen and intestine digestibility.

Autoclaved120° C (minutes)	Body gain of chickens, g	Feed efficiency kg/kg	PDI(protein dissolvent), %	Urease index (change pH)
0	450a	1.79	86.0	0.03
5	445a	1.87	76.3	0.02
10	424a	1.83	74.0	0
20	393b	1.89	65.4	0
40	316c	2.04	48.1	0
80	219d	2.55	40.8	0

Table 13. Effect of autoclaved on performance, protein dissolvent and urease activity (Araba and Dale,1990)

Product	PDI,%	Trypsin inhibitor activity, mg/g	Pectins mg/g	Antigens, mg/g
Soybean flour non toasted	90	23.9	7.3	610
Soybean flour slightly toasted	70	19.8	4.5	570
Soybean flour toasted	20	3.1	0.05	125
Concentrate of soybean extracted	6	2.5	<0.0001	<0.02

Table 14. Concentration of anti-nutritional factors in soybean products subjected to technological processes (Huisman & Tolman,1992)

Species	Soybean meal mechanical extracted	Soybean meal solvent- extracted 44%CP	Soybean meal solvent- extracted 48%CP
Pigs :			
- digestible energy ,kcal/kg	3394	3446	3776
- metabolizable energy, kcal/kg	2986	3210	3299
- net energy, kcal/kg	1903	1955	1992
Poultry:			
-apparent metabolizable energy for hens, kcal/kg	2179	2208	2464
- apparent metabolizable energy for chickens, kcal/kg	1929	1973	2147
Ruminants:			
- net energy for cows,kcal/kg	1706	1748	1826
- net energy for beef cattle,kcal/kg	1838	1847	1993

Table 15. Energy value of soybean meal obtained different technologies (Van Eys et al.2004)

In before rumen soybean meals are strong destruction and making up of source protein for microorganisms, but the part of protein which no destruction   is nonsufficient   for ruminants with high production. Energy value of soybean products depends on technique processes, which influence on chemical composition, digestibility and availability of nutrients.

The most suitable methods for protection of soybean proteins before destruction in rumen is thermal processing. The soybean products are sensitive on oxygenation, because contains the high of unsaturated fatty acids, mainly linoleic . The fatty acids composition is very important trait of fats from nutritional consideration. Lately in soybean oil the level of palmitic , stearic and oleic acids increased and linoleic and linolenic decreased.

2.4 Another high protein feeds as part of substitute of soybean meal

The withdrawal the animal meals from mixtures improved the microbiological quality of mixtures (the decrease of the Salmonella occurrence for poultry),but it simultaneously yet more worsened the balance of protein in the animals feeding, increase of prices of high protein feeds and demand on soybean meal. Soybean meal is the dominating protein feed in poultry feeding. As soybeans are only grown in North and South America, most countries have to import it. About choice of soybean meal to mixtures decide the high protein and amino acids content and accessibility big consignment of homogenous nutritional value of its feed. Increase price of soybean meal and deficiency protein source coerce to look for alternative source of protein. According Święcicki et al. (2007) is possibility of partial replacement of imported soybean meal using of protein from domestic sources (rape products and legume seeds).

Lately in Poland increased area of rapeseed cultivation from 550 to 750th.ha. This is bound up with program the component to fuel production. In 2010 in Poland the rapeseed crops nursed about 2.72mln.ton what supply about 0.5mln ton of protein. The polish cultivars and foreign registration in Poland are crossbreeding cultivars, non GMO. The Polish cultivars contain 2-3 time less of glucosinolates then foreign.

Lately in Poland was processed about 1.6mln ton of rape seeds and obtained about 700thou.ton of rapeseed meal. On the feed market increase supply rape cakes products which remaining after extraction of oil from rapeseed without chemical process. This is connected for extraction of oil, mainly to produced of bio-components by ecological methods (Nystrom et.al.1996). Lately rape cake production increased, as different technologies of processing rapeseed oil are preferred as more environment friendly. Low glucosinolate rape products are appropriate alternatives to soybean meal as a vegetable protein source in broiler diet. The rape meal contain abort 35% of protein, and about 2-3% of fat. In dependent on efficiency of press to extraction of oil the rape cakes contains 28-32% of protein and from 9 to 16% of fat.

Banaszkiewicz (2000) published that rapeseed is characterized by high protein (21-22%), fat (above 40%), total phosphorus (6-8g·kg-1) and gross energy content (26-27MJ·kg-1). About 75% of total phosphorus is the phytate form. Rapeseed contains about 8% crude fiber and 20% N-free extracts. The non-starch polysaccharides in rapeseed oil meal contain up to 35% dry matter. Total non-starch polysaccharides in rapeseed products contains about 200g (Knudsen ,1997) and have negative effect on nutrients digestibility, mainly crude fat and amino acids. Amino acid composition of rapeseed protein is beneficial:6g Lys; 2g Met; 4.6g Thr and 1.2g/16g N of Trp (Banaszkiewicz,2000).

Specification	SBM	Rape seeds	RSM	Rape cakes
Dry matter,%	92	93	92.5	92.37
Crude ash,%	6.5	5.3	7.2	5.57
Crude protein,%	44	22.5	36.5	28.82
Crude fat,%	1.9	42	2.4	25.98
Crude fibre,%	6.7	8.2	6.7	8.49
NDF,%	14.5	24.5	29.5	25.3
ADF,%	9.5	20.5	22.5	19.7
N-free-extracts,%	15.5	15.5	33.9	23,51
Lysine,g/100gCP	6.3	6.0	5.5	6.11
Methionine+ Cystine,g/100gCP	3.1	4.6	4.3	3.97
Threonine,g/100gCP	3.7	4.5	4.2	4.72
Tryptophan,g/100gCP	1.2	1.2	1.2	1.45
Ca, g/kg	3.2	3.4	6.3	5.9
P, g/kg	5.8	7.3	11.2	8.35
Mg, g/kg	2.5	2.8	4.6	3.26
Na, g/kg	0.4	0.2	0.4	0.16
Metabolizable energy, MJ/kg:				
pigs	13	19	11	15
poultry	9	16	8	13.5
ruminants	11.5	17	9	14.3

Table 16. Comparison of chemical composition and energy value soybean meal and rape products (Krasucki & Grela, 2004; Strzetelski,2006; Banaszkiewicz ,2000)

The digestibility of some amino acids in rape meal is less than that of soybean meal (Jondreville et al.2000). Digestibility of rapeseed and rape cake proteins for poultry is 70% and 76% respectively (European Table of Energy Values, 1986) and of rapeseed meal protein - 80% (Pastuszewska et al.,1987). Digestibility of rapeseed and rape cake fat is 90-98% (European Table of Energy Values, 1986). Fat digestibility stated by Banaszkiewicz (1995) was about 60-70%. Digestibility of N-free extracts ranges from 37 to 68% (Smulikowska et al.,1997) and 22-32% (European Table of Energy Values, 1986).

Amino acids	Soybean meal	Rapeseed meal
Protein	88.4	73.6
Lysine	90.5	70.7
Threonine	86.8	73.2
Methionine	93.0	86.0
Cystine	87.4	77.7
Tryptophan	89.4	74.8
Arginine	94.6	83.9

Table 17. True intestine digestibility of protein and amino acids,% (Jondreville et al.2000)

The rapeseeds products are limited by the nutritionally unfavorable substances such as glucosinolates, sinapin, tannin, phytate (Ciska & Kozłowska, 1998), but also by high content of dietary fiber and non starch polysaccharides (Kocher et al.2000).Rape products are characterized by low available energy and phytate phosphorus of about 25% (Nwokolo &

Bragg,1980). A study by Kocher et al. (2001) showed that rape meal (CM) can replace SBM in broiler diets even at high inclusion levels without any loss in performance, but according Bell (1993) the level of indigestible carbohydrates increased compared with soybean meal. The fiber is restrictive component to digestibility of protein, fat, absorption amino acids and fatty acids.

Nutrient	Soybean seeds	Soybean hulls	Rape seeds	Rape hulls
Dry matter	92.0	91.0	91.5	87.5
Crude ash	5.5	5.1	4.2	4.0
Crude protein	42.8	12.1	22.5	14.2
Crude fat	18.8	2.1	40.1	9.9
Acid Detergent Fiber	10.0	50.0	16.8	47.7

Table 18. Chemical composition of soybean and rape seeds and its hulls,% (Banaszkiewicz, 2000; Ensminger et al.,1990)

Soybean hulls contain about 12% of crude protein and rapeseed hulls about 14%. In the hulls are high of acid detergent fiber (ADF), about 50% in soybean hulls and 47.7% in rapeseed hulls compared to 10% in soybean seeds and 16.8%in rape seeds. Obtained of tree-000 of cultivars in Poland is advanced.

The nutritive value of rapeseed feeds may be improved by enzyme addition (Lesson & Caston, 1996; Kocher et al., 2001). Addition of carbohydrates to the basal wheat-rapeseed diet, may influence the nutritive value of rapeseed meal, but an inclusion of rapeseed meal to replace SBM, and an addition of Roxazyme G or Ronozyme VP to canola meal diets did not significantly affect broiler performance, but the mortality in birds fed canola meal diet was significantly reduced compared with the mortality in birds fed soybean meal (Kocher et al., 2001).

Dietary treatment	Group	Body weight g/bird	Feed intake g/bird	Feed conversion (FCR)g/g gain	Mortality %
Soybean meal	Control	1979	3520	1.779	4.1a
Canola meal	Control	1987	3525	1.779	1.2b
Canola meal	Enzyme A	2017	3579	1.775	2.9a
Canola meal	Enzyme B	2024	3603	1.781	2.8a

a,b. – means in rows followed by different letters are significantly different ($p \leq 0.05$)

Table 19. Growth performance and mortality of broiler chickens fed soybean and canola diets (Kocher et al. 2001)

Further improvement of nutritive value of diets can be obtained by the combined introduction of enzymes. Wu et al. (2004) reported that the combined addition of phytase and xylanase to wheat-based diets significantly increased the value of AME. Banaszkiewicz et al. (2009) reported that the rape cake from Kaszub cultivar supplemented with enzyme could partially replace soybean meal in wheat-based diets without any detrimental effects on chickens performance.

Supplementation with enzyme preparations can degrade dietary fiber and improve digestibility of crude fat and protein (Mikulski et al., 2000). Banaszkiewicz et al. (2009) reported that the simultaneous application of phytase and xylanase to wheat-soybean –rape diets significantly increased digestibility of crude protein, fat, fiber and N-free extracts.

Specification	Groups				SEM
	(SBM)	(RC XY)	(RC FYT)	(RCXY+FYT)	
Body weight at 1 day, g	40	40	41	40,5	1.92
Body weight at 21 day, g	672	692	679	672	9.92
Body weight gain 1-21days, g	631	649	633	628	11.27
Feed/gain ratio, kg/kg 1-21 day	1.55a	1.59a	1.67b	1.62ab	0,02

a,b. – means in columns followed by different letters are significantly different (p≤ 0.05)
SEM- pooled standard error of mean

Table 20. Body weight gain and feed efficiency of broilers (Banaszkiewicz et al.2009)

Groups	Digestibility, %				
	Crude protein	Crude fat	Crude fiber	N-free extracts	Crude phosphorus
(SBM)	88.20a	87.43a	18.06c	82.4a	57.93a
(RC XY)	87.92ab	71.24b	42.76a	79.15b	54.18a
(RC FYT)	85.82c	65.89c	37.67b	76.48c	50.10a
(RC XY+FYT)	86.30bc	67.24c	46.58a	79.49b	55.91a
SEM	0.37	2.28	2.90	0.63	1.25

a,b. – means in columns followed by different letters are significantly different (p≤ 0.05)
SEM- pooled standard error of mean

Table 21. Apparent digestibility of nutrients in experimental diets (Banaszkiewicz et al.2009)

Digestibility of protein in the control group (soybean meal) was about 88% and for diets containing rape cakes was significant higher and ranged from 85.8 to 87.9%. The highest digestibility of fat for control –soybean group was found- about 87%, when for groups contained rape cakes was from about 66 to 71%. Partial replacement of soybean meal with rape cakes supplemented with an enzyme preparations containing phytase or xylanase individually or in combination had not statistical effect on digestibility of crude phosphorus (Banaszkiewicz et al.,2009). The rape cakes with regard on high protein and fat may be good substitution of imported soybean meal particularly in mixtures where animal products (meat - bone meal and fats) were applied. Use of rape cakes on fodder aims maybe limited import of soybean even about 30-40% i.e. (about 500-600 thou. ton) a year (Strzetelski 2006).

3. Conclusion

The soybean meal characterized the highest content of protein and amino acids and its good point are big consignments on homogenous nutritional value. In the regard of big quantity of soybean meal in mixtures the small difference in nutritional value in this feed may have influence on balance of nutrients in diets and animal performance. Rapeseed may be one alternative to soybeans. If 25% of soybean meal in mixtures for poultry was replace by rapeseed meal the quantity of imported soybean meal may decrease of about 0.18-0.2mln ton. On the ground of investigations conducting with rape cakes were ascertained that rape cakes can replace about 15-20% of soybean meal, but efficiency of rape product will be at about 15-20% worse. The lower intestine digestibility of amino acids for rape cakes (70-75%) than for soybean meal (90-92%) is this cause. The rape products can completely replace soybean meal in fattening pigs and cows. It is possibility of replacing soybean meal by

domestic feeds in mixtures for poultry, mainly for broiler chickens at level of 300 thou. ton, in mixtures for pigs -180 thou. ton and for cattle 20 thou. ton. In general domestic high protein feeds can replace about 500 thou. ton of soybean meal what make about 25% of imported soybean meal. In connection with low utilization of fat from rape cake, the investigations relating of its utilization should be conducted. One ought to limit of variability in chemical composition and nutritive value of rape cake. In the regard of increasing interest of rape cultivation one should expand a system of small oil mill. These ought decrease cost of transportation of materials and products and influence of higher utilization of rape cake in animal nutrition. In Poland obtained about 3mln ton of rapeseeds. If the take cognizance of that from 100kg of rapeseeds are obtained the 40kg oil and 60kg rape meal or rape cakes, it is possible to production of about 1.8 mln ton of feeds with high protein content and about 0.6 mln ton of crude protein.

4. References

Anderson-Haferman, J.C., Zang, Y., Parsons, C.M.,& Hymowitz, T. (1992).Effect of heating on the nutritional quality of kunitz - trypsin- inhibitor-free and conventional soybeans for chickens. *Poultry Science*, Vol.71,pp.1700-1709, ISSN 0032-5791

Araba, M. & Dale, N.M. (1990).Evaluation of protein solubility as an indicator of over-processing of soybean meal. *Poultry Science*, Vol. 69, pp.76-83, ISSN 0032-5791

Banaszkiewicz, T.(1995). Nutritive value of rapeseed 00 and oil for chickens (in Polish). *Biuletyn Naukowy Przemysłu Paszowego*,Vol.1, pp. 43-54, ISSN 1231-8337

Banaszkiewicz, T. (2000). Nutritive value of new rape cultivars stated in the tests for broiler chickens (in Polish). *Thesis University of Podlasie*, No. 61, pp.1-99, PL ISSN 0860-4371, Siedlce, Poland

Banaszkiewicz, T., Borkowska, K.,& Kot, B. (2009).Effect of high rape cake content supplemented in enzymes on the nutritional value of a broiler diet and intestinal lactic acid bacteria number. *Acta Veterinaria (Beograd)*,Vol. 59, No. 5-6, (April 2009), pp.535-545, ISSN 0567-8315

Bell, JM.(1993). Factors affecting the nutritional value of canola meal. *Canadian Journal of Animal Science*, Vol.73, pp.679-97, ISSN 0008-3984

Berk, Z.(1992). Technology of production of edible flours and protein products from soybeans. In: Soymilk and related product. Chapter 5, *FAO Agricultural services Bulletin*, No 97, ISSN 0022-3166

Brzóska, F. (2009). Is it possible to replace GMO protein with other protein source, Part I (in Polish). *Wiadomości Zootechniczne*, Vol. XLVII, No.1,pp.3-9, ISSN 1731-8068

Brzóska, F., Koreleski, J., & Korol, W. (2009).Possible consequences of a ban on genetically modified (GM) soybean in animal feed (in Polish). *Wiadomości Zootechniczne*, Vol. XLVII, No.3,pp.3-10, ISSN 1731-8068

Ciska,E.,& Kozłowska, H.(1998). Glucosinolates of cruciferous vegetables. *Polish Journal of Food Nutrition Science*, Vol.48, pp. 5-22, ISSN 1230-0322

Coon, C.N., Leske, K.L., Akavanichan, O., & Cheng, T.K.(1990). Effect of oligosaccharide-free soybean meal on true metabolizable energy and fiber digestion in adult roosters. *Poultry Science*,Vol.69,pp. 787-793. ISSN 0032-6791

Douglas, J.S.(1996).Recommended compositional and nutritional parameters to test in soybeans. *Report No A59915.Technical Assessment Services*, Washington D.C.

Douglas, M.W., Parsons C.M.,& Hymowitz T.(1999).Nutritional evaluation of lectin-free soybeans for poultry. *Poultry Science*,Vol.78,pp.91-95, ISSN 0032-6791

Dudley-Cash, W.A.(n.d.). Soybean Meal Quality. American Soybean Association, *www.sbm_qual.pdf*

Ensminger, M.E., Oldfield J.E., & .Heinemann W.W. (1990). *Feeds and Nutrition*. The Ensminger Publishing Company, ISBN 0941 21 80 82,Clovis, California

ENV/JM/MONO(2001)15. Unclassified. 2001. Series on the Safety of Novel Foods and Feeds No.2, Consensus Document on Compositional Considerations for New Varieties of Soybean: Key Food and Feed Nutrients and Anti-nutrients, 30-November-2001

European Table of Energy Values for Poultry Feedstuffs. (1989). *WPSA,3ed Ed.* Beekbergen, The Niderlands

Fasina, Y.O., Classen, H.L., Garlich, J.D., Swaisgood, H.E., & Clare, D.A. (2003).Investigating the possibility of monitoring lectin levels in commercial soybean meals intended for poultry feeding using steam-heated soybean meal as a model. *Poultry Science,* Vol. 82, pp.648-656, ISSN 0032-6791

Glencross, B. (2004). Adapting lupines to use in fish diet- benefits and constrains. *Proccedings of the Third Workshop* Future for grains in aquaculture feeds.14 April 2005,Publ. Department of Fisheries, Fremantle , Western Australia, pp.42-49, ISSN 1447-2058

Glencross, B.& Carter C. (2007). Field peas and aquafeeds. *Proceedings of the Fourth Workshop* Harvesting the benefits of grain in aquaculture feeds. 13 February 2007. 42-49, Publ. Department of Fisheries Fremantle, ISSN 1447-2068, Western Australia

Hanssen, O.K. (2003). Soya is no soya: (soya proteins for feed products). *Feed International*, Vol.23,N0 9,pp.14-18 ISSN 0274-5770

Hoeck, J.A., Fehr, W.R., Murphy, P.A., & Welke, G.A.(2000). Influence of genotype and environment on isoflavone contents of soybean. *Crop Science*, Vol. 40,pp.48-51, ISSN 0011-183X

Huisman, J., & Tolman, G.H. (1992). Antinutritional factors in the plant proteins of diets for non-ruminants. In: *Recent advances in Animal Nutrition* , Garnsworthy, P.C., Haresign, W & Cole, D.J.A.(ed), Butterworth Heinemann (October 1992),3-32, ISBN 10: 0750607149, UK, Oxford

Hymowitz, T., Collins, F.I., Panczner, J., & Walker, W.M.(1972).Relationship between the content of oil, protein, and sugar in soybean seed. *Agronomy Journal*, Vol.64,pp.613-616, ISSN 0002-1962

Jiang, Z.Y. (2003). Know ME it pays. *Proceedings of the 11th annual ASA Southeast Asian Feed Technology and Nutrition Workshop.* Hanoi, 28 July

Jondreville, C.,van den Broecke, J., Gatel, F., Grosjean, F., Van Cauwenberghe, S., & Seve, B.(2000). Ileal amino acid digestibility and estimates of endogenous amino acid losses in pigs fed rapeseed meal, sunflower meal and soybean meal. *Canadian Journal of Animal Science*,Vol.80,pp.495-506, ISSN 0008-3984

Judziński,B.(2007).Śruta sojowa w polskiej przestrzeni gospodarczej. *Science Conference "* Skutki potencjalnego zakazu stosowania genetycznie modyfikowanych roślinnych surowców paszowych", Izba Zbożowo-Paszowa, Warszawa , 04.10.2007

Kakade, M., Siomons, N., Liener, I.,& Lambert, J. (1972).Biochemical and nutritional assessment of different varieties of soybeans. *Journal Agricultural Food Chemistry*, Vol.20,pp.87-90, ISSN 0021-8561

Kerley, M.S., & Allee G.L.(2003). Modification in soybean seed composition to enhance animal feed use and value; Moving from a dietary ingredient to a functional dietary component . *AgBioForum,* Vol. 6, No. 1&2,14-17, ISSN 1522936X.Guest Editors: Henry T. Nguyen & GaryStacey Available on the World Wide Web: http://www.agbioforum.org.

Knudsen, K.E.(1997).Carbohydrate and lignin contents of plant materials used in animal feeding. *Animal Feed Science and Technology*,Vol.67, pp.319-338,ISSN 0377-8401

Kocher A, Choct M, Porter MD., & Broz, J.(2000). The effect of enzyme addition to broiler diets containing high concentrations of canola or sunflower meal. *Poultry Science*, Vol. 79,pp.1767-74,ISSN 0032-5791

Kocher, A, Choct, M, Morrisroe,L., & Broz, J.(2001). Effect of enzyme supplementation of the replacement value of canola meal for soybean meal in broiler diets. *Australian Journal Agricultural Research*, Vol. 52,pp. 447-52, ISSN 0004-9409

Kocher,A., Choct, M., Porter, M.D.,& Broz ,J.(2002). Effects of feed enzymes on nutritive value of soybean meal fed to broilers. *British Poultry Science*, Vol.43,pp.54-63, ISSN 0007-1668

Krasucki W., & Grela E.R.(2004). Zastosowanie śruty poekstrakcyjnej rzepakowej w żywieniu zwierząt gospodarskich. *Ed. Polish Academy of Science, Ewico* sp.z.o.o, Lublin, Poland

Kulasek, G., Leonowicz, H., & Krzemiński, R.(1995).Bioaktywne substancje w pokarmach dla ludzi i zwierząt (cz.I). Czynniki antyżywieniowe. *Magazyn Weterynaryjny,* Vol.15, pp.39-44,ISSN 1230-4425

Lee, S.J., Ahn, J.K., Kim, S.H., Kim, J.T., Han, S.J., Jung, M.Y.,& Chung, I.M.(2003).Variation in isoflavone of soybean cultivars with location and storage duration. *Journal Agricultural Food Chemistry*,Vol.51,pp.3382-3389, ISSN 0021-8561

Lesson, S., & Caston, LJ. (1996). Adding Roxazyme to wheat diets of chicken and turkey broilers. *Journal Applied of Poultry Research*, Vol.5, pp.167-72,ISSN 1056-6171

Liener,I.E.(1994).Implications of anti-nutritional components in soybean foods. *Critical Reviews in Food Science and Nutrition*, Vol.34,pp.31-67, ISSN 1040-8398

Liener,I.E.(2000). Non-Nutritive Factors and Bioactive Compounds in Soy. *Soy in Animal nutrition Symposium* (August 4-5 Chicago, IL), (Ed. J.K. Drackley), Fed. An. Sci. Societies, Savoy, ISBN 10: 1884706010, IL,USA.

Maenz D.D., Irish G.G., Classen H.L.1999.Carbohydrate – binding and agglutinating lectins in raw and processed soybean meals. *Animal Feed Science and Technology*, Vol.76,No. 3-4, pp.335-343, ISSN 0377-8401

Messina, M., & Barnes, S.(1991).The role of soy products in reducing risk of cancer. *Journal of the National Cancer Institute*, Vol.83,pp.541-546, ISSN 0027-8874

Mikulski, D., Faruga, A., Majewska, T., Pomianowski, J., Jankowski, J.,& Mikulska, M. (2000). Effect of various NSP-hydrolysing enzymes on value of feed for slaughter turkeys (in Polish). *Zeszyty Naukowe Przeglądu Hodowlanego, Chów i hodowla drobiu*, Vol. 49, pp.343-52, ISSN 1230-5367

Moroz, L.A.,& Young, W.H. (1980). Kunitz soybean trypsin inhibitor. *The New England Journal of Medicine*, Vol. 302,pp.1126-1128, ISSN 0028-4793

National Research Council(NRC).(1998).Nutrient Requirements of Swine(Tenth revised Ed). *National Academy Press*, ISBN 0-309-05993-3, Washington DC. USA

Nyström, R., Pastuszewska, B., Buraczewska, L., Tulisalo, U., & Ochtabińska ,A. (1996).Effect of pressing technology of oil separation and heat treatment on the protein value of low-glucosinolate rapeseed cake for nonruminants. *Journal Animal Feed Science*,Vol.5,pp.235-248,ISSN 1230-1388

Nwokolo, E., & Bragg, DB. (1980). Biological availability of minerals in rapeseed meal, *Poultry Science*, Vol. 50,pp.155-8, ISSN 0032-5791

Padgette, S.R., Taylor, N.B., Nida, D.L., Bailey, M.R., MacDonald, J., Holden, L.R.,& Fuchs, R.L.(1996).The composition of glyphosate-tolerant soybean seeds is equivalent to that of conventional soybeans. *Journal of Nutrition,* Vol.126,pp.702-716, ISSN 0022-3166

Parsons,, C.M., Hashimoto, K., Wedekind, K.J., Han, Y.,& Baker, D.H. (1992).Effect of over-processing on availability of amino acids and energy in soybean meal. *Poultry Science*, Vol.71,pp.133-140,ISSN 0032-5791

Pastuszewska, B., Grala, W., & Gdala. J. (1987), Evaluation of chemical analysis and biological value of two-zero rape (in Polish). *Biuletyn Informacyjny Przemysłu Paszowego*, Vol. 3,pp. 3-9, ISSN 0137-1568

Payne, R.L., Bidner, T.D., Southern, L.L.,& McMillin, K.W.(2001).Dietary Effects of Soy Isoflavones on Growth and carcass traits of commercial broilers. *Poultry Science*, 80., 1201-1207.

Pedersen, H.E.(1988). Allergenicity of soy proteins. *Proceedings of the World Congress* Vegetable protein utilization in human foods and animal feedstuffs, Singapore. Ed. T.H. Applewhite , American Oil Chemist Society Champaign, ISBN 0-935315-25-X, Illinois ,USA

Peisker, M.(2001).Manufacturing of soy protein concentrate for animal nutrition. *Cahiers Options Mediterraneennes.* 3.Conference of Feed Manufactures of the Mediterranean, Reus (Spain) 22-24.03.2000, Vol.54,pp.103-107, ISSN 1022-1379

Poultry Feeding Standards.(2005). Ed. The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, ISBN 83-917097-7-9, Jabłonna /Warszawa, Poland

Prusiński, J. (2008). Is the needs in Poland the second PR 4. *Biuletyn Polish Association of Feed Producers* , Vol. 61, No.4/5,108-113, ISSN 0081-4539

Pusztai, A.(1991). Mitogenic stimulation of limfocytes. In: *Plant lectins (*ed. A.Pusztai). Cambridge University Press, ISBN 0-306-44057-1,Cambridge, UK

Radzikowski, Cz.(2004). Genistein: a soy isoflavone revealing a pleiotropic mechanism of action- clinical implications in the treatment and prevention of cancer. *Postępy Higieny i Medycyny Doświadczalnej*, Vol. 58,pp.128-139, ISSN 1732-2693

Ravindran,V., Cabahug G., Ravindran,G., Selle P.H., & Bryden, W.L.(2000). Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus levels. II. Effects on apparent metabolizable energy, nutrient digestibility and nutrient retention. *British Poultry Science*, Vol.41, pp.193-200, ISSN 0007-1668

Ravindran,V., Hew, L.I., Ravindran,G., &Bryden, W.L.(2005). Apparent ideal digestibility of amino acids in dietary ingredients for broiler chickens. *Animal Science*, Vol.81, pp.85-97, ISSN 1357-7298

Refstie, S., Svihus, B., Shearer, K.D., & Storebakken, T.(1999). Nutrient digestibility in Atlantic salmon and broiler chickens related to viscosity and non-starch polysaccharide content in different soybean products. *Animal Feed Science and Technology*, Vol.79,pp. 331-345, ISSN 0377-8401

Rubio, L.A., Brenes, A., Setien, I., de la Asuncion, G., Duran, N.,& Cutuli, M.T.(1998). Lactobacilli counts in crop, ileum and caecum of growing broiler chickens fed on practical diets containing whole and dehulled sweet lupin (Lupinus angustifolius) seed meal. *British Poultry Science*, Vol.39,pp. 354-359, ISSN 0007-1668

Rynek Pasz. (September,2010). Stan i perspektywy. IERiGŻ. ISSN 1428-1228

Rynek Rzepaku. (October,2010). Stan i perspektywy. IERiGŻ, ISSN 1231-269X

Salgado P., Freire, J.P.B., Mourato, M., Cabral, F., Toullec, R., & Lalles, J.P.(2002). Comparative effects of different legume protein sources on weaned piglets: nutrient digestibility , intestinal morphology and digestive enzymes. *Livestock Production Science,* Vol.74, pp.191-202, ISSN 0301-6626

Sebastian, S., Touchburn, S.P., & Chavez E.R.(1998).Implications of phytic acid and supplemental microbial phytase in poultry nutrition : a review. *World Poultry Science Journal*, Vol.54,pp.27-47, ISSN 0043-9339

Shan A.S.,Davis R.H.1994. Effect of dietary phytate on growth and selenium status of chicks fed selenite or selenomethionine. *British Poultry Science*, Vol.35,pp.725-741, ISSN 0007-1668

Shinbasaki ,M., Suzuki,S., Tajima, S., Niemoto, H., &Kuroume, T.(1980). Allergenicity of major component proteins of soybean. *International Archives of Allergy and Immunology,* Vol.61,pp.441-448,ISSN 1018-2438

Shutt, D.A.(1976).The effect of plant estrogens on animal reproduction. Endeavor ,Vol. 35,pp.110-113.

Sikorski, Z.E. (2007).*Chemia żywności*. Praca zbiorowa, t.1,WNT,.ISBN 978-83-204-3273-2, Warszawa.

Smith, KJ.,& Huyser W. (1987). World distribution and significance of soybean. In: *Soybeans: Improvement , Production , and Uses.* Wilcox J.R.(ed), Second edition. Agronomy Monographs No16: American Society of Agronomy, pp.1-22, ISBN 0-89118-090-7, Madison. WI

Smulikowska, S., Pastuszewska, B., Mieczkowska, A., & Ochtabińska, A. (1997). Chemical composition, energy value for chickens and protein utilization in rats of rapeseed expeller cakes produced by different processing technologies. *Journal of Animal Feed Science,* Vol. 6,pp.109-21, ISSN 1230-1388

Soybean meal composition. Soybean Meal INFOcenter. www.soymeal.org/sbmcomposition.html.

Strzetelski, J.(2006). The possibility of feeding cattle with by products of deep pressing of oil from oilseeds and bioethanol by products (in Polish), *Wiadomości Zootechniczne*, Vol. XLIV , no.3 ,pp.56-66, ISSN 1731- 8068

Świderska –Kiełbik, S., Krakowiak ,A., Witczak, T.,& Pałczyński, C.(2005). Occupational alergy: a major heath hazard among ford processing workers(in Polish). *Medycyna Pracy*, Vol. 56 No.6,pp.475-483, ISSN 0465-5893

Święcicki, W., Szukał, J., Mikulski, W.,& Jerzak, M.(2007).Possibilities to replace the soybean cake with domestic raw materials (in Polish). *Zeszyty Problemowe Postępów Nauk Rolniczych*, No. 522,pp.515-521, ISSN 0084-5477

Thacker, P.A.,& Kirkwood, R.N.(1990).Non-traditional Feed Sources for Use in Swine Production. Thacker (Eds.)*Butterworth,* p.441, ISBN 10 040 9901 903, Stoneham, MA., U.S.

Van Eys, J.E., Offner, A.,& Bach, A. (2004). Chemical Analysis. Manual of Quality Analysis for Soybean Products in the Feed Industry. American Soybean Association. *http//www.asa-europe. org/ Library/ library_e.htm.*

Wang, H.J., & Murphy, P.A.(1994). Isoflavone content in commercial soybean foods. *Journal of Agricultural Food Chemistry*, Vol.42,pp.1666-1673, ISSN 0004-9409

Winiarska-Mieczan, A.(2007). Bowman-Birk trypsyn inhibitors: their structure and value in human and animal feeding (in Polish). *Medycyna Weterynaryjna*, Vol.63, No.3, pp. 276-281, PL ISSN 0025-6828

Wu, YB., Ravindran, V., Thomas, DG., Britles, MJ.,& Hendriks, WH. (2004). Influence of phytase and xylanase, individually or in combination, on performance, apparent metabolizable energy, digestive tract measurements and gut morphology in broilers fed wheat-based diets containing adequate level of phosphorus. *British Poultry Science*,Vol. 45, No.1, pp.76-84, ISSN 0007-1668

Soy as a Functional Food

Jocelem Mastrodi Salgado and Carlos M. Donado-Pestana
University of São Paulo
Brazil

1. Introduction

Soybean (*Glycine max*, L.) has been part of Southeast Asia culture for almost 2 millennia. However, only in the second half of the 19th century has it started being used in the Western world coinciding with the Chinese migration to the USA. Today, USA, South America, especially Brazil, and Northwestern Europe, account for almost 90% of the world total soybean production. At first, the nutrition value of soybean was attributed to its high quality protein content thus attracting considerable interest for its use in human diet. Nowadays, it is known that soybeans are a rich source of phytochemicals, and many of those compounds have important beneficial effects on human and animal health.

Among the important phytochemicals in soybeans for human health, phytoestrogens, mainly, isoflavones (genistein and daidzein) and lignans, are the most widely studied. Nevertheless, saponins and phytosterols have also been the subject of research on soybeans. This chapter will discuss these phytochemicals compounds, their chemical structures, and their relationship with the major biological functions, scientifically proved, and their health benefits.

2. Phytoestrogens

Phytoestrogens are non-steroidal compounds found in plants. They demonstrate estrogenic and/or antiestrogenic activity and constitute a diverse group of compounds that have similar chemical structures and biological activity of estrogens.

The phytoestrogens can be divided into four main classes: isoflavonoids, flavonoids, coumestrol and lignans. Nearly all food vegetables have phytoestrogens although the amount and concentration of the compounds vary significantly. Some vegetables are high in phytoestrogens content such as flaxseed, which are a rich source of lignans; soybeans and chickpeas have high concentrations of isoflavones. Lignans can also be found in cereals, vegetables, and fruits (Kuhnle et al., 2009), whereas isoflavone-containing foods, may be specially related and limited to grains and soy products such as tofu, soy beverages, soy flour, and soy flakes among others products, due to its high consumption. Discussions about the chemical structure and major properties such as the main biological functions will focus particularly on this type of phytoestrogen.

2.1 Chemical structure and properties of phytoestrogens

Phytoestrogens are intrinsic plant compounds and their contents depend on a number of factors including the cultivar, place of production, planting or harvesting season, or growth-related factors.

Among the phytoestrogens, isoflavones are found mostly in plants, especially in the glycosides forms and are biologically inactive. Soybeans are a main source of phytoestrogens in the human diet. Flavonoids are widely distributed throughout the plant kingdom and are found in many vegetables, grains, herbs, and green tea. As for the coumestrol, its only sources are alfalfa sprouts and a variety of bean seeds. Chemical structures of the four classes of phytoestrogens are shown in Figure 1.
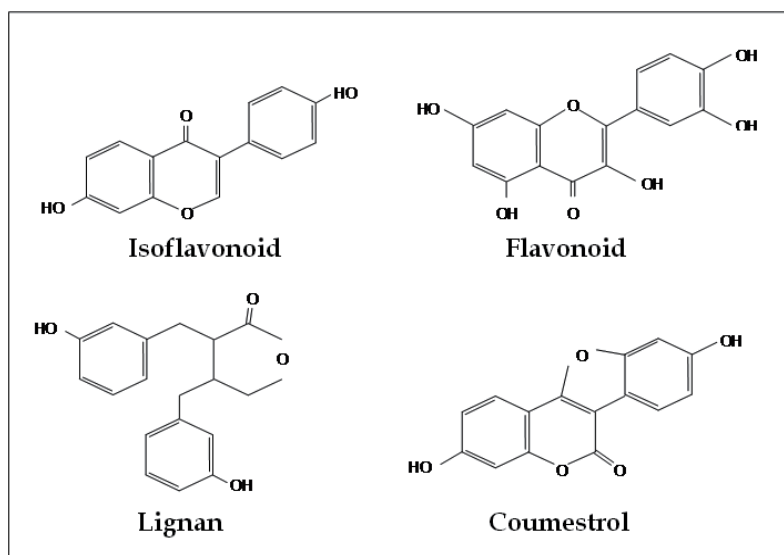
Fig. 1. Chemical structure of the major classes of phytoestrogens.

The contents of isoflavones in soybean and soy products have been extensively analyzed and studied. Those studies have demonstrated that the concentration and composition of isoflavones vary considerably, which can be explained by environmental and geographical conditions as well as by the level of industrial transformation. Daidzein, genistein, and glycitein are the most abundant isoflavones found in soy.

Isoflavones are naturally occurring compounds in foods as glycosides conjugated highly polar conjugated or non-conjugated form. For example, the textured soy protein and tofu have high contents of conjugated isoflavones such as daidzein and genistein, while fermented soy products such as miso, have approximately 90% of the isoflavones in non-conjugated form, mainly daidzein and genistein (Coward et al., 1993). Those chemical structures and their similarity with equol and estradiol (human estrogen) can be seen in Figure 2.

When ingested, phytoestrogens are metabolized by intestinal bacteria to equol (Cassidy, 1996; Setcehll et al., 1984). The metabolism of phytoestrogens in humans is probably facilitated and modulated by bacterial colonies; therefore, the colonic ecology might be associated with increased efficiency in conversion of dietary soy isoflavones to their bioactive form (Teas et al., 2009). After absorption, isoflavones undergo enterohepatic circulation and are primarily conjugated with glucuronic acid in the liver and then excreted in urine.

Numerous studies have shown that a number of factors influence the bioavailability of soy isoflavones. Xu et al. (1994) evaluated the bioavailability of daidzein and genistein from

soymilk and observed that daidzein was more bioavailable in adult women. The efficiency of absorption of soymilk isoflavones varies from 13% to 35% depending on individual gut microflora (Xu et al., 1995). Lampe et al., (1998) suggested that dietary fiber and other compounds in a diet rich in fibers might promote the growth and/or activity of bacterial colonies favorable to the conversion of daidzein to one of its catabolic products, equol, for a further absorption in the colon. Fermentation can reduce the isoflavones content in food products but increasing its bioavailability (Hutchins et al., 1995). Recently, it has been demonstrated in postmenopausal Japanese women that the bioavailability of isoflavones in fermented soy products rich in aglycones is much greater compared to the consumption of glucoside-rich non-fermented soybeans (Okabe et al., 2011).
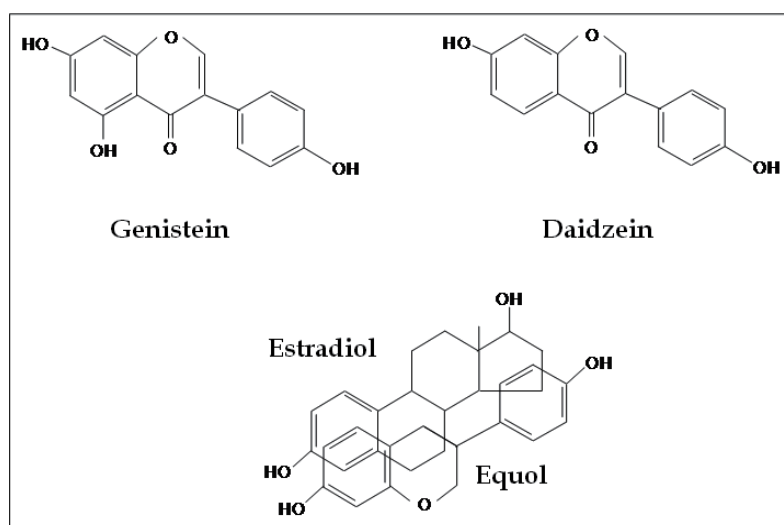
Fig. 2. Chemical structure of the major isoflavones found in soy and their similarity to equol and estradiol.

2.2 Mechanisms of action and biological functions

In recent years, it has been demonstrated that the phytoestrogens have multiple mechanisms of action. Such growing scientific evidence suggests that isoflavones are responsible for many of health benefits, which include the prevention and treatment of cardiovascular diseases, cancer, osteoporosis, and also for the relief of unpleasant pre- and post-menstrual symptoms.

2.2.1 Reduction in risk of coronary heart diseases

Coronary heart diseases are the leading cause of death especially in industrialized countries. High levels of total and LDL cholesterol are considered risk factors for these diseases. In humans, the consumption of 25g of soy protein per day may reduce the levels of total and LDL cholesterol

Preliminary results suggest that isoflavones, such as estrogens, may produce a cardioprotector effect directly on blood vessel walls and on other processes involved in the etiology of coronary heart diseases although the results are sometimes incompatible. Soy isoflavones act as potent antioxidants able to reduce the oxidation of LDL cholesterol and to induce vascular reactivity. The presence of modified LDL cholesterol in the blood vessel

walls contribute to the formation of atherosclerotic plaques and, according to studies in humans, soy isoflavones improve endothelial function and arterial relaxation.

Nagarajan (2010) suggested that soy isoflavones may inhibit the effect of endothelial cell activation associated to chronicle diseases such as atherosclerosis by blocking the activation of inflammatory cells and the adhesion to the vascular endothelium. This author concluded that the atherosclerotic protection of soy isoflavones is mediated through the regulation of monocyte activation (Figure 3). Another mechanism associated with soy isoflavones regarding cardiovascular health has been demonstrated by the reduction of vascular contraction through inhibition of the RhoA/Rho-kinase signaling pathway, which has a major role in muscle contraction (Seok et al., 2008).
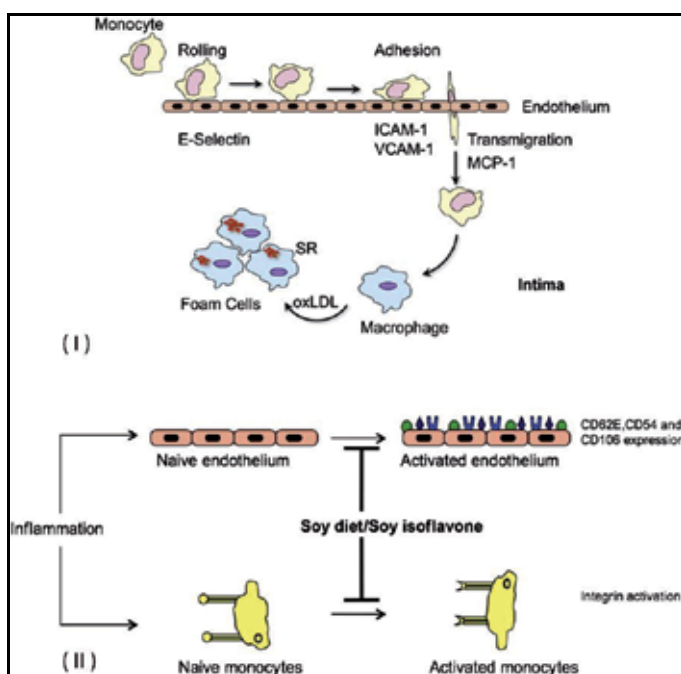
Fig. 3. (I) Inflammatory process associated with atherosclerosis. (II) Soy/isoflavone diet blocks endothelial and monocytes activation (Adapted from Nagarajan, 2010).

A prospective study carried out in Japan showed that the high consumption of isoflavones was associated with reduced risk of cerebral and myocardial infarctions in women, mainly in postmenopausal women, suggesting that the consumption of dietary isoflavones may be beneficial for the prevention of cardiovascular diseases (Kokubo et al., 2007*)*.

Isoflavones may have inhibitory effects on the adipose tissue that could help prevent obesity-associated diseases by improving the plasma lipid profile. Nevertheless, *in vivo* studies, especially in humans, have demonstrated that the mechanisms of action of soy isoflavones seem to be dependent upon interactions between some other factors, such as the presence of soy protein and intestinal bacteria *(Ørgaard & Jensen, 2008)*.

Cardiovascular health benefits of soy isoflavones are controversial. It has been suggested that a genistein supplemented diet (Villa et al., 2009) improves the glycemic and vascular reactivity indexes in postmenopausal women compared to the control. Nonetheless,

discrepancies in clinical studies may be associated with the differences in intestinal bacterial flora of subjects and, therefore, with the bioavailability of soy isoflavones metabolites, differences in dose-response effects, duration of isoflavones supplementation, and the limited number and metabolic status of subjects included in supplementation trials (Siow & Mann, 2010)

2.2.2 Anticancer effects

Most of the evidences of the phytoestrogens effects on humans are epidemiological and are based on the differences in the consumption of soy products in different areas of the world, considering soy products as the major source of isoflavones. A recent publication of a meta-analysis of a prospective study suggested that the consumption of isoflavones is associated with a reduced risk of breast cancer incidence in Asian populations (Dong & Qin, 2011). Accordingly, anticancer effects of soy isoflavones have been reported on prostate and colon cancer. The mechanisms that define the anticancer effects of isoflavones have also been reported in several studies that suggest various cellular pathways of the functional role as an anticancer agent.

Studies have demonstrated that isoflavones prevent the growth of a variety of cells including those that are not hormone-dependent, these effects are based on the capacity of isoflavones to inhibit the activity of enzymes that control cell growth. Recently, it has been proved that dietary genistein may reduce breast cancer progression via transcriptional regulation of Rho GTPases and PAK (Martínez-Montemayor et al., 2010). Genistein acts as an inhibitor of the tyrosine-kinase activity, essential enzyme in the biological control of cell growth and differentiation.

Another mechanism proposed for the anticancer activity of isoflavones was demonstrated by the inhibition of angiogenesis. Guo et al. (2007) found that soy isoflavones may inhibit prostate tumor angiogenesis through the suppression of vascular endothelial growth factor signaling pathways between tumor cells and vascular endothelial cells. Similar results were found by Su et al., (2005) in human bladder cancer cells lines. These authors demonstrated that isoflavones did not exhibit toxicity to normal bladder cells due to their angiogenic inhibitor effects.

In prostate cancer cell lines has been observed that genistein significantly decreased reactive oxygen species levels and induce the expression of antioxidant enzymes such as manganese, superoxide dismutase and catalase through AMP-activated protein kinase (AMPK) activation and increase PTEN expression (phosphatase and tensin homolog deleted from chromosome 10) (Park et al., 2010).

Based on *in vitro* studies, it has been proposed that isoflavones antagonize tumor cell growth in different cell lines by inhibiting cell cycle and inducing apoptosis. Studies have showed that isoflavones act in the activation of apoptotic pathways. Isoflavones may to induce the formation of Smad-DNA complexes and phosphorylation of Smad2 and Smad3 indicating increased TGF-β1 signaling, which has been associated with proapoptotic and antimitotic activities inducing the death of tumor cells (Davis et al., 2008; Yu et al., 2005).

2.2.3 Pre- and post- menopausal effects

Experimental and epidemiological evidences support the hypothesis that phytoestrogens have estrogenic and antiestrogenic effects in women. The biological effect varies according

to the woman's biological phase. Hence in premenopausal women, the phytoestrogens act as antiestrogens when the estrogen levels are high, and they act as estrogen in postmenopausal women when the estrogen levels are low (Messina, 2000).

2.2.3.1 Premenopause

Studies of controlled intervention in premenopausal women suggest that phytoestrogens diet may produce estrogenic effects (Cassidy et al., 1994, 1995). The interest in soy's hormonal effects on premenopausal women is based on potential antiestrogenic benefits evidenced in hormone-dependent cancers, such as breast cancer. Hence, frequent assays measure the plasma concentration of reproductive hormones and the menstrual cycle. A reduced risk of breast cancer is associated to a longer menstrual cycle, reduced estrogens, increase in sex hormone-binding globulin, and increase urinary excretion ratio of 2- to 16α-hidroxy estrogens (Kurzer, 2002). In a randomized study with 40 premenopausal women was demonstrated that a soy diet slightly increased menstrual cycle length by 1.8 ± 0.7 days and significantly increased urinary isoflavonoid excretion compared to women fed a control diet (Brown et al., 2002). In similar study, Hooper et al. (2009) found that the consumption of isoflavone-rich soy products significantly reduced concentrations of FSH and LH hormones that regulate the development, growth, puberty maturation, and reproduction processes in premenopausal women. Menstrual cycle length was increased by 1.05 days.

A recently published study, carried out in 73,223 premenopausal Chinese women demonstrated that the consumption of soy protein or isoflavones was inversely associated with the risk of breast cancer, and the association was highly statistically significant (P for trend < 0.001)). Women who frequently consume a high amount of soy foods during adolescence and adulthood had a very low risk of developing breast cancer (Lee et al., 2009). A daily intake of soy textured protein containing 45mg of isoflavones alters the menstrual cycle in healthy premenopausal women by prolonging its length, especially in the follicular phase. This effect was not observed with soy protein free of isoflavones, supporting the evidence that soy phytoestrogens act as an endocrine regulator. Similar effects of phytoestrogens on the menstrual cycle have been reported in other studies; nonetheless Phipps et al (1993) reported an increase in the length of the luteal phase. This result is not so easy to explain since changes in the luteal phase length are associated almost exclusively with changes in the follicular phase. The luteal phase is extremely constant and difficult to modify (Ferin et al., 1993).

2.2.3.2 Postmenopause

Epidemiological and clinical data indicate that postmenopausal estrogen therapy provides protection against cardiovascular diseases, reduces osteoporosis, improves the cognitive function, and relieves the menopausal symptoms related to the major loss of ovarian estrogen (Col et al., 1997). Alternative sources of exogenous estrogen have been extensively investigated due to a possible increased risk of breast cancer associated with hormonal replacement therapy (Brechkwoldt et al., 1995). Various studies have focused on verifying the potential of soy isoflavones as a source of exogenous estrogen.

During menopause, the ovarian production of estrogens decreases. A reduction of estrogen levels in the blood leads a series of characteristic symptoms of menopause, such as hot flashes, insomnia, excessive sweating, headaches, mood swings, nervous tension, irritability, depression and vaginal dryness and pain. A group of 145 postmenopausal women was fed a diet rich in phytoestrogens (soy and flaxseed) and a control diet for 12 weeks. The subgroup

of women fed a phytoestrogen diet presented a significant lower incidence of hot flashes and vaginal dryness and an increase in the serial phytoestrogen concentrations (Brzezinski et al., 1997).

A study involving 51 perimenopausal women who were fed a rich diet supplemented with isoflavones presented menopausal symptoms relief, reduction in the blood pressure, and improvements in the lipoprotein profile (Washburn et al., 1999). Similarly, a double-blind study involving 40 women who received daily doses of 100mg of isoflavones reported a decrease in menopausal symptoms and in the plasma levels of total and LDL cholesterol, which are risk factors for cardiovascular diseases (Han et al., 2002). Similar results were found in a study involving 58 postmenopausal women who consumed 45g of soy flour or 45g of wheat flour. Those who consumed the soy flour presented a significant reduction of hot flash (Warren et al., 2002).

2.2.4 Osteoporosis

By the time a woman reaches menopause, her bone density (peak bone density is reached at approximately 30 years of age) decreases rapidly along with a reduction in estrogen in the plasma. If the estrogen replacement treatment starts before the onset of menopause, it is possible to prevent bone density loss as well as the risk of cardiovascular diseases in postmenopausal women. However, estrogen hormone replacement can also cause an increase in the risk of endometrial and breast cancer.

The possibility that soy phytoestrogens may offer a natural alternative to the conventional hormone therapy for the prevention of bone loss has fostered research in animals and humans. Animal studies have used ovariectomized rats, although this is not an ideal model that simulates the influence of ovarian hormones on the reproductive physiology and bone loss in postmenopausal women; the findings are encouraging in terms of the protective effects of phytoestrogens. The consumption of soy isoflavone has demonstrated a significantly decreased in the number of osteoclasts and an inhibition of bone resorption after ovariectomy in this type of animals (Uchida et al., 2010). Analogously, it has been observed in ovariectomized rats, a reduction of urinary excretion levels of deoxypyridinoline, a specific biomarker of bone resorption, after the consumption of isoflavones with supplemental calcium (Breitman et al., 2003). Another study in rats demonstrated that genistein and moderate physical exercises prevented body weight gain and bone loss (Wu et al., 2004).

Epidemiologic studies have showed a lower incidence of osteoporosis in populations consuming diets high in soy, such as Asians, when compared to western populations. Hip fracture is 50-60% less frequent among Asian compared to western women although this benefit gradually disappears as Asians adapt a western lifestyle (Adlercreutz & Mazur, 1997; Roos et al., 1991, as cited in Lagari & Levis, 2010).

3. Saponins

In addition to the usual investigations on isoflavones as one of the main soy bioactive compounds, there has been a growing interest for investigating the functionality of soy saponins and their health benefits.

Saponins (from the Latin "*sapo*") constitute a vast group of glycosides that form foamy solutions in water exhibiting hemolytic and toxic effects in fish and invertebrates (Oleszek,

2000; Tsukamoto & Yoshiki, 2006). They are the major active constituents of many roots and corks that have been used by primitive people as a soap substitute (Fieser & Fieser, 1959).

Saponins are usually located in the seed, hulls, leaves, stems, and roots of plants (Carlson, 2009), and many of those compounds occur naturally even within a single vegetable species. Among the legumes, soy is one the main sources of saponins in the human diet (Lin & Wang, 2004).

Saponins have an antifungal activity and a major role in defenses against predators in plants. This function has been traditionally associated with an anti-nutritional factor in foods containing saponins, besides having limited their application due to their bitter taste. However, recent studies have demonstrated the role of these compounds in the prevention and control of chronic degenerative diseases.

3.1 Chemical structure and properties of saponins

Saponins are compounds that have amphiphilic structure, i.e. having polar and non-polar fractions. The polar fraction is represented by one or more hydrophilic sugars chains linked to hydrophobic aglycon, triterpen or steroidal called sapogenin.

Sapogenins are composed of carbon atoms in the form of fused rings. The sugars are in the form of oligosaccharides, linear or branched chains, although monosaccharides can also occur, such as the case of glucose and galactose. Both polar and non-polar groups are responsible for the beneficial biological effects.

The structure of saponins from different plant sources varies depending on the types and amount of sugars as well as the composition of the steroid ring (Rao & Sung, 1995). In vegetables, steroidal saponins are mainly found in monocotyledons, and triterpene saponins are predominately present in dicotyledons such as leguminous plants, in which soy is considered as one of their major food sources (Güçlü-Üstündağ & Mazza, 2007). Galactose, arabinose, rhamnose, glucose, glucuronic acid, and fructose are the most common sugars in saponin structures, and five sapogenins have been identified in soybeans (Figure 4). Soy saponins are usually classified into three groups: A, B, and E (Lin & Wang, 2004) suggesting that saponins group E are formed from group B saponins during extraction and analysis when the 22-hydroxyl group is oxidized to a ketone (Berhow et al., 2006).

As in most vegetables, the concentration of soy saponins depends on many factors, including the cultivar, age, physiological stage, geographical location, processing, and storage. Similarly, there are qualitative and quantitative variation also exists between plant parts (Oleszek, 2000).

The contents of saponins in soybeans can vary among the different genotypes. In a study conducted in the USA, 21 lines of soybean grown in five different environments were evaluated. The contents of saponins found were between 2209 and 5830 $\mu g.g^{-1}$ among the different lines and locations (MacDonald et al., 2005). The saponin content in the grain varies, and the highest amount is found in the hypocotyl, where there is a 4-fold accumulation compared to that of cotyledon and pod shell (Shimoyamada et al., 1990).

Saponins are thermal sensitive, and several studies have evaluated the characteristics and stability of saponins in a number of soy products. During processing and storage of soybeans, chemical modification of saponins can occur resulting in a change in the total content, composition, and properties/biological activity and properties, which may or may not be desirable (Güçlü-Üstündağ & Mazza, 2007).

The cooking process of legumes as soybean reduces the amount of saponins by 7-53% (Shi et al., 2004). Soy-based foods have different amounts of saponins; usually low values compared to those of raw soybeans. Soybean flour, soy protein isolate, lecithin, and tofu present reduced content of saponins, 50, 25, 73, and 37%, respectively, compared to that of the whole soybeans (Fenwick & Oakenfull, 1981). Soy-based foods obtained by ethanol processes, such as concentrated proteins, for example, present low content of saponins due to their solubility in alcohol (Murphy et al., 2008).
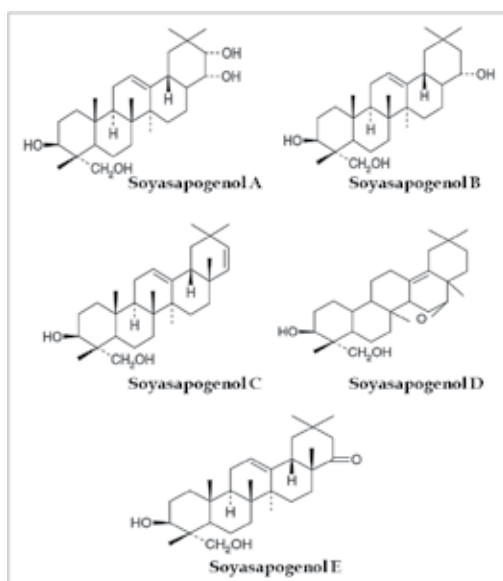
Fig. 4. Structures of five types of sapogenins identified in soybeans (Adapted from de Lin & Wang, 2004).

3.2 Mecahisms of action and biological functions
Due to recent advances in analysis and purification techniques of saponins, several studies have evaluated the functionality of these compounds influenced by the genetic polymorphism of their different structures (Tsukamoto & Yoshiki, 2006). Hence, saponins have been reported to possess a wide range of biological activities (Güçlü-Üstündağ and Mazza, 2007), mainly anticancer, antioxidant, hypocholesterolemic, and antiviral effects.

3.2.1 Anticancer and antioxidant effects
The biological oxidation caused by reactive oxygen species (ROSs) and free radicals is involved in biological processes such as aging and degenerative diseases such as cancer. The etiology and pathogenesis of cancer are multifactorial and involve multiple steps that culminate into complex disarray of cell signaling.

Today, there are no clinical studies evaluating the activity of saponins as an anticancer or antioxidant agent, and even considering the scanty experimental research in animals, information is still insufficient to provide pertinent conclusions. Most studies in the literature are limited to *in vitro* analysis with cell lines of colon, liver, and breast cancer, and several mechanisms of action are proposed, among which include direct cytotoxicity,

induction of apoptosis, antiestrogenic activity, inhibition of tumor cell metastasis, antimutagenic activity effect, bile acid binding action, and normalization of carcinogen-induced cell proliferation (Kang et al., 2010). Saponins (such as diosgenin, Figure 5) have been demonstrated to target multiple cellular and molecular pathways in their functional role as cancer chemopreventive agents.
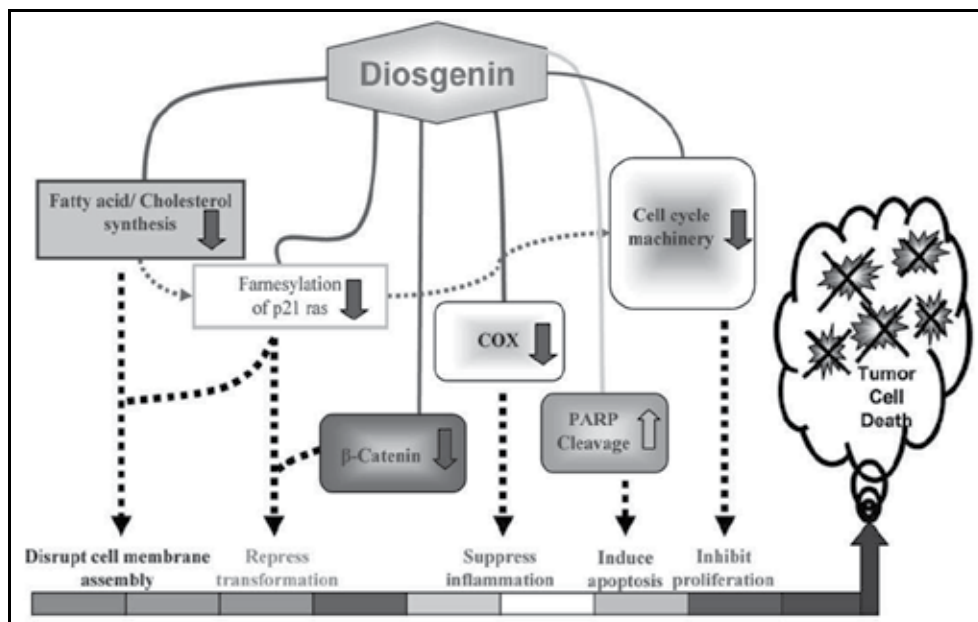
Fig. 5. Schematic representation of plausible mechanism of action(s) of diosgenin (saponin) at the cellular level as a cancer chemopreventive agent (Adapted from Raju & Mehta, 2009)

Soy saponins help preventing the development and reducing the number of tumors in rats (Konoshima & Takasaki, 2000). More recently, the effect of soy saponins on human colon cancer cells was evaluated leading to the conclusion that these compounds may be effective in preventing colon cancer by affecting cell morphology, cell growth, and cell proliferation enzymes. In this study, the saponins affected the cell growth in two different ways; by increasing the alkaline phosphatase activity while reducing protein kinase C activity to induce cell differentiation, or by inducing type II autophagic death (Tsai et al., 2010).

The antioxidant activity of saponins is also related to their capacity to bind to cholesterol and prevent cholesterol oxidation. Group B saponins, abundant in soy, linked with 2, 3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) group, are responsible for preventing lipid peroxidation or degeneration of DNA and protecting proteins from free radical attack (Ruiz et al., 1996; Shi et al., 2004; Yoshiki et al., 1994).

In addition to preventing cholesterol oxidation by the action of free radicals, group A and B saponins have antioxidant effects on rat liver microsome through the inhibition of lipid peroxidation (Nishida et al., 1993). Recently, a group of researchers isolated type I soy saponins, one of the main groups occurring in soybeans, showing that it presents a free radical scavenging activity comparable to that of α-tocopherol inhibiting lipid peroxidation. In this study was also demonstrated that the treatment with soy saponins increases the

superoxide dismutase and catalase activity, essential for the control of free radicals (Lee et al., 2010). Similarly, mice fed a soy extract rich in saponins exhibited a better profile of the antioxidants system with significant increase of superoxide dismutase, glutathione peroxidase, and glutathione S-transferase activities (Yang et al., 2011).

Saponins may have a synergistic antioxidative effect in the presence of hydrogen donors such as phenol compounds. Iron and copper ions generate hydroxyl radicals through the Fenton reaction to facilitate biological oxidation. Saponins may also have a preventive antioxidant that prevents active oxygen from being generated during the chelation of these metal ions (Tsukamoto and Yoshiki, 2006).

3.2.2 Hypocholesterolemic effects

The amphiphilic nature of saponins can explain the hypocholesterolemic activity of these compounds. This activity has been attributed to saponins for years (Sautier et al., 1979; Sidhu & Oakenfull, 1986), and, in general, is associated with their ability to interfere with cholesterol absorption through possible mechanisms of action such as the formation of insoluble complexes with cholesterol, affectation micelle formation, interference with bile acid metabolism, and/or the perturbation the unstirred water layer or brush border membrane characteristics of enterocytes due to their detergent-like properties (Cohn et al., 2010).

Afrose et al. (2010) demonstrated the hypocholesterolemic effects of saponins in laying hens suggesting that the reductions in the levels of serum and egg cholesterol are caused by the suppression of cholesterol synthesis and the promotion of cholesterol catabolism in the liver. In another study, Zhao et al., (2008) proved these effects in hamsters fed a diet supplemented with saponins, but they attributed the results to the substantial increase in fecal cholesterol loss and not to the reduction of cholesterol absorption or synthesis.

3.2.3 Antiviral effects

A glycoside saponin, glycyrrhizin, found in some roots, exhibited suppressive effects on the replication *in vitro* of HIV (Ito et al., 1988). Due to its structural similarity to soy saponins, several studies have investigated the antiviral effects of this compound on soybeans.

Soybean saponins have been evaluated for their antiviral activity against *in vitro* HIV demonstrating that group B soy saponin inhibits HIV-induced cytopathic effects and virus-specific antigen expressions (Nakashima et al., 1989). The antiviral functions of soy saponins was also demonstrated in the herpes simplex virus type 1 (Hayashi et al., 1997; Ikeda et al., 2005).

Group B saponins with arabinose as the second sugar has an inhibitory effect on human cytomegalovirus (HCMV), influenza A virus, and human immunodeficiency virus type 1. The antivirus effect of soy saponins is not limited to inhibit cell permeability and protein synthesis but also exhibit virus inactivation activity (Tsukamoto and Yoshiki, 2006).

4. Phytosterols

Phytosterols are compounds found in plants that have important functions, especially in the structure of cell membranes and in cellulose biosynthesis. Studies have suggested that due to structural and functional analogy of phytosterols and phytostanols with cholesterol, they

have properties to compete for incorporation into micelles inhibiting cholesterol absorption in the intestine and enhancing its elimination in the feces.

Phytosterols, also called plant sterols, are found naturally in plants (Schneider et al., 2009; Harrabi, 2008) in small quantities. Their hypocholesterolemic properties come from their structural similarity to cholesterol (Kaloustian et al., 2008).

Since the 1950s, beneficial effects of plant sterols have been observed due to decreases in the plasma cholesterol levels and a significant reduction in the incidence of atherosclerosis in chick fed a soybean diet; therefore, it is possible that some soy sterols interfere with cholesterol absorption in the intestine (Peterson et al., 1952). Since that time, there have been studies in animals and humans demonstrating the effects of these compounds on the reduction of plasma cholesterol.

4.1 Chemical structure and properties of phytosterols

Phytosterols are steroid alcohols derived from plants with resemblance to cholesterol, the predominant sterol found in animals, and also are similar in functions to cholesterol, especially in terms of structure and functions of cell membranes. These compounds are members of the triterpen family and, unlike cholesterol, include a methyl or ethyl group at carbon 24 (AbuMweis & Jones, 2008; Palou et al., 2005, Piironen et al., 2000).

Phytostanols are formed from the saturation of the double bond at carbon-5, and they do not occur very frequently in nature. The term plant sterols is usually used to refer to phytosterols and phytostanols. A phytosterol can be converted to its similar phytostanol by enzyme activity in plants, *in vivo,* or by industrial hydrogenation.

More than 200 phytosterols have been identified in the plant kingdom, and many are found in edible foodstuffs (Bradford & Awad, 2007). Their nomenclature is rather confusing due to the partial approval of international norms defined by the International Union of Pure and Applied Chemistry (IUPAC) and the International Union of Biochemistry (IUB) (Moreau et al., 2002).

The most common and widely studied phytosterols are sitosterol or *β*-sitosterol estigmasterol and campesterol. Other relevant phytosterols that can be found in plants in minor amounts are brassicasterol, Δ^5-avenasterol, sitostanol and campestanol (Fernandes & Cabral, 2007). Figure 6 shows the chemical structures of the common phytosterols and phytostanols and their similarity to animal cholesterol.

In addition to the free form, phytosterols can be found as conjugated or esterified compounds, in which the 3β-hidroxyl group is esterified to fatty acids, glycosides, or phenolic compounds with different chemical, technological, and nutritional properties (Figure 7). The occurrence of these classes varies between the foods and parts of these foods (Palou et al., 2005; Piironen & Lampi, 2004). Vegetable oils are, in general, rich in free phytosterols and their fatty acid esters (Piironen & Lampi, 2004) although there is a great variability in the contents of free and esterified phytosterols in many types of oils and fats (Phillips et al., 2002). Nuts also contain high amounts of phytosterols (Piironen & Lampi, 2004). Due to the wide variety of soy-based food products for human consumption, it is considered an important dietary source of phytosterol, and it can contribute significantly to the consumption of those products. A study involving 510 soybean cultivars showed that the content of phytosterols ranged from 202 and 843 µg.g-1. The highest amounts are found in soybeans with high lipid content. β-sitosterol, campesterol, and estigmasterol were the main phytosterols found in the grain at the proportions of 43-67%, 17-34%, and 10-30%, respectively (Yamaya et al., 2007). Germinated soybeans have higher levels of phytosterols,
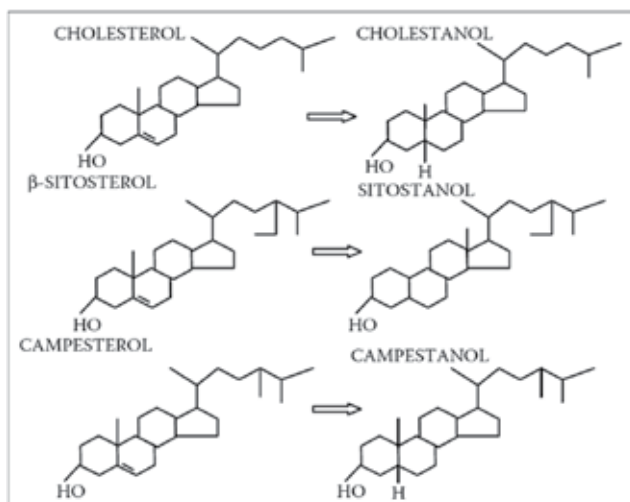
Fig. 6. Chemical structures of cholesterol, phytosterols and their saturated derivatives
(Adapted from Yankah, 2006).
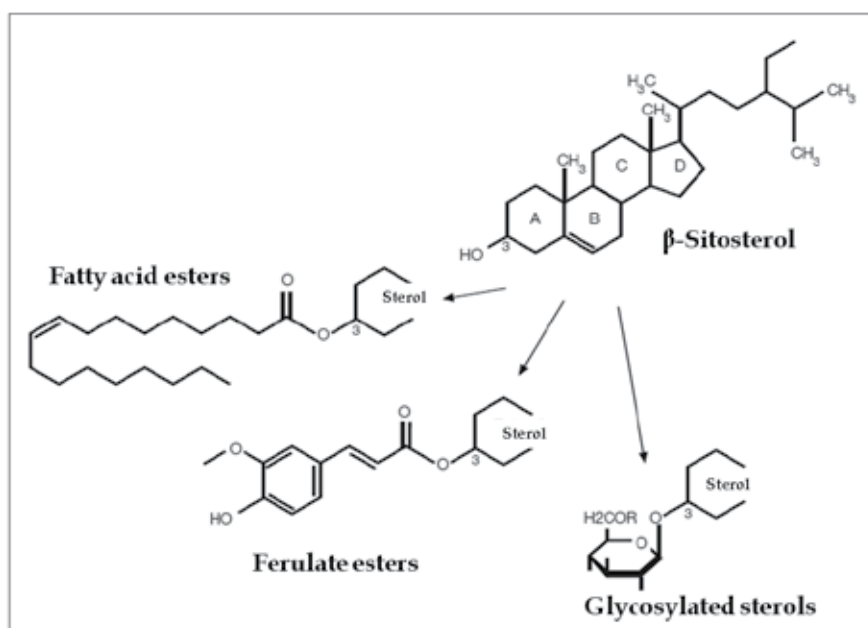
Fig. 7. Modification of 3β-OH group in phytosterols (Adapted from de Palou et al., 2005).

finding that has been corroborated recently in a study involving soybeans with 7 days of
germination with phytosterol contents of 1004 and 1987 μg.g^{-1}; the predominant phytosterol
found was β-sitosterol (Shi et al., 2010).

Soy germ is rich in phytosterols. Therefore, the soybean oil produced is an important source
of these compounds in countries where it is highly consumed. Studies cited by Piironen et
al., (2000) indicated that the contents of phytosterols in raw soybean oil vary between 2,290

and 4,490 µg.g^{-1} and between 2,210 and 3,280 µg.g^{-1} in refined soybean oil. This type of oil showed lower loss of phytosterols in a continuous frying system compared with corn and sunflower oils (Winkler et al., 2007).

4.2 Mechanisms of action and biological functions

The intake of free phytosterols, especially β-sitosterol and the esterified sources, has demonstrated properties of reducing serum cholesterol in animals and humans studies. According to the World Health Organization criteria, this biological function is fairly convincing, but further studies are necessary to demonstrate that this reduction is associated to the prevention of cardiovascular diseases. Other biological activities have also been attributed to the action of phytosterols such as antioxidant activity, cancer prevention, and immune system improvement. Nevertheless, those studies are considered insufficient to corroborate these hypotheses (de Jong et al., 2008).

4.2.1 Hypocholesterolemic effects

The functions of phytosterol in plants are similar to those of cholesterol in animals. They have an important role in the structure of the vegetable cell membranes acting as regulators of membrane fluidity and permeability by affecting the proteins associated to membranes (Alignan et al., 2009; Piironen et al., 2000; Roche et al., 2008). In addition to this structural function, phytosterols also act as precursors of a group of factors related to the growth of the plant. They act in the biosynthesis of cellulose and as substrates for secondary vegetable products such as alkaloids, cardenolides, and saponins (Palou et al., 2005; Peng et al., 2002; Piironen et al., 2000; Read & Bacic, 2002;). Phytosterols can also act as biogenetic precursors of a number of metabolites including plant steroid hormones, such as brassinosteroids and are involved in embryogenesis (Alignan et al., 2009; Breinhölder et al., 2002; Schaller, 2003).

Scientific evidences demonstrate that phytosterols interfere in the reduction of cholesterol absorption stimulating a subsequent synthesis of the endogenous cholesterol. This leads a reduction in the plasma cholesterol levels and an increase elimination of cholesterol in the feces. The way by which phytosterols reduce cholesterol absorption has not been defined yet; however, there are evidences that indicate that the phytosterols compete with cholesterol for the micelles of absorption in the intestine.

Studies have demonstrated serum cholesterol-lowering effect of phytosterols when consumed at levels of 1.5-2 g per day (Piironen & Lampi, 2004). An increase in the rate of endogenous cholesterol synthesis caused by the action of phytosterols leads to an increase LDL-receptor activity in the liver and an increase of the number of LDL receptors in order to capture the cholesterol from these lipoproteins for the bile acid synthesis thus increasing elimination of LDL from circulation. Finally, the levels of LDL cholesterol, and therefore, total cholesterol in humans decrease without affecting the levels of HDL cholesterol and triglycerides (Palou et al., 2005).

The contents of phytosterols in soy and its products are relatively too low to cause relevant effects in the reduction of cholesterol levels, which has attracted the interest of industries in the development and improvement of soy-based products.

A clinical study on the consumption of a soy drink enriched with 2.6 g of phytosterol was conducted in 50 subjects (19 men and 31 women) for eight weeks, aged between 19 and 65 years with moderate hypercholesterolemia. The study found that the regular consumption of 200 ml of soy drink enriched with plant sterols reduces significantly the level of LDL

cholesterol by approximately 0.29 mmol.L^{-1} or 7% compared to baseline. The reduction in the levels of total, LDL and non-HDL cholesterol were significant greater than in the placebo group. Nevertheless, HDL cholesterol and triglycerides were not affected by the consumption of the drink, which was sensorally accepted by the subjects (Weidner et al., 2008).

Accordingly, many studies have demonstrated and corroborated the effectiveness of phytosterols enriching various foods; margarine (AbuMweis et al., 2008), juices (Devaraj et al., 2003), milk (Plana et al., 2008; Hansel et al., 2007), and yogurt (Plat et al., 2009), evaluating cholesterol-lowering effect when consumed in the recommended dose.

Recently, a meta-analysis of 84 clinical trials was conducted evaluating the effect of the enrichment of foods with phytosterols on subjects with hypercholesterolemia. The combined results of all studies grouped indicated that the reductions in the levels of LDL cholesterol were of 0.34 mmol.L^{-1} or 8.8% compared with control, for a mean daily dose of 2.15 g phytosterol. This study evaluated the impacts of subject baseline characteristics of the grouped studies through regression analysis suggesting that groups higher baseline LDL cholesterol resulted in greater absolute LDL cholesterol reductions, which could indicate the effectiveness of those foods in subjects with hypercholesterolemia (Demonty et al., 2009). Figure 8 shows the dose/response relationship in the reduction of LDL cholesterol levels in the grouped studies.
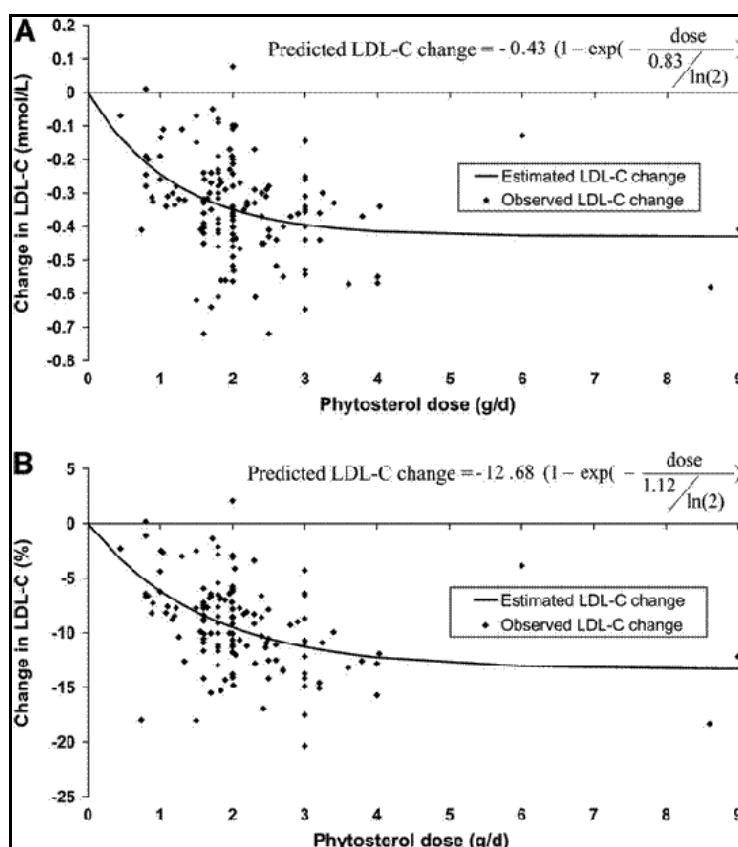
Fig. 8. Dose-response relationship for the absolute (A) and relative (B) LDL cholesterol-lowering effect of phytosterols (Adapted from Demonty et al., 2009).

Soy-based products have high potential for phytosterols enrichment. Soy is considered a very important food due to its countless benefits, and its phytosterols and high quality protein can produce a synergic effect, which also has proved to reduce the levels of LDL and total cholesterol.

4.2.2 Antioxidant effects

Some studies suggest the hypothesis that phytosterols have antioxidant activity and may help the prevention of certain types of cancer (Awad et al., 2001; Normén & Andersson, 2004; Wang et al., 2002). Nonetheless, the information available is still insufficient to corroborate those findings.

In a study in mice was founded that phytosterols modulated and reduced the growth of tumors in ovariectomized female athymic (Ju et al., 2004). On the other hand, oxidant effects of phytosterols on biomarkers, such as DNA or lipid peroxidation, were not found in clinical studies (de Jong et al., 2008). The discrepancies between the results are frequently found in the literature.

Emerging evidence supports the inhibitory actions of phytosterols on lung, stomach, ovarian and breast cancer, through multiple mechanisms of action, including inhibition of carcinogen production, cancer-cell growth, angiogenesis, invasion, metastasis and apoptosis. Phytosterol consumption may also increase the activity of antioxidant enzymes and thereby reduce oxidative stress (Woyengo et al. , 2009).

5. Conclusions

Soy is considered a rich source of proteins and lipids and contains other bioactive compounds such as isoflavones, saponins, and phytosterols. Such diversity in compounds makes it difficult to attribute an exclusive beneficial biological function to a single compound after consuming soy-based food products. For example, cholesterol-lowering effects are associated to the consumption of soy protein, but are also associated to phytosterols, isoflavones, and even saponins. Highlight the synergistic multicomponent effects of soy on biological functions would be a recommendation for further studies, as well as studies of the mechanism of action and new biomarkers for to prove the effectiveness of soy bioactive compounds in preventing and treating several symptoms and/or pathologies.

6. References

AbuMweis S. & Jones P. (2008). Cholesterol-lowering effect of plant sterols. *Curr Atheroscler Rep*, Vol. 10, No. 6, pp. 467-472.

AbuMweis S., Vanstone C., Lichtenstein A. & Jones P. (2008). Plant sterol consumption frequency affects plasma lipid levels and cholesterol kinetics in humans. *Eur J Clin Nutr*, Vol. 63, No. 6, pp. 747-755.

Afrose S., Hossain S., Salma U., Miah A. & Tsujii H. (2010). Dietary karaya saponin and *Rhodobacter capsulatus* exert hypocholesterolemic effects by suppression of hepatic cholesterol synthesis and promotion of bile acid synthesis in laying hens. *Cholesterol*, Vol. 2010, No. 1, pp. 1-7.

Alignan M., Roche J., Bouniols A., Cerny M., Mouloungui Z. & Merah O. (2009). Effects of genotype and sowing date on phytostanol-phytosterol content and agronomic traits in wheat under organic agriculture. *Food Chem*, Vol. 117, No. 2, pp. 219-225.

Awad A., Toczek J., Williams H & Kim U. (2001). *In vitro* and *in vivo* (SCID mice) effects of phytosterols on the growth and dissemination of human prostate cancer PC-3 cells. *Eur J Cancer Prev*, Vol. 10, No. 6, pp. 507-513.

Barnes S., Kim H. & Xu J. (1999). Soy in the prevention and treatment of chronic diseases. *Proceedings of Congresso Brasileiro de Soja*, pp. 295-308, Londrina-Brazil, may, 1999.

Berhow M., Kong S., Vermillion K. & Duval S. (2006). Complete quantification of group A and group B soyasaponins in soybeans. *J Agric Food Chem,* Vol. 54, No. 6, pp. 2035-2044.

Bradford P. & Awad A. (2007). Phytosterols as anticancer compounds. *Mol Nutr Food Res*, Vol. 51, No. 2, pp. 161-170.

Breckwoldt M., Keck C. & Karck U. (1995) Benefits and risks of hormone replacement therapy. *J Steroid Biochem Mol Biol*, Vol. 53, No. 1-6, pp. 205-208.

Breinhölder P., Mosca L. & Lindner W. (2002). Concept of sequential analysis of free and conjugated phytosterols in different plant matrices. *J Chromatogr B*, Vol. 777, No. 1-2, pp. 67-82.

Breitman P., Fonseca D., Cheung A. & Ward W. (2003). Isoflavones with supplemental calcium provide greater protection against the loss of bone mass and strength after ovariectomy compared to isoflavones alone. *Bone*, Vol. 33, No. 4, pp. 597-605.

Brown B., Thomas W., Hutchins A., Martini M. & Slavin J. (2002). Types of dietary fat and soy minimally affect hormones and biomarkers associated with breast cancer risk in premenopausal women. *Nutr Cancer*, Vol. 43, No. 1, pp. 22-30.

Brzezinski A., Adlercreutz H., Shaoul R., Rösler A., Shmueli A., Tanos V. & Schenker J. (1997). Short-term effects of phytoestrogen-rich diet on postmenopausal women. *Menopause*, Vol. 4, No. 2, pp. 89-84.

Burke G. (1996). The potential use of a dietary soy supplement as a post-menopausal hormone replacement therapy. *2nd International Symposium on the Role of Soy in Preventing and Treating Chronic Disease*. Brussels, September, 1996.

Carlson E. (2009). *Saponins: bioactivity and potential impact on intestinal health.* Thesis (Master Science), The Ohio State University.

Cassidy A. (1996). Physiological effects of phyto-oestrogens in relation to cancer and other human health risks. *Proc Nutr Soc*, Vol. 55, No. 1B, pp. 399-417.

Cassidy A., Binghaim S. & Setchell K. (1994). Biological effects of a diet of soy protein in rich in isoflavones on the menstrual cycle of premenopausal women. *Am J Clin Nutr*, Vol. 60, No. 3, pp. 333-340.

Cassidy A., Bingham S. & Setchell K. (1995). Biological effects of isoflavones in young women: importance of the chemical composition of soybean products. *Br J Nutr*, Vol. 74, No. 4, pp. 587-601.

Cohn J., Kamili A., Wat E., Chung R. & Tandy S. (2010). Reduction in intestinal cholesterol absorption by various food components: Mechanisms and implications. *Atherosclerosis*, Vol. 11, No. 1, pp. 45-48.

Col N., Eckman M., Karas R., Pauker S., Goldberg R., Ross E. & Wong J. (1997). Patient-specific decisions about hormone replacement therapy in postmenopausal women. *JAMA*, Vol. 277, No. 14, pp. 1140-1147.

Coward L, Barnes N, Setchel K. & Barnes S. (1993). Genistein, daidzein and their .beta.-glycoside conjugates: antitumor isoflavonas in soybased foods from American and Asian diets. *J Agric Food Chem*, Vol. 41, No. 11, pp. 1961-1967.

Davis D., Díaz-Cruz E., Landini S., Kim Y. & Brueggemeier R. (2008). Evaluation of synthetic isoflavones on cell proliferation, estrogen receptor binding affinity, and apoptosis in human breast cancer cells. *J Steroid Biochem Mol Biol*, Vol. 108, No. 1-2, pp. 23-31.

de Jong A., Plat J., Bast A., Godschalk R., Basu S. & Mensink R. (2008). Effects of plant sterol and stanol ester consumption on lipid metabolism, antioxidant status and markers of oxidative stress, endothelial function and low-grade inflammation in patients on current statin treatment. *Eur J Clin Nutr*, Vol. 62, No. 2, pp. 263-273.

de Jong N., Ros M., Ocké M. & Verhagen H. (2008). A general postlaunch monitoring framework for functional foods tested with the phytosterols/-stanol case. *Trends Food Sci Tech*, Vol. 19, No. 10, pp. 535-545.

Deluca D., Krazeisen A., Breitling R., Prehn C., Möller G. & Adamski J. (2005). Inhibition of 17beta-hydrozysteroid dehydrogenases by phytoestrogens: Comparison with other steroid metabolizing enzymes. *J Steroid Biochem Mol Biol*, Vol. 93, No. 2-5, pp. 285-292.

Demonty I., Ras R., van der Knaap H., Duchateau G., Meijer L., Zock P., Geleijnse J. & Trautwein E. (2009). Continuous dose-response relationship of the LDL-cholesterol-lowering effect of phytosterols intake. *J Nutr*, Vol. 139, No. 2, pp. 271-284.

Devaraj S., Autret B. & Jialal I. (2003). Reduced-calorie Orange juice beverage with plant sterols lowers C-reactive protein concentrations and improves the lipid profile in human volunteers. *Am J Clin Nutr*, Vol. 84, No. 4, pp. 756-761.

Dong J. & Qin L. (2011). Soy isoflavones consumption and risk of breast cancer incidence or recurrence: a meta-analysis of prospective studies. *Breast Cancer Res Treat*, Vol. 125, No. 2, pp. 315-323.

Fenwick D. & Oakenfull D. (1981). Saponin content of soya beans and some commercial soya bean products. *J Sci Food Agric*, Vol. 32, No. 3, pp. 273-278.

Ferin M., Jewelewicz R. & Warren M. (1993). *The Menstrual Cycle: Physiology, Reproductive Disorders and Infertility*. (1th edition), Oxford University Press, ISBN 0195061934, New York.

Fernandes P. & Cabral J. (2007). Phytosterols: applications and recovery methods. *Bioresour Technol*, Vol. 98, No. 12, pp. 2335-2350.

Fieser L. & Fieser M. (1959). *Steroids*, Reinhold Publishing Corporation, ISBN 0278917097. New York.

Francis C. & Hume I (1971). The relationship between lignification and flavonoid production in subterranean clover. *Aust J Biol Sci*, Vol. 24, No. 1, pp. 1-5.

Güçlü-Üstündağ Ö. & Mazza G. (2007). Saponins: properties, applications and processing. *Crit Rev Food Sci Nutr*, Vol. 47, No. 3, pp. 231-258.

Guo Y., Wang S., Hoot D. & Clinton S. (2007). Suppression of VEGF-mediated autocrine and paracrine interactions between prostate cancer cells and vascular endothelial cells by soy isoflavones. *J Nutr Biochem*, Vol. 18, No. 6, pp. 408-417.

Han K., Soares J., Haidar M., Lima G. & Baracat E. (2002). Benefits of soy isoflavone therapeutic regimen on menopausal symptoms. *Obstet Gynecol*, Vol. 99, No. 3, pp. 389-394.

Hansel B., Nicolle C., Lalane F., Tndu F., Lassel T., Donazzolo Y., Ferrières J., Krempf M., Schlienger J., Verges B., Chapman M. & Bruckert E. (2007). Effect of low-fat, fermented milk enriched with plant sterols on serum lipid profile and oxidative stress in moderate hypercholesterolemia. *Am J Clin Nutr*, Vol. 86, No. 3, pp. 790-796.

Harrabi S., St-Amand A., Sakouhi F., Sebei K., Kallel H., Mayer P. & Boukhchina S. (2008). Phytostanols and phytosterols distributions in corn kernel. *Food Chem*, Vol. 111, No. 1, pp. 115-120.

Hayashi K., Hayashi H., Hiraoka N., Ikeshiro Y. (1997). Inhibitory activity of saoyasaponin II on virus replication in vitro. *Planta Med*, Vol. 63, No. 2, pp. 102-105.

Hooper L., Ryder J., Kurzer M., Lampe J., Messina M., Phipps W. & Cassidy A. (2009). Effects of soy protein and isoflavones on circulating hormone concentrations in pre- and post-menopausal women: a systematic review and meta-analysis. *Hum Reprod Update*, Vol. 15, No. 4, pp. 423-440.

Hutchins A., Slavin J. & Lampe J. (1995). Urinary isoflavonoid phytoestrogen and lignan excretion after consumption of fermented and unfermented soy products. *J Am Diet Assoc*, Vol. 95, No. 5, pp. 545-551.

Ikeda T., Yokomizo K., Okawa M., Tsuchihashi R., Kinjo J., Nohara T. & Uyeda M. (2005). Anti-herpes virus type 1 activity of oleanane-type triterpenoids. *Biol Pharm Bull*, Vol. 28, No. 9, pp. 1779-1781.

Ito M., Sato A., Hirabayashi K., Tanabe F., Shigeta S., Baba M., De Clercq E., Nakashima H. & Yamamoto N. (1988). Mechanism of inhibitory effect of glycyrrhizin on replication of human immunodeficiency virus (HIV). *Antivirus Research*, Vol. 10, No. 6, pp. 289-298.

Ju Y., Clausen L., Allred K., Almada A. & Helferich W. (2004). β-sitosterol, β-sitosterol glucoside, and a mixture of β-sitosterol and β-sitosterol glucoside modulated the growth of estrogen-responsive breast cancer cells in vitro and in ovariectomized athymic mice. *J Nutr*, Vol. 134, No. 5, pp. 1145-1151.

Kaloustian J., Alhanout K., Amiot-Carlin M., Lairon D., Portugal H., Nicolay A. & Technical collaboration (2008). Effect of water cooking cooking on free phytosterol levels in beans and vegetables. *Food Chem*, Vol. 107, No. 4, pp. 1379-1386.

Kang J., Badger T., Ronis M. & Wu X. (2010). Non-isoflavone phytochemicals in soy and their health effects. *J Agric Food Chem*, Vol. 58, No. 14, pp. 8119-8133.

Kokubo Y., Iso H., Ishihara J., Okada K., Inoue M. & Tsugane S. (2007). Association of dietary intake of soy, beans, and isoflavones with risk of cerebral and myocardial infarctions in Japanese populations. *Circulation*, Vol. 116, No. 22, pp. 2553-2562.

Konoshima T. & Takasaki M. (2000). Anti-tumor-promoting activities (cancer chemopreventive activities) of natural products. *Studies in Natural Products Chemistry*, Vol. 24, No. 5, pp. 215-267.

Kuhnle G., Dell'Aquila C., Aspinall S., Runswick S., Joosen A., Mulligan A. & Bingham S. (2009). Phytoestrogen content of fruits and vegatbles commonly consumed in the UK based on LC-Ms and ^{13}C-labelled standard. *Food Chem*, Vol. 116, No. 2, pp. 542-554.

Kurzer M. (2002). Hormonal effects of soy in premenopausal women and men. *J Nutr*, Vol. 132, No. 3, pp. 570S-573S.

Lagari V. & Levis S. (2010). Phytoestrogens and bone health. *Curr Opin Endocrinol Diabetes Obes*, Vol. 17, No. 6, pp. 546-553.

Lampe J., Karr S., Hutchins A. & Slavin J. (1998). Urinary equol excretion with a soy challenge: influence of habitual diet. *Proc Soc Exp Biol Med*, Vol. 217, No. 3, pp. 335-339.

Lee I., Park Y., Yeo H., Han M. & Kim D. (2010). Soyasaponin I attenuates TNBS-induced colitis in mice by inhibiting NF-kB pathway. *J Agric Food Chem*, Vol. 58, No. 20, pp. 10929-10934.

Lee S., Shu X., Li H., Yang G., Cai H., Wen W., Ji B., Gao J., Gao Y. & Zheng W. (2009). Adolescent and adult soy food intake and breast cancer risk: results from the Shanghai women's health study. *Am J Clin Nutr*, Vol. 89, No. 6, pp. 1920-1926.

Lin J. & Wang C. (2004). Soybean Saponins: Chemistry, Analysis, and Potential Health Effects, In: *Soybean as Functional Foods and Ingredients*, Liu K., pp. 77-105, AOCS Press, ISBN 1-893997-33-2, Illinois.

Liu K. (2004). Soy Isoflavones: Chemistry, processing effects, health benefits, and commercial production. In: *Soybean as Functional Foods and Ingredients*, Liu K., pp. 52-73, AOCS Press, ISBN 1-893997-33-2, Illinois.

MacDonald R., Guo J., Copeland J., Browning J. & Sleper D., Rottinghaus G., Berhow M. (2005). Environmental influences on isoflavonas and saponins in soybeans and their role in colon cancer. *J Nutr*, Vol. 135, No. 5, pp. 1239-1242.

Martínez-Montemayor M., Otero-Franqui E., Martinez J., De La Mota-Peynado A., Cubano L. & Dharmawardhane S. (2010). Individual and combined soy isoflavones exert differential effects on metastatic cancer progression. *Clin Exp Metastasis*, Vol. 27, No. 7, pp. 465-480.

Messina M. (2000). Soyfoods and soybean phyto-estrogens (isoflavones) as possible alternatives to hormone replacement therapy (HRT). *Eur J Cancer,* Vol. 36, Suppl. 4, pp. S71-S72.

Moreau R., Whitaker B. & Hicks K. (2002). Phytosterols, phytostanols, and their conjugates in foods: Strutural diversity, quantitative analysis, and health-promoting uses. *Prog Lipid Res*, Vol. 41, No. 6, pp. 457-500.

Murkies A., Lombard C., Strauss B., Wilcox G., Burger H. & Morton M. (1995). Dietary flour supplementation decreases post-menopausal hot flushes: effect of soy and wheat. Maturitas, Vol. 21, No. 3, pp. 186-195.

Murphy P., Hu J., Barua K. & Hauck C. (2008). Group B saponins in soy products in the U.S. department of agriculture-Iowa State University isoflavona database and their comprasion with isoflavonas contens. *J Agric Food Chem*, Vol. 56, No. 18, pp. 8534-8540.

Nagarajan S. (2010). Mechanisms of anti-atherosclerotic functions of soy-based diets. *J Nutr Biochem*, Vol. 21, No. 4, pp. 255-260.

Nakashima H., Okubo K. & Honda Y. (1989). Inhibitory effect of glycosides like saponins from soybean on the infectivity of HIV in vitro. *AIDS*, Vol. 3, No. 10, pp. 655-658.

Nishida K., Ohta Y., Araki Y., Ito M. & Nagamura Y. (1993). Inhibitory effects of group A saponin and group B saponin fractions from soybean seed hipocotyls on radical-initiated lipid peroxidation in mouse liver microcosms. *J Clin Biochem Nutr*, Vol. 15, No. 3, pp. 175-184.

Normén L. & Andersson S. (2004). Does Phytosterols Intake Affect the Development of Cancer?. In: *Phytosterols as functional food components and nutraceuticals*, Dutta P., pp. 1-52, CRC Press, ISBN 9780824747503, Texas.

Okabe Y., Shimazu T. & Tanimoto H. (2011). Higher bioavailability of isoflavones after a single ingestion of aglycone-rich fermented soybeans compared with glucoside-rich

non-fermented soybeans in Japanese postmenopausal women. *J Sci Food Agric*, Vol. 91, No. 4, pp. 658-663.

Oleszek W. (2000). Saponins, In: *Natural Food Antimicrobial Systems*, Naidu A., pp. 295-324, CRC Press, ISBN 9780849320477, Florida.

Ørgaard A., & Jensen L. (2008). The effects of soy isoflavones on obesity. *Exp Biol Med*, Vol. 223, No. 9, pp. 1066-1080.

Palou O., Picó S., Bonet P., Oliver V., Serra V., Rodríguez G. & Ribot R. (2005). *El Libro Blanco de los Esteroles Vegetales* (2nd edition), Unilever Foods S. A., ISBN 8460958507 España.

Park C., Yun H., Lee E., Min B., Bae H., Choe W., Kang I, Kim S. & Ha J. (2010). The antioxidant effects of genistein are associated with AMP-activated protein kinase activation and PTEN induction in prostate cancer cells. *J Med Food*, Vol. 13, No. 4, pp. 815-820.

Parkin D. (1989). Cancers of the breast, endometrium and ovary: geographical correlations. *Eur J Cancer Clin Oncol*, Vol. 25, No. 12, pp. 1917-1925.

Peng L., Kawagoe Y., Hogan P. & Delmer D. (2002). Sitosterols-β-glucoside as primer for cellulose synthesis in plants. *Science*, Vol. 295, No. 5552, pp. 147-150.

Peterson D., Nichols C. & Shneour E. (1952). Some relationships among dietary sterols, plasma and liver cholesterol levels, and atherosclerosis in chicks. *J Nutr*, Vol. 47, No. 1, pp. 57-65.

Phillips K., Ruggio D., Toivo J., Swank M. & Simpkins A. (2002). Free and esterified sterol composition of edible oils and fats. *J Food Compos Anal*, Vol. 15, No. 2, pp. 123-142.

Phipps W., Martini M., Lampe J., Slavin J & Kurzer M. (1993). Effect of flax seed ingestion on the menstrual cycle. *J Clin Endocrinol Metab*, Vol. 77, No. 5, pp. 1215-1219.

Piironen V. & Lampi A. (2004). Occurrence and Levels of Phytosterols in Foods. In: *Book Phytosterols as Functional Foods Components and Nutraceuticals*, Dutta P., pp. 1-32, CRC Press, ISBN 9780824747503, Texas.

Piironen V., Lindsay D., Miettinen T., Toivo J. & Lampi A. (2000). Plant sterols: biosynthesis, biological function and their importance to human nutrition. *J Sci Food Agric*, Vol. 80, No. 7, pp. 939-966.

Plana N., Nicolle C., Ferre R., Camps J., Cos R., Villoria J., Masana L. & Danacol Group (2008). Plant sterol-enriched fermented milk enhances the attainment of LDL-cholesterol goal in hypercholesterolemic subjects. *Eur J Nutr*, Vol. 47, No. 1, pp. 32-39.

Plat J., Brufau G., Dallinga-Thie G., Dasselaar M. & Mensink R. (2009). A plant stanol yogurt drink alone or combined with a low-dose statin lowers serum triacylglycerol and non-HDL cholesterol in metabolic syndrome patients. *J Nutr*, Vol. 139, No. 6, pp. 1143-1149.

Raju J. & Mehta R. (2009). Cancer chemopreventive and therapeutic of Diosgenin, a food saponin. *Nutr Cancer*, Vol. 61, No. 1, pp. 27-35.

Rao A. & Sung M. (1995). Saponins as anticarcinogens. *J Nutr*, Vol. 125, Suppl 3, pp. 717S-724S.

Read S. & Bacic T. (2002). Plant biology. Prime time for cellulose. *Science*, Vol. 295, No. 5552, pp. 59-60.

Roche Y., Gerbeau-Pissot P., Buhot B., Thomas D., Bonneau L., Grestu J., Mongrand S., Perrier-Cornet J. & Simon-Plas F. (2008). Depletion of phytosterols from the plant

plasma membrane provides evidence for discruption of lipid rafts. *FASEB J*, Vol. 22, No. 11, pp. 3980-3991.

Rose D., Boyer A. & Wynder E. (1986). International comparison of mortality rates for cancer of the heart, ovary, prostate and colon, per capita fat consumption. *Cancer*, Vol. 58, No. 1, pp. 2363-2371.

Ruiz R., Price K., Fenwick G. & Rhodes M. (1996). Effect of processing on saponin content and composition of chickpeas, *Biochem Soc Trans*, Vol. 24, No. 3, pp. 393S-395S.

Russo A., Cardile B., Lombardo L., Vanella L. & Acquaviva R. (2006). Genistin inhibits UV light-induced plasmid DNA damage and cell growth in human melanoma cells. *J Nutr Biochem*, Vol. 17, No. 2, pp. 103-108.

Sautier C., Doucer C., Flament C. & Lemonnier D. (1979). Effects of soy protein and saponins on serum, tissue and feces steroids in rat. *Atherosclerosis*, Vol. 34, No. 3, pp. 233-241.

Schaller H. (2003). The role of sterols in plant growth and development. *Prog Lipid Res*, Vol. 42, No. 3, pp. 163-175.

Schneider K., NiNovi M., Baines J. & Schlatter J. (2009). Phytosterols, Phytostanols and Their Esters. In: *Safety Evaluation of Certain Food Additives*, WHO, pp. 117-163, WHO Press, ISBN 9789241660600, Geneva.

Seok Y., Baek I., Kim Y., Jeong Y., Lee I., Shin D., Hwang Y., Kim I. (2008). Isoflavone attenuates vascular contraction through inhibition of the RhoA/Rho-Kinase signaling pathway. *J Pharmacol Exp Ther*, Vol. 326, No. 3, pp. 991-998.

Setchell K. Borriello S., Hulme P., Kirk D. & Axelson M. (1984). Nonsteroidal estrogens of dietary origin: possible roles in hormone- dependent disease. *Am J Clin Nutr*, Vol. 40, No. 3, pp. 569-578.

Shi H., Nam P. & Ma Y. (2010). Comprehensive profiling of isoflavonas, phytosterols, tocopherols, minerals, crude protein, lipid, and sugar during soybean (*Glycine max*) germination. *J Agric Food Chem*, Vol. 58, No. 8, pp. 4970-4976.

Shi J., Arunasalam K., Yeung D., Kakuda Y., Mittal G. & Jiang Y. (2004). Saponins from edible legumes: chemistry, processing, and health benefits. *J Med Food*, Vol. 7, No. 1, pp. 67-78.

Shimoyamada M., Kudo S., Okubo K., Yamauchi F. & Harada K. (1990). Distribution of saponin constituents in some varieties of soybean plant. *Agric Biol Chem*, Vol. 54, No. 1, pp. 77-81.

Sidhu G. & Oakenfull D. (1986). A mechanism for the hypocholesterolaemic activity of saponins. *Br J Nutr*, Vol. 55, No. 3, pp. 643-649.

Siow R. & Mann G. (2010). Dietary isoflavones and vascular protection: activation of cellular antioxidant defenses by SERMs or hormesis?. *Mol Aspects Med*, Vol. 31, No. 6, pp. 468-477.

Su S., Yeh T., Chuang W., Ho C., Chang K., Cheng H., Liu H., Cheng H., Hsu P. & Chow N. (2005). The novel targets for anti-angiogenesis of genistein on human cancer cells. *Biochem Pharmacol*, Vol. 15, No. 2, pp. 307-318.

Teas J., Hurley T., Hebert J., Franke A., Sepkovic D. & Kurzer M. (2009). Dietary seaweed modifies estrogen and phytoestrogen metabolism in healthy postmenopausal women. *J Nutr*, Vol. 139, No. 5, pp. 939-944.

Tsai C., Chen Y., Chien Y., Huang W. & Lin S. (2010). Effect of soy saponin on the growth of human colon cancer cells. *World J Gastroenterol*, Vol 16, No. 27, pp. 3371-3376.

Tsukamoto C. & Yoshiki Y. (2006). Soy Saponin, In: *Soy in Health and Disease Prevention*, Sugano M., pp. 155-172, CRC Press, ISBN 9780849335952, Florida.

Uchida R., Chiba H., Ishimi Y., Uehara M., Suzuki K., Kim H. & Matsumoto A. (2010). Combined effects of soy isoflavone and fish oil on ovariectomy-induced bone loss in mice. *J Bone Miner Metab*, Vol. 12, No. 1, pp. 1-10.

Villa P., Costantini B., Suriano R., Perri C., Macri F., Ricciardi L., Panunzi S. & Lanzone A. (2009). The differential effect of the phytoestrogen genistein on cardiovascular risk factors in postmenopausal women: relationship with the metabolic status. *J Clin Endocrinol Metab*, Vol. 94, No. 2, pp. 552-558.

Wang T., Hicks K. & Moreau R. (2002). Antioxidant activity of phytosterols, oryzanol, and other phytosterols conjugates. *J Am Oil Chem Soc*, Vol. 79, No. 12, pp. 1201-1206.

Warren M., Shortle B. & Dominguez J. (2002). Use of alternative therapies in menopause. *Best Pract Res Clin Obstet Gynaecol*, Vol. 16, No. 3, pp. 411-448.

Washburn S., Burke G., Morgan T. & Anthony M. (1999). Effect of soy supplementation on serum lipoproteins, blood pressure, and menopausal symptoms in perimenopausal women. *Menopause*, Vol. 6, No. 1, pp. 7-13.

Weidner C., Krempf M., Bard J., Cazaubiel M. & Bell D. (2008). Cholesterol lowering effect of a soy drink enriched with plant sterols in a French population with moderate hypercholesterolemia. *Lipids Health Dis*, Vol. 7, No. 1, pp. 35-42.

Winkler J., Warner K. & Glynn M. (2007). Effect of deep-fat frying on phytosterols content in oils with differing fatty acid composition. *J Am Oil Chem Soc*, Vol. 84, No. 11, pp. 1023-1030.

Woyengo T., Ramprasath V. & Jones P. (2009). Anticancer effects of phytosterols. *Eur J Clin Nutr*, Vol. 63, No. 7, pp. 813-820.

Wu J., Wang X., Chiba H., Higuchi M., Nakatani T., Ezaki O., Cui H., Yamada K. & Ishimi Y. (2004). Combined intervention of soy isoflavone and moderate exercise prevents body fat elevation and bone loss in ovariectomized mice. *Metabolism*, Vol. 53, No. 7, pp. 942-948.

Xu X., Wang H., Murphy P., Cook L. & Hendrich S. (1994). Daidzein is a more bioavailable soymilk isoflavone than is genistein in adult women. *J Nutr*, Vol. 124, No. 6, pp. 825-832.

Xu X., Harris K., Wang H., Murphy P., Hendrich S. (1995). Bioavailability of soybean isoflavones depends upon gut microflora in women. *J Nutr*, Vol. 125, No. 9, pp. 2307-2315.

Yamaya A., Endo Y., Fujimoto K. & Kitamura K. (2007). Effects of genetic variability and planting location on the phytosterols content and composition in soybean seeds. *Food Chem*, Vol. 102, No. 4, pp. 1071-1075.

Yang X., Dong C. & Ren G. (2011). Effect of soyasaponins-rich extract from soybean on acute alcohol-induced hepatotoxicity in mice. *J Agric Food Chem*, Vol. 59, No. 4, pp. 1138-1144.

Yankah V. (2006). Phytosterols and Human Health. In: *Handbook of Functional Foods*, Akoh C., pp. 403-418, CRC Press, ISBN 9780849321627, Florida.

Yoshiki Y., Jin-Hyeong K. & Okubo K. (1994). Saponins conjugated with 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one from phaseolus coccineus. *Phytochemistry*, Vol. 36, No. 4, pp. 1009-1012.

Yu Z., Tang Y., Hu D. & Li J. (2005). Inhibitory effect of genistein on mouse colon cancer MC-26 cells involved TGF-β1/Smad pathway. *Biochem Biophys Res Commun*, Vol. 333, No. 3, pp. 827-832.

Zhao H., Harding S., Marinangeli C., Kim Y. & Jones P. (2008). Hypocholesterolemic and anti-obesity effects of saponins from *Platycodon grandiflorum* in hamsters fed atherogenic diets. *J Food Sci*, Vol. 73, No. 8, pp. H195-H200.

Use of Soybean in Cereal Based Food Formulation and Development of Nutritionally Improved Foods

Silvina R. Drago[*], María S. Lassa, Roberto L. Torres,
Dardo M. De Greef and Rolando J. González
Instituto de Tecnología de Alimentos - Facultad de Ingeniería Química -U.N.L.,
[*]*CONICET*
Argentina

1. Introduction

Since the 1960s, soy protein products have been used as nutritional and functional food ingredients in several food categories available to the consumer. Currently the food industry has incorporated soy protein, either as flours, textured, concentrates or protein isolates in the manufacture of numerous products (infant formulas, bakery products, dairy and meat) because it is a good quality protein, low cost and good functionality (Singh et al., 2008).

Cereal foods are an important source of energy in the diet of a large part of the world population. However, the protein quality of grain is low compared to that of animal origin. The nutritional benefits of adding legumes protein to grain are well known (Gomez, 1985; Messina, 1999).

Cereals have gained value, not only because they are the most important starch source in the diet and their content of other nutrients is not negligible, but also because a wide variety of products novel and convenient can be obtained from them. These last characteristics are ones of the most important for modern consumers. Cereal food industry has demonstrated their speed in exploiting these changes in food consumption model, and the large variety of cereals products existing nowadays is eloquent. Regarding nutritional aspects, soybean could be used in cereal based products in order to improve nutritional quality of these types of foods (González, 2009).

It has been shown (Pérez et al., 2008) that until 12% replacement of corn grits by soybean grits a good extrudate texture is maintained. This mixture have nutritional benefits, since the protein content and quality are superior to those of a traditional "snack", because the addition of soy cannot only increase the protein level of corn extrudate (10% vs. 7%), but also improves the profile of amino acids by the known effect of complementation between the proteins of cereals and legumes, allowing maize chemical score increased from approx. 46% to 76.5% (without taking into account the digestibility).

Moreover, soybean flour is a rich source of iron, zinc and calcium (Liener, 1972), so it could when it is added improve the content of these minerals when added to foods formulated with other components. However, the bioavailability of iron is markedly reduced, not only because of the effect of the same protein (Lynch et al., 1994), but also for its high content of

phytates (inositol hexa-phosphate) (Cook et al., 1981; Hallberg & Rossander, 1982; Hurrell et al., 1992). Phytates also exert an inhibitory effect on zinc (Fairweather-Tait, 1992; Lönnerdal et al., 1988) and calcium absorption (Greger, 1988).

Some authors have observed that phytate can be degraded by the extrusion process to a different extent depending on process conditions (Ummadi et al., 1995; Abd El-Hady & Habiba, 2003), releasing phosphates and inositol pentaphosphate and other compounds with less content of phosphorus. In fact, forms of inositol phosphates with 6 and 5 phosphates groups are responsible for the inhibitory action on mineral absorption (Sandberg et al., 1989). Few papers study the mineral bioavailability from extruded products in humans. Fairweather-Tait et al., (1989) showed that Fe and Zn retention from extruded mixtures made with bran and flour and consumed with milk did not differ from that of the product, used in the same way, but not extruded.

Furthermore, the effect of inhibitors can be minimized by the addition of adequate absorption enhancers such as ascorbic acid, citric acid and Na_2EDTA (South & Miller, 1998).

Besides that, micronutrient fortification is becoming an almost generalized practice; however in most cases nutrient bioavailability is ignored. One of the most important points in iron-fortification is the selection of an appropriate iron compound (Pizarro et al., 2002). Soluble compounds, despite their low cost and high iron bioavailability, could induce organoleptic changes in the food vehicle. In comparison, insoluble compounds are more stable and do not create adverse effects in foods, but they have a lower rate of absorption (Hurrell, 1997).

The use of soybean in food formulation and development of nutritionally improved corn: soybean based food are presented.

The objective of this work was to analyze the effects of the addition of different mineral sources and several absorption enhancers on physicochemical properties and mineral availability of corn-soybean expanded product fortified with minerals.

2. Materials and methods

2.1 Soybean grits

A commercial sample of "Don Mario 4.400" soybean variety was selected to obtain the soybean grits. The beans were previously treated to inactivate lipoxygenase, by immersing them in boiling water during two minutes and soon after cooling them with tap water. Treated beans were dried in an oven at 50 °C until they reached 9 to 10 % of moisture. The grains were dehulled and ground using an air classifier and a roll mill (Vario Miag Germany), avoiding the production of fine particles. Final grits particle size was between 420-250 μm, less than 1% of particle size being below 250 μm. Moisture content, crude protein, petroleum ether extract and ash content were determined by AOAC methods (1995). The composition in dry base was: protein: 374.0 g/kg, lipids: 155 g/kg, ash: 51.5 g/kg, and moisture: 77 g/kg.

2.2 Corn grits

A grits sample taken from a hard red corn (*Zea mays*) (supplied by Litex S.H, Santa Fe), with a particle size between 1190-420 μm was used in the experiments. The composition in dry base was: protein: 70 g/kg, lipids: 3.8 g/kg, ash: 2.4 g/kg, and moisture: 122 g/kg.

2.3 Corn: Soybean blend

The grits mixture corn:soy (88:12) was selected as adequate to improve protein value without impairing sensory attributes of the expanded product (Fritz et al., 2006). The composition in dry base was: protein: 106 g/kg, lipids: 22.6 g/kg, ash: 8.5 g/kg, and moisture: 125.7 g/kg.

2.4 Mineral sources

Mineral sources used were: ferrous bisglycinate (Ferrochel, Albion Lab donated by Parmalat, Argentina), ferric sodium EDTA –FeNaEDTA- (Sigma) and ferrous sulfate (Cicarelli). The zinc and calcium salts ($ZnSO_4$ and $CaCO_3$) were from Cicarelli. In all cases, analytical grade reagents were used.

2.5 Absorption enhancers

Ascorbic Acid, Sodium Citrate and Na_2EDTA from Cicarelli were evaluated as enhancers of mineral absorption. In all cases, analytical grade reagents were used.

2.6 Extrusion experiments

The corn: soybean blends were conditioned to the extrusion moisture level, 2 hours before each run. The other ingredients (mineral sources and enhancers) were added to the water of hydration.

Sample	Code
Corn: Soy (88:12) (blank)	CS
CS+ Ascorbic acid	AA
CS+$FeSO_4$	FS
CS+$FeSO_4$+ Ascorbic acid	FS+AA
CS+ Sodium Citrate	Citr
CS+ Na_2EDTA	EDTA
CS+Na_2EDTA+$FeSO_4$	FS+EDTA
CS+ Ferrous Bisglicinate	FB
CS+FeNaEDTA	FE
CS+ FB+ Ascorbic acid	FB+AA
CS+FeNaEDTA+ Ascorbic acid	FE+AA
CS+FB+ Sodium Citrate	FB+Citr
CS+FB+Na_2EDTA	FB+EDTA
CS+FeNaEDTA+Citrate	FE+Citr
CS+$FeSO_4$+$ZnSO_4$	FS+Zn
CS+$CaCO_3$+$ZnSO_4$	Ca+Zn
CS+$CaCO_3$+$FeSO_4$	FS+Ca
CS+$FeSO_4$+$ZnSO_4$+$CaCO_3$	M
CS+$FeSO_4$+$ZnSO_4$+$CaCO_3$+Citrate	M+Citr
CS+ $FeSO_4$+$ZnSO_4$+$CaCO_3$+Na_2EDTA	M+EDTA
CS+$CaCO_3$+Citrate	Ca+Citr

Table 1. Samples and codes.

Fortification was made in order to have: 40 mg/kg, 30 mg/kg, and 1400 mg/kg for Fe, Zn and Ca, respectively. This level of iron is usually used in fortification of corn meal. The level of zinc was selected in order to have a molar ratio Fe: Zn not greater than 2:1 and the calcium level, so that the contribution to be approximately 400 mg/1000 kcal.

For enhancers, the amounts were as follows: Na_2EDTA: 266 mg/kg (BS); Citrate Sodium: 10.53 g/kg (BS) and ascorbic acid: 1 g/kg (BS), corresponding to Fe molar ratios: AA: (1:8), Fe: citrate: (1:50) and Fe: EDTA: (1:1).

21 different mixtures were prepared, including the blank sample (corn/soybean alone), whose codes are shown in Table 1.

Each mixture was prepared using a Brabender planetary mixer P-600 L (Germany) at a rotation speed of 60 rpm, 2 h before extrusion.

The extrusion process was carried out according to Pérez et al., (2008), with a Brabender 20 DN single screw extruder, using the following conditions: 4:1 compression ratio screw, 3/20-mm (diameter/length) die and screw speed of 150 rpm, temperature 170°C and moisture 14%. The feeding rate of the extruder was at full capacity. While the extruder feeding section was maintained cool by circulating water through the jacketed device, the metering and die sections were both kept at the same temperature by using the heat control device of the extruder. The extruded samples (obtained in duplicate) were air-dried in an oven at 50 °C until moisture content of 6%. This moisture level is adequate for texture evaluation (González et al., 2004). Each dried sample was divided in several portions and kept in polypropylene bag hermetically sealed until their evaluation.

2.7 Extrusion response evaluation

Samples were obtained as soon as the stationary condition was reached, torque and mass output being simultaneously measured. The latter values were used to determine the specific mechanical energy consumption (SMEC) (González et al., 2002; González et al., 2006), using the following formula: $SMEC$ (J g^{-1}) = $k. T. N. Q^{-1}$, where k is: 61.6 10^{-3}; T is torque in Brabender units (BU); N is screw rpm and Q (g/min) is the mass output, referred to feeding moisture level. The value of k takes into account unit conversion and constants.

2.8 Product response evaluation
2.8.1 Expanded samples

Diameters were measured with a Vernier caliper on ten pieces of sample and radial expansion (E) was determined as the ratio $E = D. d^{-1}$, where D is the extrudate diameter (average of ten determinations) and d is the die diameter. Extrudate specific volume (SV) was obtained by calculating the volume/d.b. weight ratio (cm^3.g^{-1}), corresponding to an extrudate piece of about 15 cm long. This procedure was applied to ten pieces and the average is reported.

Product texture was evaluated by a trained panel (three judges), according to Fritz et al., (2006). The score given to each sample was obtained by consensus among the judges. A hardness scale from 1 to 9 point was used (from soft to hard). In order to have the two scale extremes (1 and 9) two additional samples were obtained by extruding corn:soybean blend 88:12, at 185 °C -14% moisture and at 155 °C -18% moisture. The first is the softer one and the second the hardest one.

The compressive strength was measured on extruded pieces of 6 cm in length, using an Instron Universal Testing Machine (model 4411, Norwood, Massachusetts, USA), with a load cell of 4,905 N and a compression speed of 10 mm/min according to Park et al. (1993). Each determination was performed in quintuplicate.

2.8.2 Precooked flours

For obtaining the precooked flours, an amount (150 g) representative of each extrudate sample was first ground with a laboratory hammer mill (Retsch-Muhle- Germany) using a 2 mm sieve, and then with a Ciclotec mill (UD Corp Boulder Colorado-USA) using a 1 mm sieve.

2.8.2.1 Total soluble solids

Total soluble solids from extrudates were determined by the method of Anderson et al., (1969) modified by Gonzalez et al., (2002).

2.8.2.2 Determination of mineral dialyzability (DFe%, DZn%, DCa%)

All measurements were performed using the precooked flour. A modification of the widespread *in vitro* Miller et al., (1981) method, according to Wolfgor et al., (2002) was followed. The samples were prepared to 3% protein concentration (W/W) using distilled water at 70ºC. Aliquots (25 g) of homogenized samples were adjusted to pH 2.0 with 6 N HCl and after addition of 0.8 mL pepsin digestion mixture (16% pepsin (Sigma P-7000) solution in 0.1 N HCl), were incubated at 37°C during 2 h in a shaking water bath. At the end of pepsin digestion, dialysis bags containing 20 mL 0.19 M PIPES (piperazine-N,N'-bis[2-ethane-sulfonic acid] disodium salt) buffer (Sigma P-3768) were placed in each flask and were incubated for 50 min in a shaking water bath at 37°C. Pancreatin-bile mixture (6.25 mL of 2.5% bile (Sigma B-8631), 0.4% pancreatin (Sigma P-1750) solution in 0.1N NaHCO$_3$) was then added to each flask and the incubation continued for another 2 h. Then, bag contents were weighed and analyzed for its mineral content by flame atomic absorption spectroscopy (AAS). Assessment of minerals in precooked flour samples was made by AAS after dry ashing (AOAC, 1995).

Mineral dialyzability was calculated from the amount of each dialyzed mineral expressed as a percentage of the total amount present in each sample.

$$\text{Dialyzable Mineral (\%)} = [D / (W \times A)] \times 100$$

Where: D is the total amount of dialyzed mineral (μg); W is the weight of sample (g) and A is the concentration of each mineral in the sample (μg/g).

2.9 Statistical analysis

Analysis of Variance was carried out using the software Statgraphics Plus 3.0, and the statistical differences among samples were determined using the LSD test.

3. Results and discussion

3.1 Evaluation of extrusion process and physical properties

Table 2 shows the average values for each of the responses: those related to the process: Torque (T), mass flow rate (Q) and Specific Mechanical Energy Consumption (SMEC), and those related to the physical properties: Expansion (Exp), Specific Volume (SV), Total soluble solids (S), mechanical resistance (Res) and sensory evaluation of hardness.

These average values show dispersion between 2 and 10 %. The range of variation for T was 4 to 8 %; for Q: 2 to 4 %; for SMEC: 4 to 9 %; for Expansion: 4 to 8 %; for SV: 3 to 5 %, for S: 2 to 4 % and Res: 5 to 10 %. With respect to the sensory evaluation, no dispersion is given, because values were obtained by consensus.

Sample	Torque (UB)	Q (g/min)	SMEC (J/g)	Exp.	SV (cm³/g)	S (%)	Sensorial hardness	Res (kgf)
CS	5825^{h,i}	84.05^{b,c,d}	637^{h,i}	4.02^{h}	10.10^{j}	43.0^{h}	3	3.02^{a,b}
AA	5825^{h,i}	83.70^{a,b,c}	640^{i}	4.03^{h}	9.74^{h,i}	42.0^{g,h}	3	3.20^{b,c}
FS	5737.5^{h,i}	83.90^{a,b,c}	629^{g,h,i}	3.82^{g}	9.63^{h}	42.0^{g,h}	4	3.80^{d,e}
FS+AA	5675^{g,h,i}	83.10^{a,b}	628^{g,h,i}	3.61^{f}	8.86^{e,f}	38.0^{c,d}	3	3.50^{c,d}
Citr	5600^{f,g,h}	82.25^{a}	626^{g,h,i}	3.98^{h}	9.85^{i}	45.0^{i}	3	2.95^{a,b}
EDTA	5562.5^{f,g,h}	82.50^{a,b}	620^{f,g,h}	3.82^{g}	8.95^{f}	37.0^{c}	4	4.12^{e,f}
FS+EDTA	5675^{g,h,i}	84.20^{b,c,d,e}	620^{f,g,h}	3.82^{g}	8.92^{e,f}	40.0^{e,f}	3	4.30^{f,g}
FB	5525^{e,f,g}	82.50^{a,b}	616^{f,g}	3.61^{f}	9.18^{g}	40.5^{f}	2	2.70^{a}
FE	5512.5^{e,f,g}	83.25^{a,b}	610^{f,g}	3.83^{g}	8.93^{e,f}	38.0^{c,d}	3	3.08^{a,b}
FB+AA	5475^{e,f,g}	83,15^{a,b}	605^{f}	3.80^{g}	8.90^{e,f}	34.0^{b}	3	4.10^{e,f}
FE+AA	5412.5^{d,e,f}	82,50^{a,b}	603^{f}	3.50^{d,e,f}	8.74^{e}	35.0^{b}	3	3.70^{d,e}
FB+Citr	5500^{e,f,g}	83.87^{a,b,c}	601^{f}	3.74^{g}	9.15^{g}	39.0^{d,e}	3	3.15^{b,c}
FB+EDTA	4850^{a}	85.30^{c,d,e}	522^{a,b}	3.30^{b,c}	6.91^{a}	32.0^{a}	5	5.70^{h}
FE+Citr	5425^{d,e,f}	85,85^{e}	580^{e}	3.55^{e,f}	7.81^{d}	37.0^{c}	4	4.60^{g}
FS+Zn	5225^{b,c,d}	85.15^{c,d,e}	563^{d}	3.45^{d,e}	7.40^{c}	37.0^{c}	4	3.90^{d,e,f}
Ca+Zn	5500^{e,f,g}	90,25^{f}	560^{d}	3.29^{b,c}	7.40^{c}	40.0^{e,f}	7	6.20^{i}
FS+Ca	5180^{b,c}	85.62^{d,e}	557^{d}	3.41^{c,d}	7.20^{b}	34.0^{b}	4	4.10^{e,f}
M	5412.5^{d,e,f}	91.00^{f,g}	547^{c,d}	3.25^{b}	7.42^{c}	41.0^{f,g}	6	5.80^{h}
M+Citr	5250^{b,c,d}	90,32^{f}	535^{b,c}	3.29^{b,c}	7.50^{c}	41.0^{f,g}	5	5.50^{h}
M+EDTA	5350^{c,d,e}	92.25^{g}	533^{b,c}	3.27^{b}	7.40^{c}	39.0^{d,e}	5	5.90^{h,i}
Ca+Citr	5125^{b}	92.02^{g}	511^{a}	3.02^{a}	7.10^{b}	42.0^{h,h}	8	7.05^{j}

Table 2. Average values for: Torque, Mass Flow (Q), Specific Mechanical Energy Consumption (SMEC), Expansion (Exp), Specific Volume (SV), Total soluble solids (S), Sensorial hardness, Mecanical Resistence (Res)

Table 2 also shows the results of ANOVA for the 21 samples. It is observed that there are significant differences between samples for all properties evaluated. The values of torque and Q were affected differently according to the composition of the sample. In general, the torque values varied in a narrow range for most samples: 5825 and 4850 BU, and Q, between 82.25 and 85.90 g/ min, except for those samples containing Ca. In this case, Q was significantly higher (between 90.25 and 92.25 g/ min), while the torque values were lower than those expected for these Q levels (between 5500 and 5125 BU). The samples containing citrate showed intermediate values of torque and Q: between 4850 and 5500 BU and between 85.15 and 90.25 g/ min, respectively.

When working with corn meal, SMEC and S are direct indicators of degree of cooking (Gonzalez et al., 1987; Mercier & Feillet, 1975). However, low SMEC values obtained for samples with Ca would not correspond to the high values of the S that these samples have. This apparent discrepancy could be explained considering that the alkalinity produced by the $CaCO_3$ would generate a greater amount of soluble compounds during extrusion, ie the soluble compounds produced by the thermo-mechanical action, plus those produced by chemical action.

The lower S values corresponding to citrate containing samples are attributed to the decrease in thermo-mechanical action (less SMEC), which directly influences the degree of cooking.

Another feature of the samples with Ca is that they have lower values of Exp. and SV. While samples containing Citr presented low values for SV and intermediate values for Exp.

Figure 1 shows the direct relationship between the Exp and SV. At higher expansion, higher is the product porosity, and therefore higher specific volume the product has. Figure 1 also shows two distinctive groups: the samples with Ca and citrate, with lower SMEC and Exp values and the rest of the samples, which maintain the aforementioned relationship between the Exp and SV.
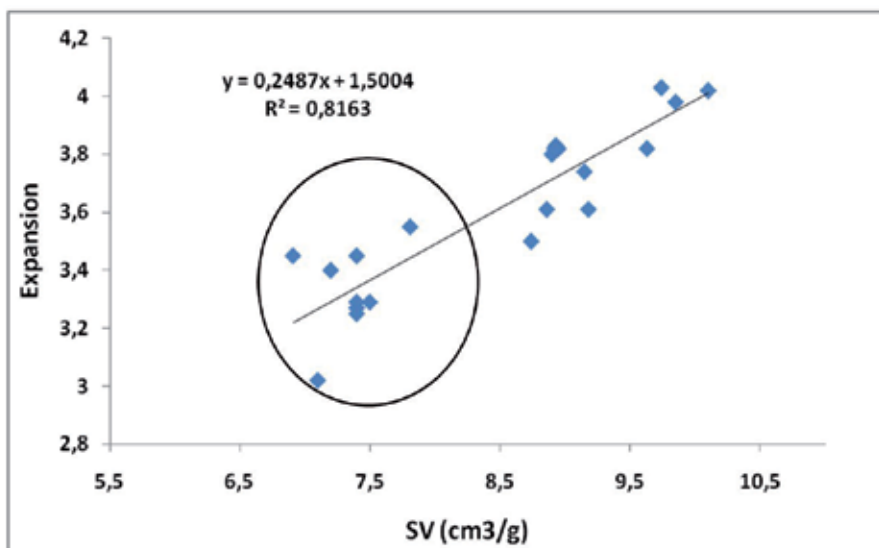
Fig. 1. Relationship between the expansion and specific volume (SV). Circle includes samples containing Ca and Citr.

Figure 2 shows the direct relationship between SV and SMEC as expected, since both are direct indicators of degree of cooking. It is also observed the two different sample groups: those with Ca and / or citrate and the rest of the samples.
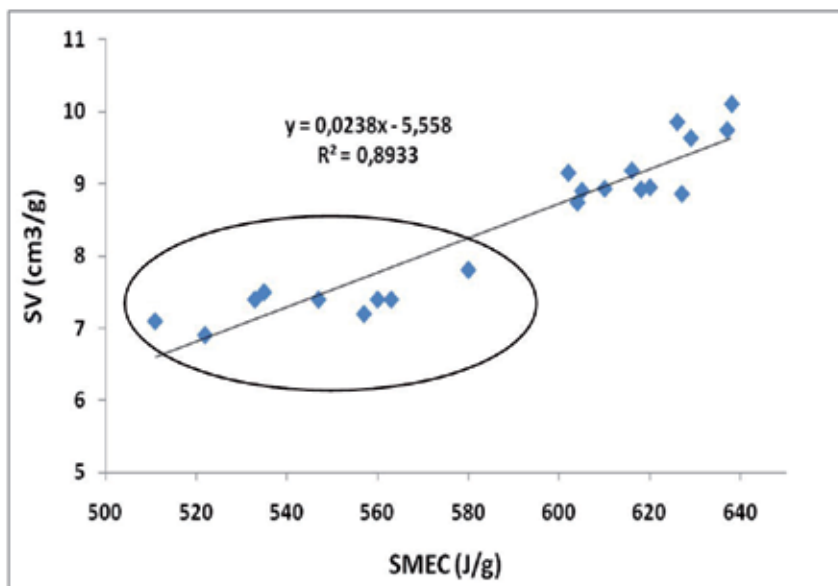
Fig. 2. Relationship between the specific volume (SV) and specific mechanical energy consumption (SMEC). Ellipse includes samples containing Ca and Citr.

Figure 3 shows the relationship between Exp and SMEC.
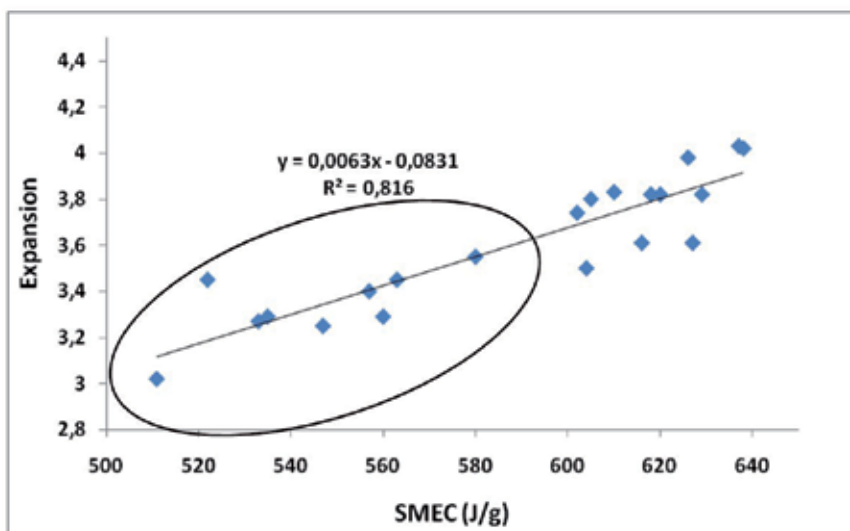
Fig. 3. Relationship between expansion and specific mechanical energy consumption (SMEC). Ellipse includes samples containing Ca and Citr.

It is noted that, even with some degree of dispersion, the direct relationship between these two responses is maintained, such as when working with corn meal (González et al., 2002). A direct relation between S and SV was also observed. However, correlation coefficient is not very good (r < 0.505). As already mentioned, S values corresponding to samples containing Ca are higher than expected according to the values of SMEC and SV, so greater data dispersion is obtained which would be the cause for the lower r value obtained. Figure 4 shows the relationship between the mechanical and sensorial hardness. There is a very good correlation between both responses confirming that the chosen methodology is appropriate to establish differences between samples.
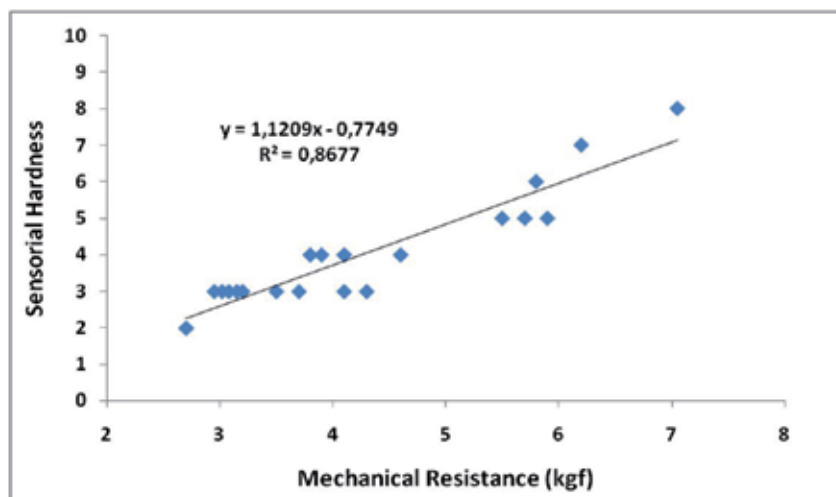
Fig. 4. Relationship between sensorial hardness and mechanical resistance.
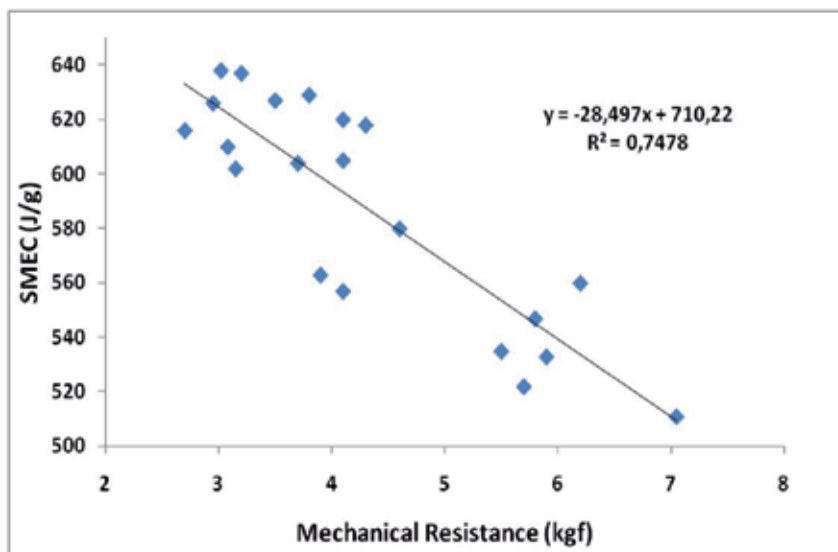
Fig. 5. Relationship between specific mechanical energy consumption (SMEC) and mechanical resistance.

Figure 5 shows the inverse relationship between the mechanical resistance and SMEC, which is expected, since as degree of cooking increases extrudate structure became lighter, as a consequence of the reduction of wall thickness of the extrudate pores (González et al., 1987). Similar tendency was also found for other materials such as beans and corn-bean mixtures (Balandrán-Quintana et al., 1998; Ruíz-Ruíz et al., 2008).

3.2 Study of mineral availability
3.2.1 Evaluation of the content of mineral and its dialyzability
Table 3 shows the results of total iron, zinc and calcium content and the corresponding percentage of dialysis, both for raw materials and extruded control samples.

Samples	Fe (mg/kg)	DFe%	Zn (mg/kg)	DZn%	Ca (mg/kg)	DCa%
Corn grits	11.2 ± 0.8	ND	3.7 ± 0.2	33.5 ± 2.9	26 ± 3	87.0 ± 4.0
Soybean grits	48.0 ± 0.2	2.2 ± 0.1	22.5 ± 0.6	34.5 ± 1.4	2130 ± 40	20.8 ± 1.1
Extruded Corn: soybean (88:12)	21.9 ± 1.0	12.5 ± 0.8	6.5 ± 0.4	62.0 ± 2.0	271 ± 4	30.1 ± 1.2

ND: Fe non detected in dialyzates.

Table 3. Mineral content in dry base and dialyzability from raw materials and extrude corn soybean blend

It is observed that the iron content of corn grits is very low, while that of soybean is about 4 times higher. The addition of 12% soy grits increased the content of this mineral. However, the value reached after extrusion is higher than that obtained from a simple mixture of raw materials. This effect has been observed by other authors, i.e. the extrusion increases the iron content from extruded product (Ummadi et al., 1995; Guy, 2001), which can be attributed to the contribution caused by wear on the barrel and screw during extrusion. This is possible because, for the extrusion conditions corresponding to expanded products, friction levels are high (SMEC values greater than 500 J/ g).

It is noted that DFe% from extruded corn:soybean (88:12) is higher than those from corn or soy grits. The low values of dialysis obtained for raw materials may be due to the presence of inositol hexaphosphate (phytate) in soybean meal, which is a strong inhibitor of Fe absorption (Davidsson et al., 2002). In the case of maize both, the low Fe initial content and the presence of residual phytate (as the corn meal is dehulled) could be involved in DFe% low values. Both, the decreasing content of phytate soybean by dilution with corn in the blend and the effect of extrusion (Ummadi et al., 1995; Guy, 2001) caused DFe% increasing in corn: soybean blend. Another important factor to take into account to explain the higher DFe% is the protein denaturation during extrusion-cooking. It could affect solubility, which could exert variable effects on DFe%, depending on process conditions (Ummadi et al, 1995).

Regarding zinc and calcium content, the values reported in the bibliography vary within a certain range due, not only to the difference of the sample used for analysis, but also because the measurements can be performed on whole meal or peeling and most of the

reports does not explain how they were obtained. For example, Senser & Scherz, (1999) reported values of 180 mg Ca/ kg and 24 mg Fe/ kg for corn meal and 1950 mg/ kg of Ca and 120 mg/ kg of Fe for whole soy flour, respectively.

DZn% from the extruded blend is greater than that of raw materials. The dilution of inhibitors, the possible decrease in the phytate content and protein denaturation during extrusion, could contribute to this increase, as was explained for DFe%. However, this result differs to that observed in corn: *Vigna unguiculata* (85:15) blends (Drago et al., 2007), where other extrusion conditions, appropriate to the raw material used were employed. The degradation of phytate to lesser forms of inositol phosphate (tetra-, tri-or di-phosphate) depends on the extrusion process conditions used and does not follow a definite pattern (Ummadi et al., 1995).

In the case of DCa%, although the dialysis value from corn grits is very high, the content of this mineral is very low. The addition of soybean increased the calcium content of the mixture, yielding an acceptable availability.

3.2.2 Effect of absorption enhancer addition on mineral dialyzability

In Figure 6 it is observed that the addition of EDTA or Citr increased the dialysis of Fe, Zn and Ca endogenous with respect to the blank (CS), but this was not observed for ascorbic acid addition.
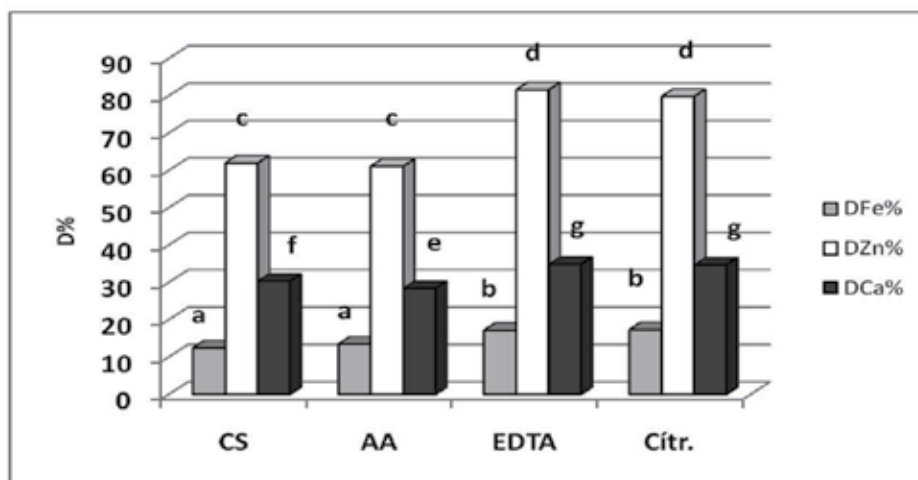
Fig. 6. Mineral availability (DFe%, DZn% and DCa%) from extruded samples with the addition of different absorption enhancers. Different letters mean significant differences between samples (P <0.05). a-b: for DFe%; c-d: for DZn%; e-g: DCa%

EDTA is a chelating agent that combines stoichiometrically with Fe, forming more stable complexes with Fe^{3+} than with Fe^{2+}, which promotes absorption. Thus, its use as an enhancer promises to be an effective strategy to increase iron absorption (Nayak & Nair, 2003).

Probably citrate forms soluble complexes with minerals that would facilitate the dialysis of Fe, Zn and Ca. Miller & Berner, (1989) suggest that the citrate and EDTA are strong complexing anions that improve mineral availability by competing for cations with other products of digestion which tend to insolubilize them at duodenum level.

Although no significant differences in mineral dialyzability were observed by using Citr or EDTA, when sensory evaluations were conducted, it was felt a gentle citric acid taste in the samples with Citr. However, even if not a study of acceptability of these samples was made, it is estimated that this effect will not be noticed with the addition of salt, oil and flavorings normally done, for the final formulation of a commercial product.

Regarding AA addition, the results obtained in relation to Fe availability differ from those reported by other authors (Clydesdale & Nadeau, 1985; Gorman & Clydesdale, 1983; Wolfgor et al., 1996). It should be noted that in these cases the foods were obtained under different processing conditions. The AA is a labile compound and probably degraded during the extrusion process. On the other hand, Siegenberg et al., (1991) suggest that ascorbic acid prevents the inhibitory effect of phytates when administered to a certain dose (30 mg AA to 10-58 mg phytate P). It would be necessary to measure phytate content and residual AA in these samples, in order to determine whether the lack of AA effect is due to insufficient level of AA.

3.2.3 Effect of iron sources on mineral dialyzability

Figure 7 shows mineral dialyzability from extruded products made using the selected iron sources: ferrous sulfate (FS), ferrous bisglycinate (FB) and FeNaEDTA (FE).

It was noted that the DFe% from FS and FB fortified samples was lower than that from the CS, and the DFe% from FB is less than that of FS. This last result is different from that reported by other researchers who observed that FB has better bioavailability than FS, especially in foods containing inhibitors of non-heme iron absorption (Bovell-Benjamin et al., 2000; Layrisse et al., 2000; Olivares et al., 1997; Pizzarro et al., 2002).
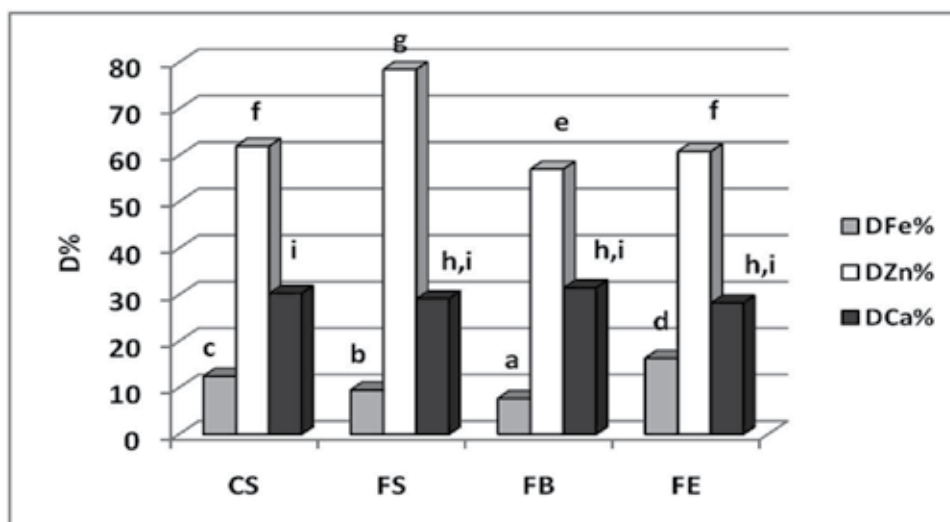
Fig. 7. Mineral availability (DFe%, DZn% and DCa%) from extruded samples with the addition of different iron sources. Different letters mean significant differences between samples (P <0.05). a-d: for DFe%; e-g: for DZn%; h-i: DCa%

FS is the reference iron source for fortification (Hallberg & Rossander, 1984). However, it is likely to cause organoleptic changes in foods in which it is used (Pizzarro et al., 2002; Wolfgor

et al., 1996) and susceptible to the inhibitory effect of food matrices (Hallbreg & Rossander, 1984). On the other hand, it is known that at pH 2 (pH of the stomach), iron from either FS, FB or FE is fully ionized. When the pH increase to 6 (pH close to the duodenum), the iron from FB and FE is soluble, while the solubility of iron from the FS decreases by 64% compared with the amount of soluble iron at pH 2, showing thus, the solubility of iron from the FB and FE sources is not affected by changes in physiological pH, probably because iron remained associated with the respective compounds (Garcia-Casal & Layrisse, 2001) and could promote iron availability.

Bovell-Benjamin et al., (2000) and Pizarro et al., (2002) suggest that iron from ferrous bisglycinate is absorbed similarly to FS and it is ionized in the acidic environment of the stomach, releasing the cations of iron (as Fe^{2+}), which are then absorbed in the duodenum following the path of non-heme iron absorption and is, like the FS, subjected to the action of inhibitors.

However, in our study, the DFe% from FB was slightly lower than that of FS, which could be due to an interaction of Fe amino-chelate with the food matrix during extrusion processes (high temperature and pressure).

The highest value of DFe% was obtained with the sample fortified with FE, possibly due to the complexing ability of EDTA which reduces the effect of intrinsic inhibitors of cereals (Bothwell & MacPhail, 2004; García-Casal & Layrisse, 2001). The FE is what is called "protected iron compound".

Another important fact to consider is that the use of FE did not produce sensory changes in the extruded samples, which agree with the statement made by Davidsson et al., (2002), who suggest that there is evidence that supports the use of FE to fortify cereals. The use of FE in fortification programs is very recent and limited, because only in 1999, the Expert Committee on Food Additives of FAO / WHO, declared it safe to use on human health and EF can be used in supervised fortification programs, providing about 2 mg Fe / kg body weight/ day (JECFA, 1999).

DZn% was affected by using different sources of fortification. Thus, when FS was used, the maximum value of dialysis (78.46%) was reached. It could be supposed that the ionic iron in excess relative to zinc (in the case of FS fortification), releases zinc from possible interactions with inhibitory compounds.

Regarding FE fortification, there was no promoting effect of EDTA on the availability of Zn, as observed in other food matrices such as milk and yogurt (Drago & Valencia, 2008), or bread rolls (Davidsson et al., 1994; Hurrell, 1997).

The DCa% was about the same (p: 0.17) regardless of the source of iron used.

Davidsson et al., (2002) and Walter et al., (2003) suggested that FE is a good alternative to replace ferrous sulfate, as it has a low interaction with the food matrix. However, it could interact with some other compounds present in foods (like cereal with banana extract, or cocoa-based foods, etc), which could cause unacceptable sensory disturbances (Hurrell, 1997).

3.2.4 Effect of iron absorption enhancers on mineral dialyzability from corn: soybean blends fortified whit different iron sources

The results of FS fortification and iron enhancers addition is shown in Figure 8.

It can be seen that the use of enhancers, in all cases increased DFe%, being the most important, that obtained when EDTA was used (2.6 times compared to the sample without enhancers). Ascorbic and citric acids enhanced DFe%, but their effect was less than that of
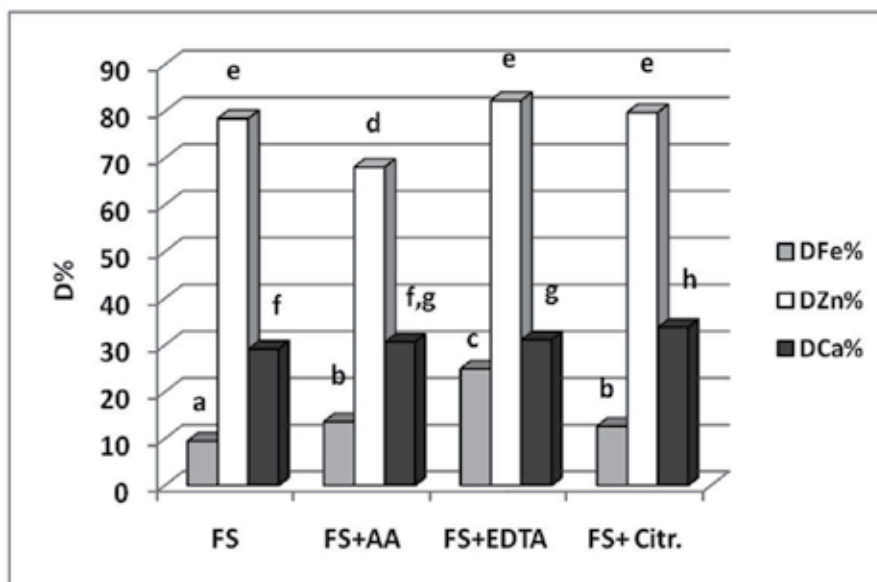
Fig. 8. Mineral availability (DFe%, DZn% and DCa%) from ferrous sulfate (FS) fortified extruded samples with the addition of different iron absorption enhancers. Different letters mean significant differences between samples (P <0.05). a-c: for DFe%; d-e: for DZn%; f-h: DCa%
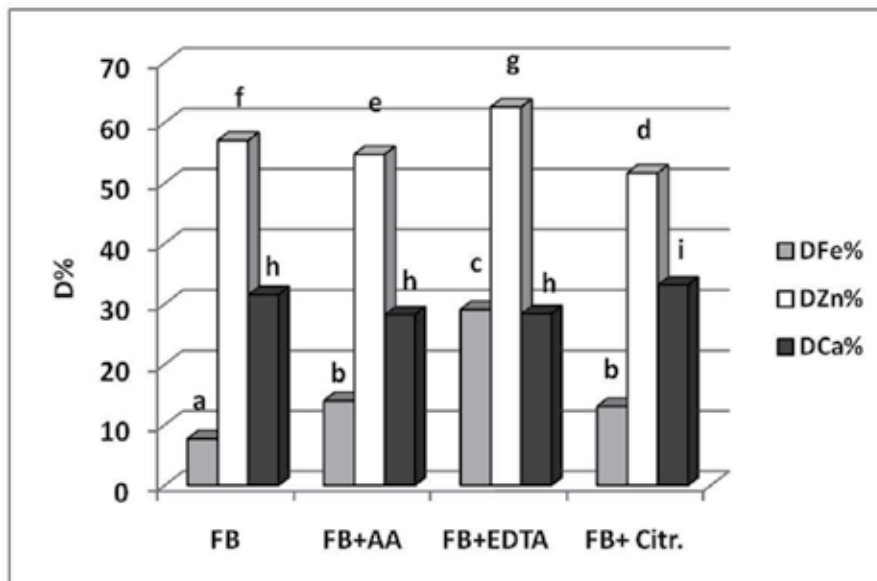
Fig. 9. Mineral availability (DFe%, DZn% and DCa%) from ferrous bisglycinate (FB) fortified extruded samples with the addition of different iron absorption enhancers. Different letters mean significant differences between samples (P <0.05). a-c: for DFe%; d-g: for DZn%; h-i: DCa%

EDTA. These results are consistent with those reported by Walter et al., (2003). They conducted trials in humans who ate corn tortillas fortified with ferrous fumarate, ferrous sulfate, ferrous bisglycinate with and without the addition of EDTA. They found that EDTA improved the bioavailability of iron from samples fortified with the three iron sources.

The already high DZn% did not improve by the addition of enhancers (EDTA and Citr) and decreased in the case of AA addition. However, Hurrell et al., (1994) observed in studies conduced in rats, that the addition of EDTA increased the bioavailability of Zn in diets based on soy isolate.

Both the use of citric and EDTA slightly increased the DCa%.

Thus, when using ferrous sulfate as a source of fortification, the best enhancer proved to be the EDTA, followed by citrate, because not only favored the Fe, but also the Ca availability.

Figure 9 shows the results obtained when using the ferrous bisglycinate (FB), with and without the addition of iron absorption enhancers.

Regarding DFe% the best enhancer proved to be EDTA, although AA and Citr also showed a promoting effect. The results obtained for iron, is consistent with that reported by Walter et al., (2003) cited above, in relation to the fact that EDTA increases the bioavailability of FB.When this source of iron was used, it was observed that EDTA promoted DZn% but Citr and AA were unfavorable. Only Citr increased DCa%.

In Figure 10 it is observed the effect of enhancers: AA and Citr, when FE was the iron source used. It is seen that AA did not modify DFe% or DCa%, but was the best enhancer for DZn%. In turn, Citr increased the dialysis of Fe and Ca, but decreased the DZn%, still below than that obtained in the absence of any enhancer.

These results show that depending on the source of iron, calcium or zinc that is used in the fortification, it should be selected suitable enhancers, or may be convenient not use anyone.
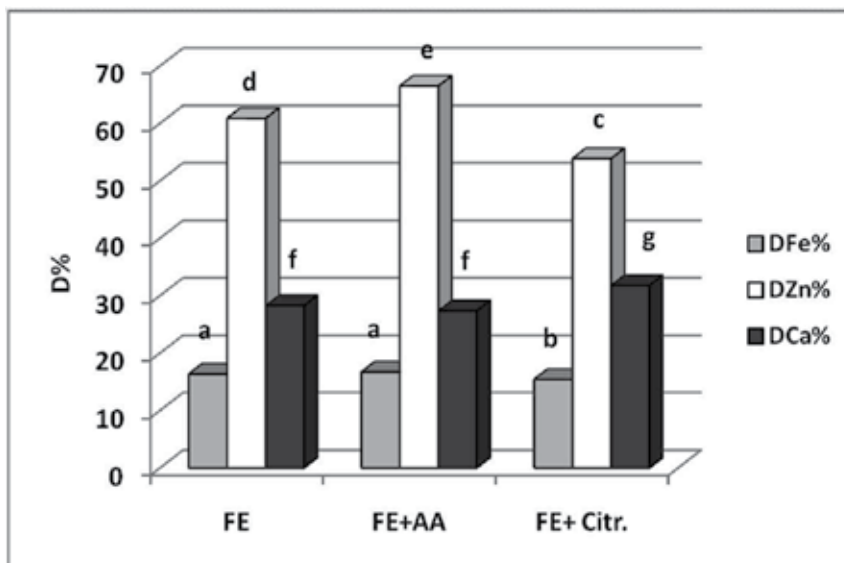
Fig. 10. Mineral availability (DFe%, DZn% and DCa%) from ferric sodium EDTA (FE) fortified extruded samples with the addition of different iron absorption enhancers. Different letters mean significant differences between samples (P <0.05). a-b: for DFe%; c-e: for DZn%; f-g: DCa%.

3.2.5 Fe, Zn and Ca availability from multi-fortified samples

Figure 11 shows the results obtained from studies on the availability of minerals from extruded corn: soybean fortified with FS as a source of iron and also adding Zn and / or Ca, with and without the addition of absorption enhancers. The enhancers used were selected based on the results of previous experiences, so we rejected the use of AA.

The addition of Zn in the presence of FS (FS vs. FS+Zn) did not affect DFe%, but the addition of Ca markedly decreased (FS vs. FS+Ca). This effect was even greater when Zn was added (FS vs. M, M: combination of three sources: $FeSO_4$, $ZnSO_4$ and $CaCO_3$). The simultaneous addition of Zn and Ca impaired DFe% of endogenous iron, even in the absence of fortification with FS (CS vs. Zn+Ca).

The use of enhancers, like Citr and EDTA for multi-fortified samples (with Fe, Zn and Ca) was very favorable, being EDTA the best enhancer.
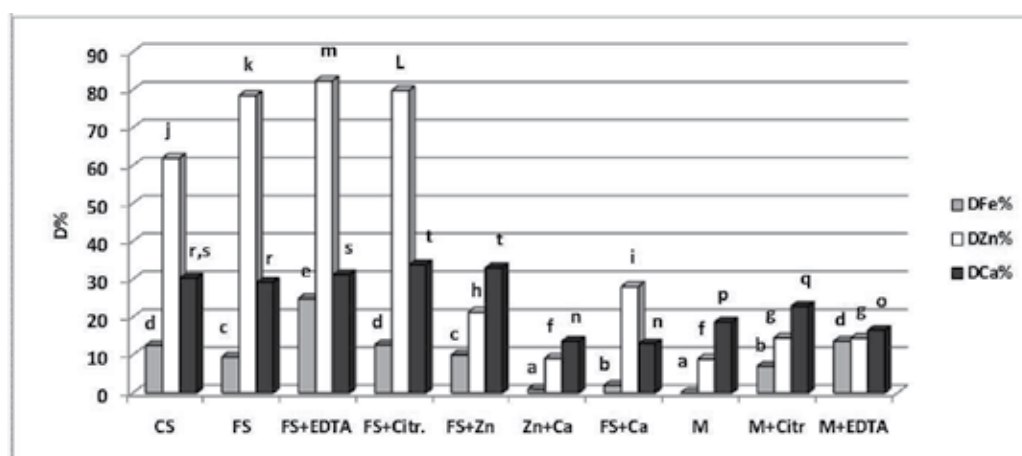
Fig. 11. Mineral availability (DFe%, DZn% and DCa%) from multi-fortified extruded samples with the addition of different iron absorption enhancers. Different letters mean significant differences between samples (P <0.05). a-e: for DFe%; f-m: for DZn%; n-t: DCa%.

In presence of FS, endogenous Zn availability is very high and even higher than the blank (CS), but adding $CaCO_3$ (FS+Ca) decreases significantly and is even lower when Zn as $ZnSO_4$ is added (FS vs. FS+Zn). Probably the added Zn interacts with the food matrix by forming complexes unable to dialyze for its insolubility and molecular size. Moreover, the addition of Ca in the presence or absence of FS caused a significant decrease in DZn% (Zn+Ca and M). This decrease may be due to complex formation between calcium, zinc and phytates, which are insoluble and cannot dialyze, nor absorbed (O'Dell, 1989; Wise, 1995).

Enhancer addition (Citr. or EDTA) to samples multi-fortified with Fe, Zn and Ca improves the availability of Zn, although the level reached is significantly lower than those reach for the control and the sample with only FS added.

These results indicate that Ca interacts with both Fe and Zn, endogenous and from fortification. The technique used to determine the mineral availability is an *in vitro* method. This implies that for these minerals, the inhibition by physicochemical interaction play an important role in the inhibition of *in vivo* absorption. Besides that, Ca inhibits Fe at mucosal level, as it was demonstrated by Hallberg et al., (1991, 1992).

In the presence of FS, endogenous Ca availability is high and equal to control. Also, the addition of Zn is beneficial for dialysis (CS vs. FS+Zn). However, the Ca added as $CaCO_3$ has lower availability (FS vs. FS+Ca). Something similar happens with the addition of Ca in the absence of FS and the presence of Zn (Zn+Ca). The Ca added interacts with the food matrix forming complexes unable to dialysis because of their insolubility or molecular size.

The increased availability of Ca, is observed with the addition of the three minerals FS+Zn+Ca (M vs. FS+Ca vs. Zn+Ca). This suggests that other minerals (Zn and Fe) have slightly more affinity for inhibitory ligands, compete for binding with them, and consequently the conditions for the Ca dialyzability are more favorable. The addition of Citr is a good option in the case of a product multi-fortified with Fe, Zn and Ca, because it improves the availability of Ca and Zn, but the use of EDTA for the same purpose is a better option, as also favors the DFe%. However, the high values of dialyzability for Fe, Zn and Ca from the sample with FS+EDTA were not reached.

4. Conclusions

The source of mineral and enhancers affected the characteristics of the extruded products. From all sources and enhancers selected, samples with the addition of Ca and Citr were the ones that differ from the rest, in terms of their physical properties. With $CaCO_3$, the extruded products had lower values of Exp and SV, which could cause lower product acceptability. When citrate was used as an enhancer, the extruded products showed intermediate values of Exp and low values of SV. The addition of Ca and / or Citr, involved a lower degree of cooking. Thus, these samples were the hardest, both for sensory and mechanical test.

The addition of soy grits (12% replacement) not only improve the protein value (protein content and quality) without impairing the sensory attributes of the expanded, but also improved the supply of iron and zinc (more content and better availability) and calcium (increased supply) of these products.

Although endogenous mineral availability is higher than that from mineral fortification, the final supply from the fortified product is higher. This is related to the iron source that is used in the fortification and the use or not of proper enhancer.

The use of EDTA may be an appropriate strategy to enhance the contribution of intrinsic minerals (Fe, Zn and Ca) of these products without fortification.

Of the three iron sources evaluated, FeNaEDTA had the highest availability of iron compared to ferrous sulfate and ferrous bisglicinate. Its use did not affect the availability of endogenous Zn and Ca. However, the sample fortified with FS had a better availability of Zn. This fact, together with the high cost of FE makes the FS the most appropriate iron source to fortify this kind of products.

When ferrous sulfate was used as a source of fortification, the best enhancer proved to be the EDTA, because not only favored the availability of Fe, but also Ca availability. The addition of AA or Citr showed no advantage when FeNaEDTA was used. AA was not a good enhancer for this kind of matrix and process.

Interactions were demonstrated between the sources of minerals. Ca fortification impaired Zn availability and most significantly Fe availability. This negative effect was greater in the presence of Zn and Ca.

The use of EDTA as an enhancer improved the availability of minerals in multi-fortified products.

When a multi-fortified product is going to be formulated, it is very important to evaluate the interactions among different minerals and to select the combination of mineral sources and absorption enhancers more appropriate for the elaboration of the product.

Calcium is an essential mineral whose daily requirement expressed in terms of mass is several times higher than the requirements of iron and zinc. Thus Ca can interfere with absorption of these micronutrients. For this reason, calcium, iron and zinc fortification would not be desirable in the same food, being much more advantageous to choose different foods to which they can be fortified with micronutrients, selecting those which have little or no interference between them.

Each micro-nutrient has its own chemical and technological characteristics that must be carefully considered when planning a fortification strategy. It is also important to consider the technological challenges that come with industrial fortification with more than one micronutrient, because the negative effect that could cause on the sensory characteristics of fortified foods.

5. Acknowledgment

6. References

Abd El-Hady, E.A. & Habiba, R.A. (2003). Effect of soaking and extrusion conditions on antinutrients and protein digestibility of legume seeds. *Lebensm.-Wiss. U.-Technol,* Vol.36, pp. 285-293, ISSN: 0023-6438

Anderson, R. A.; Conway, H. F.; Pfeifer, V. F. & Griffin, E. L. (1969). Gelatinization of corn grits by roll and extrusion – cooking. *Cereal Sciences Today,* Vol.14, pp. 1-12. ISSN: 0009-0360

A.O.A.C. (1995). Official Methods of Analysis, (16th ed.), Horowitz, Washigton DC, USA.

Balandrán-Quintana, R.R.; Barbosa-Cánovas, G.V.; Zazueta-Morales, J.J.; Anzaldúa-Morales, A. & Quintero-Ramos, A. (1998). Functional and nutritional properties of extruded whole Pinto bean meal (Phaseolus vulgaris L.). *J Food Sci,* Vol.63, No.1, pp. 113-116, ISSN: 0022-1147

Bovell-Benjamin, A.C.; Viteri, F.E. & Allen, L.H. (2000). Iron absorption from ferrous bisglycinate and ferric trisglycinate in whole maize is regulated by iron status. *Am J Clin Nutr,* Vol.71, pp. 1563–1569. ISSN: 0002-9165

Bothwel, T.H. & MacPhail, A.P. (2004). The Potential role of NaFeEDTA as an iron fortificant. *International Journal for Vitamin and Nutrition Research,* Vol.74, pp. 421–434, ISSN: 0300-9831

Clydesdale, F.M. & Nadeau, D.B. (1985). Effect off acid pretreatament on the stability of ascorbic acid complexes with various iron sources in a wheat flake cereal. *J Food Sci,* Vol.50, pp. 1342-1347, ISSN: 0022-1147

Cook, J.D.; Morck, T.A. & Lynch, S.R. (1981). The inhibitory effect of soy products on nonheme iron absorption in man. *Am J Clin Nutr*, Vol.34, No.12, pp. 2622-2629, ISSN: 0002-9165

Davidsson, L.; Kastenmayer, P. & Hurrell, R.F. (1994). Sodium iron EDTA [NaFe(III)EDTA] as a food fortificant: the effects on the absorption and retention of zinc and calcium in women. *Am J Clin Nutr,* Vol.60, pp. 231-237, ISSN: 0002-9165

Davidsson, L.; Dimitriou, T.; Boy, E.; Walczyk, T. & Hurrell, R. F. (2002). Iron bioavailavility from iron-fortified Guatemalan meals based on corn tortillas and black bean paste. *Am J Clin Nutr*, Vol.75, pp. 535-9, ISSN: 0002-9165

Drago, S.R.; González, R.J.; Chel-Guerrero, L. & Valencia, M.E. (2007) Evaluación de la Disponibilidad de Minerales en Harinas de Frijol y en Mezclas de Maíz/Frijol Extrudidas. *Revista de Información Tecnológica*, Vol.18, No.1, pp. 41-46, ISSN 0718-0764

Drago, S.R. & Valencia, M.E. (2008). Mineral dialyzability in milk and fermented dairy products fortified with FeNaEDTA. *J Agric Food Chem*, Vol.56, pp. 2553–2557, ISSN: 0021-8561

Fairweather-Tait, S.J.; Portwood, D.E.; Symss, L.L.; Eagles, J. & Minski, M.J. (1989). Iron and zinc absorption in human subjects from a mixed meal of extruded and non-extruded wheat bran and flour. *Am J Clin Nutr.* Vol.79, pp. 151-158, ISSN: 0002-9165

Fairweather-Tait, S.J. (1992). Bioavailability of trace elements. *Food Chem*. Vol.43, pp. 213-217, ISSN: 0308-8146

Fritz, M.; González, R.; Carrara, C.; De Greef, D.; Torres R. & Chel, L. (2006). Selección de las condiciones de extrusión para una mezcla maíz-frijol: aspectos sensoriales y operativos. *Brazilian Journal of Food Technology*, Edición Especial III JIPCA. (Janeiro), pp. 3-7, ISSN: 1519-0900

García-Casal, M.N. & Layrisse, M. (2001). The effect of change in pH on the solubility iron bis-glycinate chelate and other iron compounds. *Archivos Latinoamericanos de Nutrición*, vol.51, No.1, pp. 35-36, ISSN 0004-0622

Gómez, M. H. (1985). Development of a food of intermediate moistness from extracts of corn and soybean. *Archivos Latinoamericanos de Nutrición,* Vol.35, No.2, pp. 306-314, ISSN: 0004-0622

González, R.J.; De Greef, D.M.; Torres, R.L. & Gordo, N.A. (1987). Efectos de algunas variables de extrusión sobre la harina de maíz. *Archivos Latinoamericanos de Nutrición,* Vol.37, pp. 578-591, ISSN 0004-0622

González, R.J.; Torres, R.L, & De Greef, D.M. (2002). Extrusion-Cocción de Cereales. *Bol. Sociedade Brasileira de Ciencia e Tecnologia de Alimentos.*, Campinas, Vol.36, No.2, pp. 104-115, ISSN: 0101-2061

González, R. J.; Robutti, J. L.; Borras, F. S.; Torres, R. L. & De Greef, D. M. (2004). Effects of endosperm hardness and extrusion temperature on properties obtained with grits from commercial maize cultivars. *Food Science and Technology*, Vol.37, pp. 193-198, ISSN: 0023-6438

González, R.J.; Torres, R.L; De Greef, D.M.; Bonaldo A.G. (2006) Effects of extrusion conditions and structural characteristics on melt viscosity of starchy materials. *Journal Food Engineering*, Vol.74, pp. 96-107, ISSN: 0004: 0260-8774

González, R.J. (2009) Cereales listos para consumir a base de arroz y maíz: procesos de obtención y aspectos de la comercialización. *Ingenieria Alimentaria Sección Procesos*. (Nov/Dic.). Vol.83, pp. 16-26, ISSN: 0328-865

Gorman, J.E. & Clydesdale, F.M. (1983). The behaviour and stability of iron ascorbate complexes in solution. *J Food Sci*, Vol.48, pp. 1217-1220, 1225, ISSN: 0022-1147

Greger, J.L. (1988). Calcium availability. *Cereal Food World*, Vol.33, No.9, pp. 796-800, ISSN 0146-6283

Guy, R. (2001). Extrusión y calidad nutritiva. In: *Extrusión de Alimentos. Tecnología y Aplicaciones*, pp. 107-122, Ed. Acribia, ISBN: 84-200-0981-4., Zaragoza, España.

Hallberg, L. & Rossander, L. (1982). Effect of soy protein on non-heme iron absorption in man. *Am J Clin Nutr*, Vol.36, pp. 514-520, ISSN: 0002-9165

Hallberg, L. & Rossanser, L. (1984). Improvement of iron nutrition in developing countries: comparison of adding meat, soy protein, ascorbic acid, citric acid and ferrous sulphate on iron absorption from a simple Latin America-type of meal. *Am J Clin Nutr*, Vol.39, pp. 577-583, ISSN: 0002-9165

Hallberg, L.; Brune, M.; Erlandsson, M.; Sandberg, A.S. & Rossander-Hultén, L. (1991). Calcium: effect of different amounts on nonheme- and heme-iron absorption in humans. *Am J Clin Nutr*, Vol.53, pp. 112–119, ISSN: 0002-9165

Hallberg, L.; Rossanser-Hultén, L.; Brune, M. & Gleerup, A. (1992). Calcium and iron absorption: mechanism of action and nutritional importance. *Eur J Clin Nutr*, Vol.46, pp. 317-327, ISSN: 0954-3007

Hurrell, R.F.; Juillerat, M.A.; Reddy, M.B.; Lynch, S.P.; Dassenko, A.A. & Cook, J.D. (1992). Soy protein, phytate, and iron absorption in humans. *Am J Clin Nutr*, Vol.56, pp. 573-578, ISSN: 0002-9165

Hurrell, R. (1997). Preventing iron deficiency through food fortification. *Nutr Rev*, Vol.55, pp. 210-222, ISSN: 0029-6643

Hurrell, R.F.; Ribas, S. & Davidsson L. (1994). NaFeEDTA as a food fortificant: influence on zinc, calcium and copper metabolism in the rat. *British Journal of Nutrition*, Vol.71, No.1, pp. 85-93, ISSN: 0007-1145

JECFA. Joint FAO/WHO Expert Committee on Food Additives. Fifty third meeting, Rome, June 1-10, (1999). http://www.who.int/pcs/jecfa/Summary53revised.pdf

Layrisse, M.; García-Casal, M.N. & Solano, L. (2000). Bioavailability in humans from breakfasts enriched with iron glycine chelate, phytates and polyphenols. *Journal of Nutrition*, Vol.130, pp. 2195–2199, ISSN: 0022-3166

Liener, I.E. (1972). Nutritional value of food proteins products. In: *Soybeans: Chemistry and Technology*. Smith AK and Circle SJ (Ed.), pp. 203-277, The Avi Publishing Company INC, Westport, ISBN: 0-87055-111-6, Connecticut, USA.

Lönnerdal, B.; Bell, J.G.; Hendrickx, A.G.; Burns, R.A. & Keen, C.L. (1988). Effect of phytate removal on zinc absorption from soy formula. *Am J Clin Nutr*, Vol.48, pp. 1301-1306, ISSN: 0002-9165

Lynch, S.R.; Dasenko, S.A.; Cook, J.D.; Juillerat, M.A. & Hurrell, R.F. (1994) Inhibitory effect of a soybean-protein-related moiety on iron absorption in humans. *Am Clin Nutr*, Vol.60, pp. 567-572, ISSN: 0002-9165

Messina, M. J. (1999). Legumes and soybeans: overview of their nutritional profile and health effects. *Am J Clin Nutr*, Vol.70, pp. 439-450, ISSN: 0002-9165

Mercier, C. & Feillet, P. (1975). Modification of carbohydrate components by extrusion cooking of cereal products. *Cereal Chemistry*, Vol.52, pp. 283-297, ISSN: 0009-0352

Miller, D.D. & Berner, L.A. (1989). Is solubility in vitro a reliable predictor of iron bioavailability? *Biological Trace Element Reserch,* Vol.19, pp. 11-24, ISSN: 1559-0720

Miller, D.D.; Schricker, B.R.; Rasmussen, R.R. & Van Campen, D. (1981). An in Vitro method for estimation of iron availability from meals. *Am Clin Nutr*, Vol.34, pp. 2248-2256, ISSN: 0002-9165

Nayak, B. & Nair, K.M. (2003). In vitro bioavailability of iron from wheat flour fortified with ascorbic acid, EDTA and sodium hexametaphosphate, with or without iron. *Food Chem*, Vol.80, pp. 545-550, ISSN: 0308-8146

O'Dell, B.L. (1989). Mineral interactions relevant to nutrient requirements. *Journal of Nutrition*, Vol.119, No.12, pp. 1832-1838, ISSN: 0022-3166

Olivares, M.; Pizarro, F.; Pineda, O.; Name, J.J.; Hertrampf, E. & Walter, T. (1997). Milk inhibits and ascorbic acid favors ferrous bis-glycine chelate bioavailability in humans. *Journal of Nutrition,* Vol.127, pp.1407–11, ISSN: 0022-3166

Pérez, A.; González, R.J.; Drago, S.R.; Carrara, C.; De Greef, D.M. & Torres, R.L. (2008). Extrusion cooking of a maize-soybean mixture: Factors affecting expanded product characteristics and flour dispersion viscosity. *Journal of Food Engineering*, Vol.87, No.3, pp. 333–340, ISSN 0004: 0260-8774

Pizarro, F.; Olivares, M.; Hertrampf, E.; Mazariegos, D.I.; Arredondo, M.; Letelier, A. & Gidi, V. (2002). Iron bis-glycine chelate competes for the nonheme-iron absorption pathway. *Am J Clin Nutr,* Vol.76, No.3, pp. 577-581, ISSN: 0002-9165

Ruiz-Ruiz, J.; Martínez-Ayala, A.; Drago, S.R.; González, R.J.; Betancur-Ancona, D. & Chel-Guerrero, L. (2008). Extrusion of a hard-to-cook bean (*Phaseolus vulgaris L.)* and quality protein maize (*Zea mays L.*) flour blend. *Food Sci Tech*. Vol.41, pp. 1799-1807, ISSN: 0023-6438

Sandberg, A.S.; Carlsson, N.G. & Svanberg, U. (1989). Effects of inositol tri-tetra-, penta-, and hexaphosphates on in vitro estimation of iron availability. *J Food Sci,* Vol.54, pp.159-161, ISSN: 0022-1147

Senser, F. & Scherz, H. (1999). *El pequeño "Souci-Fachmann-Kraut". Tablas de composición de alimentos*. Ed. Acribia, ISBN: 84-200-0865-6, Zaragoza (España).

Siegenberg, D.; Baynes, R.D.; Bothwell, H.; Macfarlane, B.J.; Lamparelli, R.D.; Car, N.G.; MacPhail, P.; Schmidt, U.; Tal, A. & Mayet, F. (1991). Ascorbic acid prevents the dose-dependent inhibitory effects of polyphenols and phytates en no heme-iron absorption. *Am J Clin Nutr,* Vol.53, pp. 537-541, ISSN: 0002-9165

Singh, P.; Kumar, R.; Sabapathy, S.N. & Bawa, A.S. (2008). Functional and edible uses of soy protein products. *CRFSFS*, Vol.7, pp. 14-28, ISSN: 1541-4337

South, P.K. & Miller, D.D. (1998). Iron binding by tannic acid: effects of selected ligands *Food Chem,* Vol.63, No.2, pp. 167-172, ISSN: 0308-8146

Ummadi, P.; Chenoweth, W.L. & Uebersax, M.A. (1995). The influence of extrusion processing on iron dializability, phytates and tannins in legumes. *Journal of Food Processing and Preservation*, Vol.19, pp. 119-131, ISSN: 0145-8892

Walter, T.; Pizarro, T. & Olivares, M. (2003). Iron bioavailability in corn-masa tortillas is improved by the addition of disodium EDTA. *Journal of Nutrition,* Vol.133, pp. 3158-3161, ISSN: 0022-3166

Wise, A. (1995). Phytate and zinc bioavailability. *International Journal of Food Science and Nutrition*, Vol.46, pp. 53-63, ISSN 0963-7486

Wolfgor, R.; Drago, S.; Rodríguez, V.; Pellegrino, N. & Valencia, M. (2002). In vitro measurement of available iron in fortified foods. *Food Research International*, Vol.35, pp. 85-90, ISSN: 0963-9969

Wolfgor, R., Rodriguez, V., Pellegrino, N. & Valencia, M.E. (1996) Evaluación de Cereales Fortificados como aportadores de hierro. *Revista de la Sociedad Argentina de Nutrición*, Vol.7, No.2, pp. 33-37, ISSN: 0327-7925

4

Phytase: An Enzyme to Improve Soybean Nutrition

Xin Gen Lei[1] and Jesus M. Porres[2]
[1]Department of Animal Science, Cornell University, Ithaca, NY
[2]Department of Physiology, School of Pharmacy, Institute of Nutrition
and Food Technology, University of Granada
[1]USA
[2]Spain

1. Introduction

Soybean (*Glycine max*) serves as a major human food and animal feed component due to its nutritional and health values. As an important dietary source of protein, fat, fiber, minerals and vitamins, soybean also provides many bioactive components such as phytoestrogens with potential benefits for human health (Messina, 1999). Meanwhile, other components present in soybean like trypsin inhibitors and phytate can act as anti-nutritional factors that interfere with protein digestion or chelate nutritionally essential elements including Ca, Zn and Fe (Liener, 1994; Hurrell, 2003). While trypsin inhibitors are heat labile and are usually inactivated in the production of soybean meal or soy protein isolate, phytate is heat stable and needs phytases for its hydrolysis. Phytases are phosphohydrolytic enzymes that initiate the stepwise removal of phosphates from inositol hexaphosphate (Lei & Porres, 2007).

Phytase supplementation has become an efficient tool to improve bioavailability of P present in feedstuffs and to reduce the amount of phytate-derived P excreted to the environment by animals. Phytase-mediated hydrolysis of phytate also releases several other essential minerals (Lei et al., 1993a,b,c). Soybean meal is a common ingredient to be mixed with corn and other cereals for the swine and poultry ration. Various sources of plant and microbial phytases, along with other feed supplements such as citric acid, vitamin D, and strontium, have been tested to enhance utilization of P and other nutrients in the corn-soybean meal based diets (Han et al., 1998; Snow et al., 2004; Pagano et al., 2007b). Findings from these experiments have been used to improve performance and health of commercial herds and spare costly non-renewable sources of inorganic P.

Soybean serves as an important component of many dishes oriented to human nutrition. It is consumed as cooked, sprouted, and processed into soy milk, tofu, miso, tempeh or natto. Industrial processing of soybean is derived not only by its nutritional properties, but also by its chemical characteristics. Soy proteins contain lipophilic, polar, non-polar, and negatively and positively charged groups that enable them to be associated with many different types of compounds (Endres, 2001). Representing the major industrial products, soy oil and soybean meal are produced through a solvent extraction process. Crude soybean oil is further processed in to a variety of products, whereas soybean meal can be further processed to protein concentrates, protein isolates, or textured protein products for

preparations of comminuted meat products or meat analogs. Although phytase may be used to improve the nutritive utilization of soybean by humans, much less research in this regard has been done than that in animals.

2. Nutrient and non-nutrient composition of soybean

Soybeans are important dietary sources of protein, lipids, minerals, vitamins, fiber, and bioactive compounds. The chemical compositions of soybean and most of its derived products are characterized by high protein content that ranges from 33 to 43% (Grieshop et al., 2001; Karr-Lilienthal et al., 2004; Rani et al., 2008; Saha et al., 2008). After soy oil and hull are removed during processing of soybean meal, protein contents of the resultant products may rise to 47-59% (NRC, 1998; Grieshop et al., 2003). Higher protein contents may be achieved in specific extraction products like soybean protein concentrate or soybean protein isolate (64 and 85%, respectively; NRC, 1998). Soybean manifests an excellent amino acid profile, with only lysinine and methionine as the limiting amino acids (for swine). The potential availability of soybean amino acids can be affected by the extent of thermal processing conditions (Grieshop et al., 2003). Parameters like KOH protein solubility, protein dispersibility index (solubility in water) or urease activity (trypsin inhibitor activity) are used to assess the quality of soybean protein and the processing appropriateness of soybean products (Grieshop et al., 2003; Karr-Lilienthal et al., 2004). Oil and fiber are the other two major components of soybean. Acid-hydrolyzed fat is in the range of 13-15%, and may reach 22% (Achouri et al., 2008; Rani et al., 2008; Saha et al., 2008; Yuan et al., 2009). Soybean oil is mainly composed of polyunsaturated fatty acids followed by monounsaturated and saturated fatty acids. The major fatty acid is linoleic acid, although soybean oil has considerable amounts of oleic and linolenic acids (Nwokolo, 1996; NRC, 1998; Yuan et al., 2009). The presence of lipoxygenase can give rise to the appearance of off-flavors and aroma at different stages of processing, which may negatively affect the organoleptic properties of soybean oil. The major food uses of soybean oil are as salad or cooking oil, part of mayonnaise and dressings, and margarine or shortenings. Dietary fiber in soybean comprises from 11.3 up to 30% of the total seed content (NRC, 1998; Grieshop & Fahey, 2001; Karr-Lilienthal et al., 2004; Jiménez-Escrig et al., 2010). The fiber content of soybean meal is considerable, unlike lipids which are in very low proportion due to an initial extraction process. Soybean and its by-products are also a good source of nutritionally essential macro- and micro-minerals (Raboy et al., 1984; NRC, 1998; Giami, 2002; Karr-Lilienthal et al., 2004; Rani et al., 2008), although their availability can be seriously compromised by the presence of phytic acid, polyphenols, and oxalate, or by the specific structure of soybean proteins (Lynch et al., 1994).

A variety of non-nutritional components in soybean may interfere with its nutrient availability (Liener, 1994). Among these components, protease inhibitors and lectins decrease protein digestion, cause systemic effects on the digestive tract, and inhibit animal growth. Heat processing for the production of soybean meal and reduction of disulphide bonds using a NADP-thioredoxin system can inactivate such components and alter the compact structure of soybean proteins, thus improving the nutritional value of soybean containing foods (Liener, 1994; Kakade et al.,. 1972; Marsman et al., 1997; Giami et al., 2002; Olguin et al., 2003; Karr-Lilienthal et al., 2004; Faris et al., 2008). Meanwhile heat-stable components including phytate (Raboy et al., 1984; Han et al., 1988; Kumar et al., 2005; Yuan et al., 2009), polyphenols, saponins (Giami, 2002), oxalate (Ilarsan et al., 1997; Al-Whash et

al., 2005) or α-galactoside oligosaccharides may interfere with the bioavailability of protein, lipids or minerals present in the diet or induce flatulence (Suarez et al., 1999). Al-Whash et al. (2005) have reported that a strong correlation exists between oxalate and phytate content of soy foods and a significant correlation, based on molar basis, between the divalent ion binding potential of oxalate plus phytate and Ca plus Mg content. Nevertheless, some of these so-called anti-nutritional factors like saponins, phytic acid and polyphenols are also responsible for certain beneficial health effects related to soybean consumption (Rao & Sung, 1996; Porres et al., 1999; Kerwin, 2004; Kang et al., 2010; Zhang & Popovich, 2010). In general, the proximate composition of soybean or soybean meal is highly dependent on genetic, environmental, and processing conditions (Grieshop & Fahey, 2001; Karr-Lilienthal et al., 2004; Kumar et al., 2006). Improvement of soybean cultivars for specific characteristics such as early maturity, high yield, desired seed quality and resistance to pests has been carried out through plant breeding, and new lines of soybean have been developed (Giami, 2002). In addition, contents and properties of the major anti-nutritional components in soybean like trypsin inhibitors, polyphenols or phytic acid are not altered in the advanced lines tested.

3. Phytase: Enzymology and dietary efficacy

Phytases are phosphohydrolytic enzymes that initiate the stepwise removal of phosphate groups from *myo*-inositol hexakis phosphate. Four different classes of phosphatase activity are known to degrade phytic acid and to exhibit different catalytic efficiencies, structure, mechanism of action and biochemical properties (Lei et al., 2007). Histidine acid phosphatases are the most widely used phytases in animal feeds. The three remaining phytase groups include β-propeller phytases, cysteine phosphatases, and purple acid phosphatases. A phytate-degrading enzyme belonging to the last group has been reported in the cotyledons of germinating soybeans by Hegeman & Grabau (2001). Phytase efficacy in releasing phytate-P from corn-soybean meal diets has been reported (Lei et al., 1993a,b; Stahl & Lei, 2000; Auspurger et al., 2003; Applegate et al., 2003; Gentile et al., 2003). Estimated inorganic P/phytase equivalence in animal diets is that 300-600 phytase units/kg of diets can release 0.8 g of digestible P and replace either 1.0 or 1.3 g of P from mono- and dicalcium phosphate, respectively (Ravindram et al., 1995; Yi et al., 1996; Radcliffe and Kornegay, 1998; Esteve-Garcia et al., 2005). Supplemental phytase also improves the availability to farm animals of Ca, Zn or Fe in the soybean meal (Lei et al., 1993c; Lei et al., 1994; Stahl et al., 1999; Jondreville et al., 2005; Lei & Stahl, 2000, 2001). Dephytinization of soy formulas or soybean-derived food products intended for human consumption has also improved bioavailabilities of Fe and Zn (Hurrell, 2003).

The stomach seems to be the major site of action for the histidine acid phosphatases isolated from *Aspergillus niger* (Jongbloed et al., 1992; Yi and Kornegay, 1996) or *Escherichia coli* (Pagano et al., 2007a). Because *E. coli* phytase has a higher pepsin resistance than *A. niger* phytase (Rodriguez et al., 1999), pigs fed the *E. coli* phytase retained similar phytase activity in digesta among the stomach, duodenum and upper jejunum, whereas little phytase activity was found in the distal small intestine of *A. niger* phytase-fed animals. Interestingly, Pagano et al. (2007a) found an inverse relationship between colonic phytase activity and the amount of phytase supplemented to the diet. In a similar way, the major sites of phytase activity in poultry are the crop, gizzard and proventriculus, whereas little activity is found in the small intestine (Yu et al., 2004). There are several determinants of phytase efficacy to

improve the nutritional value of soybean derived products (Lei & Porres, 2007). The most important factor appears to be the Ca/P ratio that should be lower than 2:1 (Lei et al., 1994). While an excess of Ca in the diet inhibits phytate-P hydrolysis and decreases P availability (Tamin & Angel, 2003), an excessive amount of inorganic P can also negatively affect the effectiveness of phytase. Meanwhile, 1α-hydroxycholecalciferol and organic acid supplementation have shown synergistic effects on the phytase function in improving mineral bioavailability (Li et al., 1998; Snow et al., 2004; Han et al., 1998; Omogbenigum et al., 2003). Combined supplementations of phytase and other feed enzymes have been shown to improve the nutrient utilization of animal feeds (Ravindram et al., 1999; Wu et al., 2004; Juanpere et al., 2005). Likewise, combination of microbial phytase with ingredients like wheat middlings with high intrinsic phytase activity reduces the need for supplemental microbial phytase (Han et al., 1998). However, no major benefit was seen from the combination of different microbial phytases (Stahl et al., 2001; 2004; Auspurger et al., 2003; Gentile et al., 2003).

4. Synergism of soybean and phytase in nutrition

Due to its excellent protein quality, soybean meal has been extensively used as a common protein supplement in swine and poultry ration (Lei et al., 1993a,b; Fernandez-Figares et al., 1997; Stahl et al., 2003; Boling et al., 2000). Different strategies have been employed to improve its nutritional value. These include supplementing its limiting amino acids or mixing with corn, and wet feeding (Liu et al., 1997), supplementing organic acid (Ravindram & Kornegay, 1993), or treating with enzymes. Supplementations of exogenous carbohydrases, proteases or phytases enhance the dietary utilization of essential nutrients that otherwise would be lost to the animal and excreted to the environment. Soybean is also being increasingly used in aquaculture to replace the scarce and expensive fishmeal protein in diets for fish, crustaceans, and shellfish (Yan et al., 2002; Pham et al., 2010; Brinker & Reiter, 2011). In addition, substitution of 50% or even 100% of fish meal by a mixture of soybean meal and wheat gluten in trout diets counteracted the pathological alterations in the liver that were often related to highly-energetic fish meal diets (Brinker & Reiter, 2011).

Addition of phytase to the corn-soybean meal based diets for farm animals (Lei & Porres, 2007) improves phosphorus retention and bone metabolism not only in P-deficient, but also in P-adequate pigs. Pagano et al. (2007b) has observed increments in bone breaking strength (11-20%), mineral content (6-15%) and mineral density of metatarsals and femur of pigs fed P-adequate diets supplemented with the *E. coli* phytase and strontium. Supplemental phytase also resulted in larger bone areas and larger cross-sectional area of femur. Such findings could be of enormous interest in developing strategies to prevent and improve the recovery of hip fractions associated with osteoporosis in the elderly. Furthermore, phytase supplementation may enhance the availability of other minerals like Ca, Zn, Fe, Cu or Mn that are present in soybean meal but are currently added to swine and poultry diets (Adeola et al., 1995; Lei et al., 1993c; Lei et al., 1994; Rimbach et al., 1997; Stahl et al., 1999). Beneficial effects of phytase on Fe availability from soybean are quite a special issue since soybean consumption appears to affect differently the absorption of heme or non-heme Fe. A significant proportion of the Fe content in soybean is present in the seed coat with potentially good availability due to the lack of polyphenols in this seed constituent (Moraghan, 2004). However, consumption of soybean-derived products negatively affects non-heme Fe absorption (Derman et al., 1987) mainly due to the presence of phytic acid

(Hurrell et al., 1992; Davidson et al., 2001; Hurrell, 2003), and to a lesser extent due to the glycinin fraction (11S) of soybean protein (Lynch et al. 1994).

Although several low-phytate barley or corn lines have been developed and tested for nutritional applications (Sugiura et al., 1999; Baxter et al., 2003; Applegate et al., 2003; Overturf et al., 2003), the low-phytate soybean line has shown a reduced seedling emergence (Meis et al., 2003; Oltmans et al., 2005; Trimble & Fehr, 2010). In contrast, Yuan et al. (2009) have recently developed two new low phytic acid mutants and studied their nutritional properties. Their mutants showed good agronomic performance, reduced phytic acid, and increased inorganic P concentration in all tested environments without changes in the crude protein content, amino acids, total oil, and individual saturated fatty acids despite variations in oleic and linoleic acid contents. Furthermore, low phytic acid lines had a higher content of isoflavones than their parental wild-type lines.

Nevertheless, phytase supplementation is still the most feasible method for improving the phytate-mediated low availability of essential minerals in the corn soybean-based diets. In fact, Stahl et al. (1999) found that phytase was effective in releasing phytate-bound Fe and P from soybean meal *in vitro*, and in improving dietary Fe bioavailability for hemoglobin repletion in young anemic pigs fed a standard corn-soybean diet. On the other hand, Lynch et al. (1985) reported that partial substitution of beef with soy flour reduced the availability of non-heme Fe but significantly improved the percentage of heme Fe absorption, although the net effect appeared to be a modest reduction in the total amount of Fe absorbed. Furthermore, Beard et al. (1996) reported that Fe-deficiency anemia was overcome in rats fed diets containing soybean ferritin, and suggested that a considerable amount of Fe present in soybeans was associated with ferritin of high bioavailability. Davila-Hicks et al. (2004) found that Fe was equally well absorbed from ferritin and ferrous sulphate by non-anemic healthy young women, independent of the phosphate moieties of the ferritin Fe mineral (high phosphate Fe mineral of plant origin or low phosphate Fe mineral from animal origin). Hurrel et al. (1998) and Davidsson et al. (2001) reported that phytase-catalyzed dephytinization of soy or pea infant formula produced a significant improvement in Fe bioavailability, whereas Porres et al. (2001) supplemented different phytase enzymes to whole wheat bread and observed a significant phytic acid degradation, free P release and improvement of *in vitro* Fe availability. Phytase may also be applied in the industrial processing of soybean to prepare certain foods for human consumption. Saito et al. (2001) have developed a novel method for separating the major soybean storage proteins β-conglycinin and glycinin using phytase that was added to defatted soymilk at pH 6 followed by incubation at 40°C. Dephytinization helped to achieve an optimum separation of soluble and insoluble soybean storage proteins without the need for using a reducing agent or cooling.

Developing phytase transgenic crops represents another strategy to improve the availability of P and other minerals in soybean. Li et al (1997) have shown the secretion of active recombinant phytase from soybean cell suspension cultures that displayed biochemical properties indistinguishable from the commercially available fungal phytase. Denbow et al. (1998) have observed an improved bioavailability of phytate-P from soybeans transformed with a fungal phytase gene to broilers. Bilyeu et al. (2008) have reported that the cytoplasmic expression of an active *appA* phytase enzyme in developing soybean seeds resulted in the conversion of nearly all seed phytic acid to inorganic P and produced abundant active enzyme in mature seeds capable of releasing significant amounts of phytate-P from soybean meal.

5. Future perspective

The demonstrated and potential health benefits of soybean foods have rendered these products as functional foods. Numerous new health claims associated with these products are being evaluated worldwide. Several non-nutritional components in soybean have proven to be beneficial in the prevention and nutritional treatment of chronic diseases. Phytic acid is considered to be antioxidative due to its ability to chelate transition metals like Fe that may induce oxidative stress (Porres et al., 1999). Fiber and isoflavones represent other major beneficial components of soybean. Dietary intakes of soy isoflavones may be associated with lower incidences of atherosclerosis, type 2 diabetes, and coronary heart diseases, decreased risk of certain types of carcinogenesis, improved bone health, and relieved menopausal symptoms (Blum et al., 2003; Xiao, 2008; Messina et al., 2009). It is interesting to mention that those components are metabolized in the large intestine by specific bacterial populations that are present in a relatively low percentage of Westerners (Lambe, 2009; Messina et al., 2009). Such metabolism gives rise to products like equol that are just as effective as or even more effective than daidzein intrinsically present in soybean (Setchell et al., 2002). Novel benefits of soybean protein hydrolyzates have been recognized in the treatment of hypertension and hypertension-derived renal injury (Yang and Chen, 2008). Another new finding is the ability of soy isoflavones to up-regulate the expression of genes critical for drug transport and metabolism. Of especial interest is the stimulation of several phase I and II metabolizing enzymes that may act in the chemoprevention of cancer, or the activation of CYP family of enzymes that play an important role in bile acid metabolism (Appelt & Reicks, 1997; Li et al., 2007; Bolling & Parkin, 2008). Therefore, it will be fascinating to explore potential synergism between phytase and soybean in improving human and animal health beyond nutrition.

6. References

Achouri, A., Boye, J. I., & Zamani, Y. (2008). Soybean variety and storage effects on soy milk flavor and quality. International Journal of Food Science and Technology, Vol 43, pp. 82-90.

Adeola, O., Lawrence, B. V., Sutton, A. L., & Cline, T. R. (1995). Phytase-induced changes in mineral utilization in zinc-supplemented diets for pigs. *Journal of Animal Science*, Vol 73, pp. 3384-3391.

Al-Whash, I. A., Horner, H. T., Palmer, R. G., Reddy, M. B., & Massey, L. K. (2005). Oxalate and phytate in soy foods. *Journal of Agricultural and Food Chemistry*, Vol 53, pp. 5670-5674.

Appelt, L. L., & Reicks, M. M. (1997). Soy feeding induces phase II enzymes in rat tissue. *Nutrition and cancer*, Vol 28, pp. 270-275.

Applegate, T. J., Webel, D. M., & Lei, X. G. (2003). Efficacy of a phytase derived from *Escherichia coli* and expressed in yeast on phosphorus utilization and bone mineralization in turkey poults. *Poultry Science*, Vol 82, pp. 1726-1732.

Augspurger, N. R., Webel, D. M., Lei, X. G., & Baker, D. H. (2003). Efficacy of an *E. coli* phytase expressed in yeast for releasing phytate-bound phosphorus in young chicks and pigs. *Journal of Animal Science*, Vol 81, pp. 474-483.

Baxter, C.A., Joern, B.C., Ragland, D., Sands, J. S., & Adeola, O. (2003). Phytase, high available phosphorus corn, and storage effects on phosphorus levels in pig excreta. *Journal of Environmental Quality,* Vol 32, pp. 1481-1489.

Beard, J. L., Burton, J. W., & Theil, E. C. (1996). Purified ferritin and soybean meal can be sources of iron for treatinf iron deficiency in rats. *Journal of Nutrition*, Vol 126, pp. 154-160.

Biehl, R., & Baker, D.H. (1996). Efficacy of supplemental 1α-hydroxycholecalciferol and microbial phytase for young pigs fed phosphorus- or amino acid-deficient corn-soybean meal diets. *Journal of Animal Science*, Vol 74, pp. 2960-2966.

Biehl, R.R., Baker, D.H., & Delucca, H.F. (1995). 1-α-hydroxylated cholecalciferol compounds act additively with microbial phytase to improve phosphorus, zinc and manganese utilization in chicks fed soy-based diets. *Journal of Nutrition* Vol 125, pp. 2407-2416.

Bilyeu, K. D., Zeng, P., Coello, P., Zhang, Z. J., Krishnan, H. B., Bailey, A., Beuselinck, P. R., & Polacco, J. C. (2008). Quantitative conversion of phytate to inorganic phosphorus in soybean seeds expressing a bacterial phytase. *Plant Physiology*, Vol 146, pp. 468-477.

Blum, S. C., Heaton, S. N., Bowman, B. M., Hegsted, M., & Miller, S. C. (2003). Dietary soy protein maintains some indices of bone mineral density and bone formation in aged ovariectomized rats. *Journal of Nutrition*, Vol 133, pp. 1244-1249.

Boling, S.D., Webel, D.M., Mavromichalis, I., Parsons, C.M., & Baker, D.H. (2000). The effects of citric acid on phytate-phosphorus utilization in young chicks and pigs. *Journal of Animal Science*, Vol 78, pp. 682-689.

Bolling, B. W., & Parkin, K. L. (2008). Phenolic derivatives from soy flour ethanol extract are potent *in vitro* quinone reductase (QR) inducing agents. *Journal of Agricultural and Food Chemistry*, Vol 56, pp. 10473-10480.

Brinker, A., & Reiter, R. (2011). Fish meal replacement by plant protein substitution and guar gum addition in trout feed, Part 1: Effects on feed utilization and fish quality. *Aquaculture*, Vol 310(3-4), pp. 350-360.

Cheryan, M. (1980). Phytic Acid Interactions in Food Systems. *Critical Reviews in Food Science and Nutrition*, Vol 13, pp. 297-335.

Davidsson, L., Dimitriou, T., Walczyk, T., & Hurrell, R. F. (2001). Iron absorption from experimental infant formula based on pea (*Pisum sativum*)-protein isolate: the effect of phytic acid and ascorbic acid. *British Journal of Nutrition*, Vol 85, pp. 59-63.

Davila-Hicks, P., Theil, E. C., & Lönnerdal, B. (2004). Iron in ferritin or in salts (ferrous sulfate) is equally bioavailable in nonanemic women. *American Journal of Clinical Nutrition*, Vol 80, pp. 936–40.

Denbow, D. M., Grabau, E. A., Lacy, G. H., Kornegay, E. T., Russell, D. R., & Umbeck. P. F. (1998). Soybeans transformed with a fungal phytase gene improve phosphorus availability for broilers. *Poultry Science*, Vol 77, pp. 878-881.

Derman , D., T., Ballot, D., Bothwell, T. H., Macfarlane, B. J., Baynes, R. D., Macphail, A. P., Gillooly, M., Bothwell, J. E., & Bezwoda, W. R. (1987). Factors influencing the absorption of iron from soya-bean protein products. *British Journal of Nutrition*, Vol 57, pp. 345-353.

Eeckhout, W., & De Paepe, M. (1994). Total phosphorus, phytate-phosphorus and phytase activity in plant feedstuffs. *Animal Feed Science and Technology*, Vol 47, pp. 19-29.

Endres, J. G. (Ed). (2001). *Soy protein products. Characteristics, nutritional aspects, and utilization*, AOAC Press, ISBN 1-893997-27-8, Champaign, IL, USA.

Egli, I., Davidsson, L., Juillerat, M. A., Barclay, D., & Hurrell, R. F. (2002). The influence of soaking and germination on the phytase activity and phytic acid content of grains and seeds potentially useful for complementary feeding. *Journal of Food Science*, Vol 67, pp. 3484-3488.

Faris, R. J., Wang, H., & Wang, T. (2008). Improving digestibility of soy flour by reducing disulfide bonds with thioredoxin. *Journal of Agricultural and Food Chemistry*, Vol 56, pp. 7146-7150.

Fernandez-Figares, I., Nieto, R., Aguilera, J. F., & Prieto, C. (1996). The use of the excretion of nitrogen compounds as an indirect index of the adequacy of dietary protein in chickens. *Animal Science*, Vol 63, pp. 307-314.

Gentile, J. M., Roneker, K. R., Crowe, S. E., Pond, W. G., & Lei, X. G. (2003). Effectiveness of an experimental consensus phytase in improving dietary phytate-phosphorus utilization by weanling pigs. *Journal of Animal Science*, Vol 81(11), pp. 2751-2757.

Giami, S. Y. (2002). Chemical composition and nutritional attributes of selected newly developed lines of soybean (*Glycine max* (L) *Merr*). *Journal of the Science of Food and Agriculture*, Vol 82, pp. 1735-1739.

Grieshop, C. M., & Fahey, Jr., G. C. (2001). Comparisons of quality characteristics of soybeans from Brazil, China, and the United States. *Journal of Agricultural and Food Chemistry*, Vol 49, pp. 2669-2673.

Grieshop, C. M., Kadzere, C. T., Clapper, G. M., Flickinger, E. A., Bauer, L. L., Frazier, R. L., & Fahey, Jr., G. C. (2003). Chemical and nutritional characteristics of United States soybeans and soybean meals. *Journal of Agricultural and Food Chemistry*, Vol 51, pp. 7684-7691.

Han, Y.M., Roneker, K.R., Pond, W.G., & Lei, X.G. (1998). Adding wheat middlings, microbial phytase, and citric acid to corn-soybean meal diets for growing pigs may replace inorganic phosphorus supplementation. *Journal of Animal Science*, Vol 76, pp. 2649-2656.

Han, Y.W. (1988). Removal of phytic acid from soybean and cottonseed meals. *Journal of Agricultural and Food Chemistry*, Vol 36, pp. 1181-1183.

Hegeman, C. E., & Grabau, E. A. (2001). A novel phytase with sequence similarity to purple acid phosphatases is expressed in cotyledons of germinating soybean seedlings. *Plant Physiology*, Vol 126, pp. 1598-1608.

Hurrell, R. F. (2003). Influence of vegetable protein sources on trace element and mineral bioavailability. *Journal of Nutrition*, Vol 133(9), pp. 2973S-2977S.

Hurrell, R. F., Davidsson, L., Reddy, M., Kastenmayer, P., & Cook, J. D. (1998). A comparison of iron absorption in adults and infants consuming identical infant formulas. *British Journal of Nutrition*, Vol 79, pp. 31-36.

Hurrell, R. F., Juillerat, M. A., Reddy, M. B., Lynch, S., Dassenko, S. A., & Cook, J. D. (1992). Soy protein, phytate, and iron absorption in humans. *American Journal of Clinical Nutrition*, Vol 56(3), pp. 573-578.

Ilarsan, H., Palmer, R. G., Imsande, J., & Horner, H. T. (1997). Quantitative determination of calcium oxalate and oxalate in developing seeds of soybean (*Leguminosae*). *American Journal of Botany*, Vol 84(9), pp. 1042-1046.

Jiménez-Escrig, A., serra, M., & Rupérez, P. (2010). Non-digestible carbohydrates in

Brazilian soybean seeds [*Glycine max* (L.) *Merril*]. *International Journal of Food Science and Technology*, Vol 45, pp. 2524-2530.

Jondreville, C., Hayler, R., & Feuerstein, D. (2005). Replacement of zinc sulphate by microbial phytase for piglets given a maize-soya-bean meal diet. *Journal of Animal Science*, Vol 81, pp. 77-83.

Jongbloed, A. W., Mroz, Z., & Kemme, P. A. (1992) The effect of supplementary *Aspergillus niger* phytase in diets for pigs on concentration and apparent digestibility of dry matter, total phosphorus, and phytic acid in different sections of the alimentary tract. *Journal of Animal Science*, Vol 70, pp. 1159-1168.

Juanpere, J., Perez-Vendrell, A.M., Angulo, E., & Brufau, J. (2005). Assessment of potential interactions between phytase and glycosidase enzyme supplementation on nutrient digestibility in broilers. *Poultry Science*, Vol 84, pp. 571-580.

Kakade, M. L., Simons, N. R., Liener, I. E., & Lambert, J. W. (1972). Biochemical and nutritional assessment of different varieties of soybeans. *Journal of Agricultural and Food Chemistry*, Vol 30, pp. 87-90.

Kang, J., Badger, T. E., Ronis, M. J. J., & Wu, X. (2010). Non-isoflavone phytochemicals in soy and their health effects. *Journal of Agricultural and Food Chemistry*, Vol 58, pp. 8119-8133.

Karr-Lilienthal, L. K., Grieshop, C. M., Merchen, N. R., Mahan, D. C., & Fahey, Jr., G. C. (2004). Chemical composition and protein quality comparisons of soybeans and soybean meals from five leading soybean-producing countries. *Journal of Agricultural and Food Chemistry*, Vol 52, pp. 6193-9199.

Kerwin, S. M. (2004). Soy saponins and the anticancer effects of soybeans and soy-based foods. *Current Medical Chemistry-Anti Cancer Agents*, Vol 4, pp. 263-272.

Kornegay, E.T., & Qian, H. (1996). Replacement of inorganic phosphorus by microbial phytase for young pigs fed on a maize-soybean meal diet. *British Journal of Nutrition*, Vol 76, pp. 563-578.

Kumar, V., Rani, A., Rajpal, S., Srivastava, G., & Ramesh, A. (2005). Phytic acid in Indian soybean: genotypic variability and influence of growing location. *Journal of the Science of Food and Agriculture*, Vol 85, pp. 1523–1526.

Kumar, V., Rani, A., Solanki, S., & Hussain, H. M. (2006). Influence of growing environment on the biochemical composition and physical characteristics of soybean seed. *Journal of Food Composition and Analysis*, Vol 19, pp. 188-195.

Lambe, J. W. (2009). Is equol the key to the efficacy of soy foods? *American Journal of Clinical Nutrition*, Vol 89, pp. 1664S-1667S.

Lei, X. G., Ku, P. K., Miller, E. R., & Yokoyama, M. T. (1993a). Supplementing corn-soybean meal diets with microbial phytase linearly improves phytate phosphorus utilization by weanling pigs. *Journal of animal Science*, Vol 71, pp. 3359-3367.

Lei, X. G., Ku, P. K., Miller, E. R., Yokoyama, M. T., & Ullrey, D. E. (1993b). Supplementing corn-soybean meal diets with microbial phytase maximizes phytate phosphorus utilization by weanling pigs. *Journal of Animal Science*, Vol 71, pp. 3368-3375.

Lei, X. G., Ku, P. K., Miller, E. R., Ullrey, D. E., & Yokoyama, M. T. (1993c). Supplemental microbial phytase improves bioavailability of dietary zinc to weanling pigs. *Journal of Nutrition*, Vol 123, pp. 1117-1123.

Lei, X. G., Ku, P. K., Miller, E. R., Yokoyama, M. T., & Ullrey, D. E. (1994). Calcium level affects the efficacy of supplemental microbial phytase in corn-soybean meal diets of weanling pigs. *Journal of Animal Science*, Vol 72, pp. 139-143.

Lei, X. G., Porres, J. M., Mullaney, E. J., & Brinch-Pedersen, H. (2007). Phytase source, structure and applications, In: *Industrial enzymes. Structure, Function and Applications*, Polaina, J., MacCabe, A. P., Eds, pp. 505-529, Springer, ISBN -78-1-4020-5376-4, Dordrecht, The Netherlands.

Lei, X.G., & Porres, J.M. (2007). Phytase and inositol phosphates in animal nutrition: Dietary manipulation and phosphorus excretion by animals, In: *Inositol Phosphates. Linking Agriculture and the Environment*, Turner, B. L., Richardson, A.E., Mullaney, E. J., pp. 133-149, CAB International, ISBN 1-84593-152-1, UK.

Lei, X.G., & Stahl, C.H. (2000). Nutritional benefits of phytase and dietary determinants of its efficacy. *Journal of Applied Animal Research*, Vol 17, pp. 97-112.

Lei, X.G., & Stahl, C.H. (2001). Biotechnological development of effective phytases for mineral nutrition and environmental protection. *Applied Microbiology and Biotechnology*, Vol 57, pp. 478-481.

Li, D. F., Che, X. R., Wang, Y. Q., Hong, C., & Thacker, P. A. (1998). Effect of microbial phytase, vitamin D3, and citric acid on growth performance and phosphorus, nitrogen and calcium digestibility in growing swine. *Animal Feed Science and Technology*, Vol 73(1-2), pp. 173-186.

Li, J., Hegeman, C.E., Hanlon, R.W., Lacy, G.H., Denbow, D.M., & Grabau, E.A. (1997). Secretion of active recombinant phytase from soybean cell-suspension cultures. *Plant Physiology*, Vol 114, pp. 1103-1111.

Li, Y., Mezei, O., & Shay, N. F. (2007). Human and murine hepatic sterol-12-α-hydroxylase and other xenobiotic metabolism mRNA are upregulated by soy isoflavones. *Journal of Nutrition*, Vol 137, pp. 1705–1712

Liener, I. E. (1994). Implications of antinutritional components in soybean foods. *Critical Reviews in Food Science and Nutrition*, Vol 34(1), pp. 31-67.

Liu, J., Bollinger, D.W., Ledoux, D.R., Ellersieck, M.R., & Veum, T.L. (1997). Soaking increases the efficacy of supplemental microbial phytase in a low-phosphorus corn-soybean meal diet for growing pigs. *Journal of Animal Science*, Vol 75, pp. 1292-1298.

Liu, J., Bollinger, D.W., Ledoux, D.R., & Veum, T.L. (1998). Lowering the dietary calcium to phosphorus ratio increases phosphorus utilization in low phosphorus corn-soybean meal diets supplemented with microbial phytase for growing pigs. *Journal of Animal Science*, Vol 76, pp. 808-813.

Lynch, S. R., Dassenko, S. A., Cook, J. D., Juillerat, M. A., & Hurrell, R. F. (1994) Inhibitory effect of a soy-bean protein-related moiety on Fe absorption in humans. *American Journal of Clinical Nutrition*, Vol 60, pp. 567-572.

Lynch, S. R., Dassenko, S. A., Monck, T. A., Bear, J. L., & Cook, J. D. (1985). Soy protein products and heme iron absorption in humans. *American Journal of Clinical Nutrition*, Vol 41, pp. 13-20.

Marsman, G. J. P., Gruppen, H., Mul, A. J., & Voragen, A. G. J. (1997). *In vitro* accessibility of untreated, toasted and extruded soybean meals for proteases and carbohydrases. *Journal of Agricultural and Food Chemistry*, Vol 45, pp. 4088-4095.

Meis, S. J., Fehr, W. R., & Schnebly, S. R. (2003). Seed source effect on field emergence of soybean lines with reduced phytate and raffinose saccharides. *Crop Science*, Vol 43(4), pp. 1336-1339.

Messina, M. J. (1999). Legumes and soybeans: overview of their nutritional profiles amd health effects. *American Journal of Clinical Nutrition*, Vol 70(suppl), pp. 439S-450S.

Messina, M. J., Watanabe, S., & Setchell, K. D. R. (2009). Report on the 8th International Symposium on the role of soy in health promotion and chronic disease prevention and treatment. *Journal of Nutrition*, Vol 139, pp. 796S–802S.

Moraghan, J. T. (2004). Accumulation and within-seed distribution of iron in common bean and soybean. *Plant and Soil*, Vol 264, pp. 287–297.

National Research Council. (1998). *Nutrient Requirements of Swine, 10th Revised Edition*, National Academy Press, ISBN 0-309-05993-3, Washington, D. C., USA.

Nwokolo, E. (1996). Soybean, In: *Food and Feed from Legumes and Oilseeds*, E. Nwokolo & J. Smartt, Eds, pp. 90-102, Chapman & Hall, ISBN 0 412 45930 2, UK.

Olguin, M. C., Hisano, N., D'Ottavio, A. E., Zingale, M. I., Revelant, G. C., & Calderari, S. A. (2003). Nutritional and antinutritional aspects of an Argentinian soy flour assessed on weanling rats. *Journal of Food Composition and Analysis*, Vol 16, pp. 441-449.

Olmants, S. E., Fehr, W. R., Welke, G. A., Raboy, V., &Peterson, K. L. (2005). Agronomic and seed traits of soybean lines with low-phytate phosphorus. *Crop Science*, Vol 45(2), pp. 593-598.

Omogbenigum, F.O., Nyachoti, C.M., & Slominski, B.A. (2004). Dietary supplementation with multienzyme preparations improves nutrient utilization and growth performance in weaned pigs. *Journal of Animal Science*, Vol 82, pp. 1053-1061.

Overturf, K., Raboy, V., Cheng, Z.J., & Hardy, R.W. (2003). Mineral availability from barley *low phytic acid* grains in rainbow trout (*Oncorhynchus mykiss*) diets. *Aquaculture Nutrition*, Vol 9, pp. 239-246.

Pagano, A. R., Roneker, K. R., & Lei, X. G. (2007a). Distribution of supplemental *Escherichia coli* AppA2 phytase activity in digesta of various gastrointestinal segments of young pigs. *Journal of Animal Science*, Vol 85, pp. 1444-1452.

Pagano, A. R., Yasuda, K., Roneker, K. R., & Lei, X. G. (2007b). Supplemental *Escherichia coli* phytase and strontium enhance bone strength of young pigs fed a phosphorus-adequate diet. *Journal of Nutrition*, Vol 137(7), pp. 1795-1801.

Peter, C. M., Parr T. M., Parr, E. M., Webel, D. M., & Baker, D. H. (2001). The effects of phytase on growth performance, carcass characteristics and bone mineralization on late-finishing pigs fed maize-soybean meals containing no supplemental phosphorus, zinc, copper and manganese. *Animal Feed Science and Technology*, Vol 94, pp. 199-205.

Pham, M., Hwang, G., Kim, Y., Seo, Y., & Lee, S. (2010). Soybean meal and wheat flour, proper dietary protein sources for optimal growth of snail (*semisulcospira coreana*). *Aquaculture International*, Vol 18, pp. 883-895.

Porres, J. M., Stahl, C., Cheng, W., Fu, Y., Roneker, K., Pond, W., & Lei, X. G. (1999). Dietary intrinsic phytate protects colon from lipid peroxidation in pigs with a moderately high dietary iron intake. *Proceedings of the Society for Experimental Biology and Medicine*, Vol 221, pp. 80-86.

Porres, J. M., Etcheverry, P., Miller, D. D., & Lei, X. G. (2001). Phytase and citric acid supplementation in whole-wheat bread improves phytate-phosphorus release and iron dialyzability. *Journal of Food Science*, Vol 66(4), pp. 614-619.

Raboy, V., Dickinson, D. B., & Below, F. E. (1984). Variation in seed total phosphorus, phytic acid, zinc, calcium, magnesium, and protein among lines of *Glycine max* and *Glycine soja*. *Crop Science*, Vol 24, pp. 431-434.

Radcliffe, J.S., & Kornegay, E.T. (1998). Phosphorus equivalency value of microbial phytase in weanling pigs fed a maize-soyabean meal based diet. *Journal of Animal and Feed Sciences,* Vol 7, pp. 197-211.

Radcliffe, J.S., Zhang, Z., & Kornegay, E.T. (1998). The effects of microbial phytase, citric acid, and their interaction in a corn-soybean meal-based diet for weanling pigs. *Journal of Animal Science*, Vol 76, pp. 1880-1886.

Rani, V., Grewal, R. B., & Khetarpaul, N. (2008). Physical characteristics, proximate and mineral composition of some new varieties of soybean (*Glycine max*, L). *Legume Research*, Vol 31, pp. 31-35.

Rao, A. V., & Sung, M. K. (1995). Saponins as anticarcinogens. *Journal of Nutrition*, Vol 125, pp. 717S-724S.

Ravindram, V., & Kornegay, E. T. (1993). Acidification of weaner pig diets: A review. *Journal of the Science of Food and Agriculture*, Vol 62, pp. 313-322.

Ravindran, V., Kornegay, E.T., Denbow, D.M., Yi, Z., & Hulet, R.M. (1995). Response of turkey poults to tiered levels of Natuphos® phytase added to soybean meal-based semi-purified diets containing 3 levels of nonphytate phosphorus. *Poultry Science*, Vol 74, pp. 1843-1854.

Ravindran, V., Selle, P. H. and Bryden, W. L. (1999). Effects of phytase supplementation, individually and in combination, with glycanase, on the nutritive value of wheat and barley. *Poultry Science*, Vol 78, pp. 1588-1595.

Rimbach, G., Walter, A., Most, E., & Pallauf, J. (1997). Effect of supplementary microbial phytase to a maize-soya diet on the availability of calcium, phosphorus, magnesium and zinc: *in vitro* dialysability in comparison with apparent absorption in growing rats. *Journal of Animal Physiology and Animal Nutrition*, Vol 77, pp. 198-206.

Rodriguez, E., Porres, J. M., Han, Y., & Lei, X. G. (1999). Different sensitivity of recombinant *Aspergillus niger* phytase (r-PhyA) and *Escherichia coli* pH 2.5 acid phosphatase (r-AppA) to trypsin and pepsin *in vitro*. *Archives of Biochemistry and Biophysics*, Vol 365, pp. 262-267.

Saha, S., Gupta, A., Mahajan, V., Kundu, S., Gupta, H. S. (2008). Physicochemical and nutritional attributes in 20 black soybean lines (*Glycine max*, L.) of himalayan region, India. *Journal of Food Quality*, Vol 31(1), pp. 79-95.

Saito, T., Kohno, M., Tsumura, K., Kugimiya, W., & Kito, M. (2001). Novel method using phytase for separating soybean ß-conglycinin anf glycinin. *Bioscience Biotechnology and Biochemistry*, Vol 65, pp. 884-887.

Setchell K. D. R., Brown, N. M., & Lydeking-Olsen, E. (2002). The clinical importance of the metabolite equol-a clue to the effectiveness of soy and its isoflavones. *Journal of Nutrition*, Vol 132, pp. 3577-3584.

Spencer, J.D., Allee, G.L., & Sauber, T.E. (2000). Phosphorus bioavailability and digestibility of normal and genetically modified low-phytate corn for pigs. *Journal of Animal Science*, Vol 78, pp. 675-681.

Snow, J. L., Baker, D. H. and Parsons, C. M. (2004). Phytase, citric acid, and 1α-hydroxycholecalciferol improve phytate phosphorus utilization in chicks fed a corn-soybean meal diet. *Poultry Science*, Vol 83, pp. 1187-1192.

Stahl, C. H., Roneker, K. R., Thornton, J. R., & Lei, X. G. (2000). A new phytase expressed in yeast effectively improves the bioavailability of phytate phosphorus to weanling pigs. *Journal of Animal Science*, Vol 78, pp. 668-674.

Stahl, C.H., Han, Y.M., Roneker, K.R., House, W.A., & Lei, X.G. (1999). Phytase improves iron bioavailability for hemoglobin synthesis in young pigs. *Journal of Animal Science*, Vol 77, pp. 2135-2142.

Stahl, C.H., Roneker, K.R., Pond, W.G., & Lei, X.G. (2004). Effects of combining three fungal phytases with a bacterial phytase in plasma phosphorus status of weanling pigs fed a corn-soy diet. *Journal of Animal Science*, Vol 82, pp. 1725-1731.

Stahl, C.H., Roneker, K.R., Thornton, J.R., & Lei, X.G. (2000). A new phytase expressed in yeast effectively improves the bioavailability of phytate phosphorus to weanling pigs. *Journal of Animal Science*, Vol 78, pp. 668-674.

Stahl, C. H., Wilson, D. B., & Lei, X. G. (2003). Comparison of extracellular *Escherichia coli AppA* phyases expressed in *Streptomyces lividans* and *Pichia pastoris*. *Biotechnology Letters*, Vol 25(10), pp. 827-831.

Suarez, F. L., Springfield, J., Furne, J. K., Lohrmann, T. L., Kerr, P. S., & Devitt, M. D. (1999). Gas production in humans ingesting a soybean flour derived from beans naturally low in oligosaccharides. *American Journal of Clinical Nutrition*, Vol 69, pp. 135-139.

Sugiura, S. H., Raboy, V., Young, K. A., Dong, F. M., & Hardy, R. W. (1999). Availability of phosphorus and trace elements in low-phytate varieties of barley and corn for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, Vol 170(3), pp. 285-296.

Tamin, N. M., & Angel, R. (2003). Phytate phosphorus hydrolysis as influenced by dietary calcium and micro-mineral source in broiler diets. *Journal of Agricultural and Food Chemistry*, Vol 51, pp. 4687-4693.

Trimble, L. A., & Fehr, W. R. (2010). Genetic improvement of seedling emergence of low-phytate soybean lines. *Crop Science*, Vol 50(1), pp. 67-72.

Wu, Y. B., Ravindram, V., Thomas, D. G., Birtles, M. J. and Hendriks, W. H. (2004) Influence of phytase and xylanase, individually or in combination, on performance, apparent metabolizable energy, digestive tract measurements and gut morphology in broilers fed wheat-based diets containing adequate levels of phosphorus. *British Poultry Science*, Vol 45, pp. 76-84.

Xiao, C. W. (2008). Health effects of soy protein and isoflavones in humans. *Journal of Nutrition*, Vol 138, pp. 1244S-1249S.

Yan, W. B., Reigh, R. C., & Xu, Z. M. (2002). Effect of fungal phytase on utilization of dietary protein and minerals , and dephosphorylation of phytic acid in the alimentary tract of channel catfish *Ictalurus punctatus* fed an all-plant-protein diet. *Journal of the World Aquaculture Society*, Vol 33, pp. 10-22.

Yang, H., & Chen, J. (2008). Renoprotective effects of soy protein hydrolysates in Nʷ-Nitro-L- Arginine methyl ester hydrochloride-induced hypertensive rats. *Hypertension Research*, Vol 31, pp. 1477-1483.

Yi, Z., Kornegay, E. T., Ravindran, V., & Denbow, D. M. (1996). Improving phytate phosphorus availability in corn and soybean meal for broilers using microbial phytase and calculation of phosphorus equivalency values for phytase. *Poultry Science*, Vol 75, pp. 240-249.

Yi, Z., & Kornegay, E.T. (1996). Sites of phytase activity in the gastrointestinal tract of young pigs. *Animal Feed Science and Technology*, Vol 61, pp. 361-368.

Yi, Z., Kornegay, E.T., Ravindram, V., Lindemann, M.D., & Wilson, J.H. (1996). Effectiveness of Natuphos® phytase in improving the bioavailabilities of phosphorus and other nutrients in soybean meal-based semipurified diets for young pigs. *Journal of Animal Science*, Vol 74, pp. 1601-1611.

Yu, B., Jan, Y. C., Chung, T. K., Lee, T. T., & Chiou, P. W. S. (2004). Exogenous phytase activity in the gastrointestinal tract of broiler chickens. *Animal Feed Science and Technology*, Vol 117, pp. 295-303.

Yuan, F., Zhu, D., Deng, B., Fu, X., Dong, D. K., Zhu, S., Li, B., & Shu, Q. Y. (2009). Effects of two low phytic acid mutations on seed quality and nutritional traits in soybean (*Glycine max* L. *Merr*). *Journal of Agricultural and Food Chemistry*, Vol 57, pp. 3632–3638.

Zhang, W., & Popovich, D. G. (2010). Group B Oleanane Triterpenoid extract containing soyasaponins I and III from soy flour induces apoptosis in Hep-G2 cells. *Journal of Agricultural and Food Chemistry*, Vol 58, pp. 5315–5319.

5

Utilization of Soybean as Food Stuffs in Korea

Shin, Dong Hwa
Shin Dong Hwa Food Research Institute
Republic of Korea

1. Introduction

1.1 Origin of soybean

The origin of soybean is considered as Manchuria and Korean Peninsula and these areas are also the living base of ancient Dongyi tribes. In general, in the estimation of the origin of crops, the presence of its wild species is the most important index in which lots of wild soybeans are found at the present time in these areas. In archaeology, the cultivation year of soybeans is estimated to be about 4,000 years ago. In Korean Peninsula, carbonized soybeans were found at some historic sites in the Bronze Age and that provides evident for such cultivation (Kwon et al., 2005 [a]). Table 1 shows the historic sites of soybean excavated in Korean Peninsula according to their era.

Remains	Kind	State	Period (Earthenware)
Ohdong, Heryong County, Hambuk	Each grain of soybean, red bean as carbonization	Bottom of habitat site, bronze age	Bronze-Iron age (minmun pottery)
Honam, Samseok section, Pyongyang,	Carbonization grain of foxtail millet, proso millet, sorghum bicolor, soybean	Habitat site of No. 36	Bronze age (top type pottery)
Submerged area, Gyeonggi Paldang	Soybean, red beans		Bronze age
Buwon-dong, Gimhae county, Gyeongnam	Rice, wheat, and husk of soybeans (3 each)	A district, 11th floor	

Table 1. Unearthed remains of soybean in Korean peninsula

It has been known that the cultivation of soybean was originated from the era of the middle period in the agricultural age of the New Stone Age to the Bronze Age (around BC 1,500). Some pieces of earthenware studded with soybean in the Bronze Age were found at the Paldang submerged area in the suburb of Seoul.

Lee Sung-Woo (1988) proposed that the first people that cultivated soybean as a food in the history of mankind is around BC 4000~2000. Also, it is assumed that the first cultivation of soybeans was started in arable fields at the hub of Mountain Baekdu.

It is considered that soybean have been contributed to the table of Dongyi tribes as an important protein source in such primitive ages that show insufficient nutrients. Thus, it can be seen that soybean play an important role in forming the early countries in Northeast Asia due to the increase in military powers on the basis of improving their nutrients (Kwon et al., 2005a).

1.2 Strain of soybeans and its production

The strains of soybean cultivated in Korea are classified according to purpose that includes soybean for soybean sauces and pastes, soybean sprouts, boiled rice, early-ripening beans, unripe beans, and premium strain.

The strains of soybean for soybean sauces and pastes are a total of 43 varieties and distributed from Jangeup in 1978 to Joongmo 3003 in 2009 (National Institute of Crop Science, Rural Development Administration, Korea). As new strains of soybean have been distributed every year as a similar way to that of red peppers it is necessary to continuously conduct studies on the proper strains of soybean for soybean sauces and pastes. The production of soybean is about 130~180 thousand tons per year even though it is varied according to its harvest (Table 2).

Year	Area(ha)	Production(ton)
2004	85,270	138,570
2006	105,421	183,338
2008	75,242	132,674
2009	70,264	139,251

Table 2. Soybean production and cultivation area by year

The essential ingredients of soybean are protein (30~50%) and fat (14~24%) where the protein covers the largest portion of such ingredients. About 63~90% of the soybean protein are glycinin included in globulin, and the other components are phaseolin (17%) and legumelin. These are a type of water insoluble protein that is solved in a salt solution and represents no specific taste (Snyder et al., 1987a; Liu, 1997a). Although a sulfur containing amino acid is a limiting factor in an amino acid that is an ingredient of protein, it has abundant lysine, which is insufficient in cereal protein, and helps to intake balanced nutrients as it is taken with some cereals due to their interaction.

Although the protein of soybean has no specific component in presenting its own taste, it represents several savory tastes as it is dissolved into the types of amino acid and peptide and that may be used as various seasoning sources. A fermentation process that multiplies molds or bacteria after cooking soybean dissolves soybean protein using protease in order to obtain amino acids and peptides for resenting such savory tastes.

Regarding the uses of soybean in Korea, these uses are divided into two large different ways such as a direct use and a dissolving process of the protein of soybean through a fermentation process. In the dietary life in Southeast Asian countries, the soybean fermentation technology that obtains seasonings are already developed very well.

As mentioned above, the fermentation technology using soybean has been used since a great while ago and that represents a rich variety of products in Southeast Asian countries. The fermented soybean products in Korea are largely classified as soybean sauces and pastes such as Ganjang(soy sauce), Doenjang(fermented soybean paste), Gochujang(fermented red pepper soybean paste), Dambukjang(admixture of soybean paste with other seasonings), and Cheonggukjang(fermented soybean paste by *Bacillus*). Thus, such fermented soybean

products are fairly-well known as a common name in the foods of soybean sauces and pastes. Fig. 1 shows the classification of the uses of soybean in Korea.
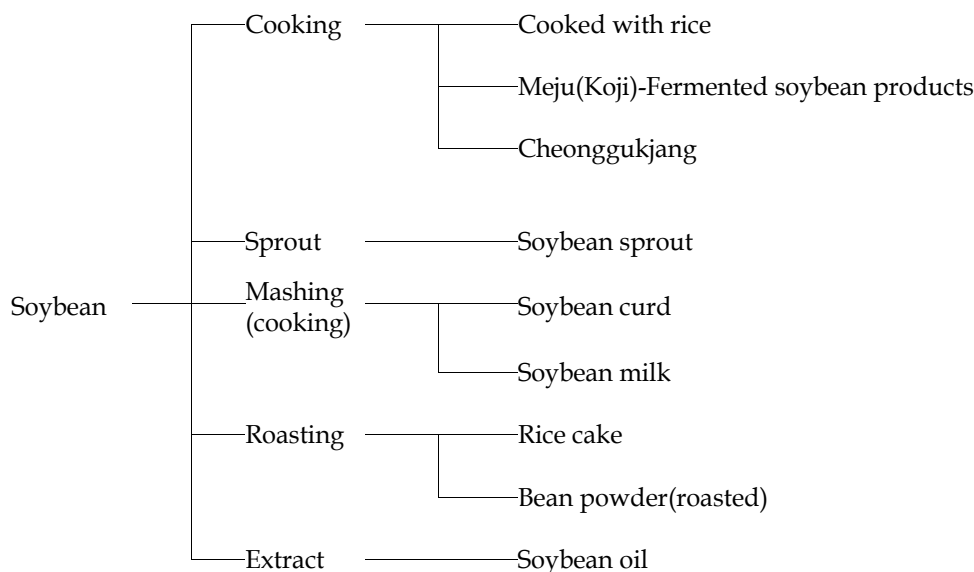
Fig. 1. Classification of soybean products in Korea

2. Non fermented soybean products

2.1 Soybean sprout

Soybean sprout are a type of vegetable that cultivates soybean in a dark place after soaking in water and sprouting soybean as a unique manner. In general, soybean sprouts represent soft tissue and yellowish white color because they are grown in a dark place. The history of soybean sprouts for food use goes back to before the age of the Three States (at ancient Korean history). Although the record presented in the Hyangyakgugeupbang, which was published in King Gojong, Goryeo Dynasty, it is estimated that the period goes back to long before the Century (Kwon et al., 2005[b]; Jang, 1993 [a]).

As soybean sprout (Photo. 1.) represent a characteristic of vegetable, it can be used in various Korean cuisines such as soybean sprout soup, boiled rice with soybean sprout, boiled rice with assorted mixtures (bibimbap), and soybean sprout salad including their flavoring roles in various stews.

As mentioned above, as soybean sprout in various cuisines show excellent nutritional characteristics, it represents several advantages as low caloric, low saturated fatty acid, non-cholesterol, and high dietary fiber foods (Hwang, 1995).

Regarding the characteristics of soybean sprout, it can be cultivated without sunlight and shows a short period of growth within seven days at 20~22℃. Also, soybean sprout can be cultivated regardless of locations and season if possible growth conditions are configured. In particular, soybean sprout have been used as one of the most familiar vegetables for Koreans due to its low price and nutritional aspects, which include abundant protein, fat, vitamins, active minerals, and so on (Park, 1991; Kim et al., 1993). Table 3 shows the comparison of the nutrition facts in soybean sprout.

Photo. 1. Growing of soybean sprout

	Ingredients	Soybean	Soybean sprout
Moisture(g)		9.7	89.3
Protein(g)		36.2(40.1)	5.3(49.5)
Fat(g)		17.8(19.7)	1.2(11.2)
Carbohydrate(g)	Sugar	25.7(28.5)	2.8(26.2)
	Fiber	5.0(5.5)	0.6(5.6)
Ash(g)		5.6(6.2)	0.8(7.5)
Calcium(mg)		245(271)	26(243)
Phosporus(mg)		620(687)	81(757)
Iron(mg)		6.5(7.2)	0.8(7.5)
Vitamin	A(I.U)	0	0
	β-carotein(μg)	0	
	Thiamine(mg)	0.53(0.6)	0.14(1.3)
	Riboflavin(mg)	0.28(0.3)	0.11)1.0)
	Niacin(mg)	2.2(2.4)	0.6(5.6)
	C(mg)	0	13(121)

* weight / 100g ( ) : weight/100g dry base

Table 3. Nutritional value of soybean and soybean sprout (Kim, et al, 1993)

The Vitamin C contents in the special nutrition facts of soybean and soybean sprout are presented in Fig. 2 (Kim et al., 1993).

As shown in Fig. 2, the vitamin C contents largely increase during the growth period of soybean sprout in which it is not detected in the early stage of the growth but shows the maximum level at the seventh day as 18㎎/100g. In the results, it shows the vitamin C can be newly produced through its entire growth period.

In addition, changes in the generation of asparagines, which is one of the particular ingredients in soybean sprout, are presented in Fig. 3 (Yang et al., 1977).
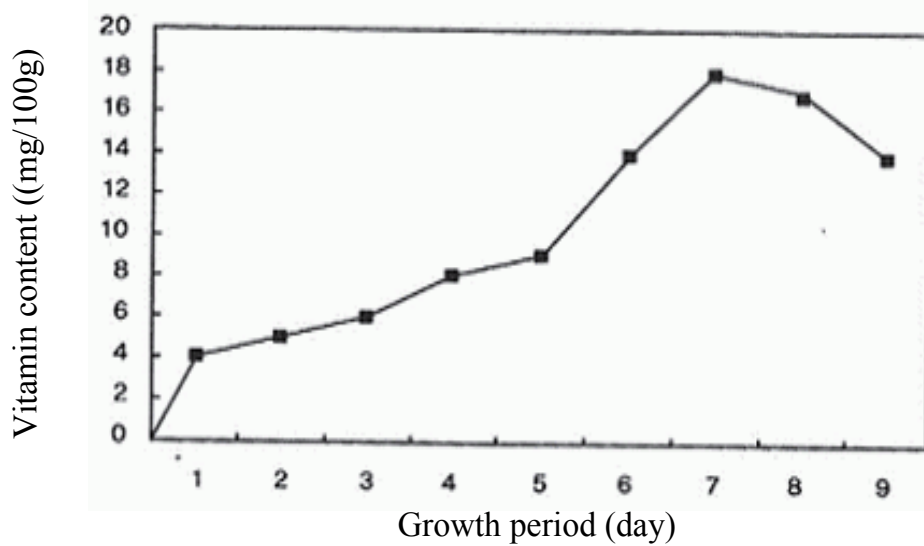
Fig. 2. Vitamin C content during sprouting of soybean (Kim, et al, 1963)
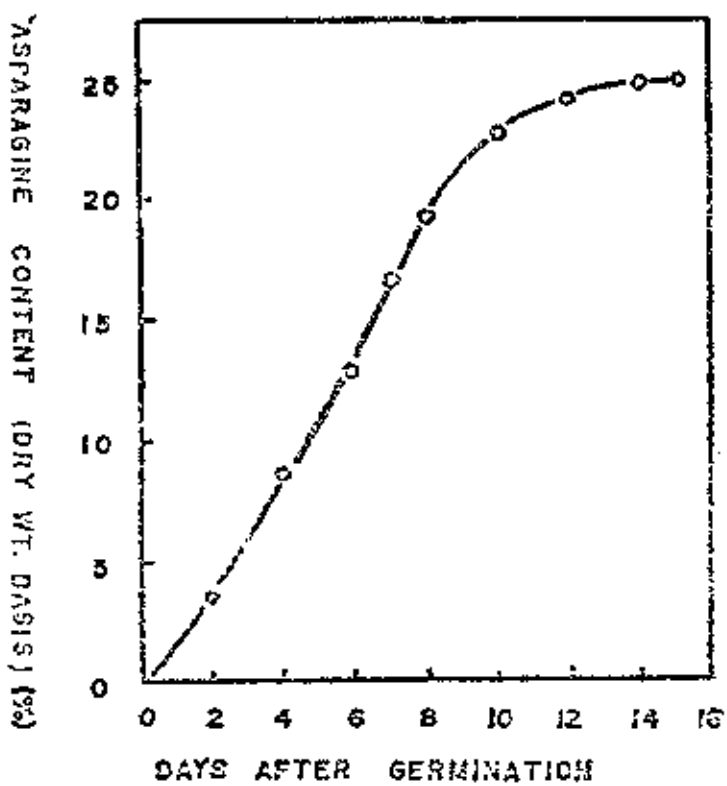
Fig. 3. Asparagines formation during soybean sprouting

As illustrated in Fig. 3, the asparagines contents in the growth period of soybean sprout significantly increase after six days of starting the growth and show up to 22% of the amount of dried one after 10 days of starting the growth.

In addition, the isoflavone contents largely increase during the sprouting period of soybean sprout (Kim et al., 2004). Based on these results, it shows that the special functional ingredients in soybean are newly created during its growth period.

Soybean sprout in Korea have been used as sub-materials in various cuisines, such as boiled rice with assorted mixtures, boiled rice ball with soybean sprouts, stews, and soups, as exquisite foods (Lee, 2005).

2.2 Soybean curd (Tofu)

Soybean curds show a long history in its dietary uses in Korea, China, and Japan and have currently been sold in western markets. In particular, soybean curds have been playing roles in supplying excellent protein sources to the consumers who are lack of animal proteins in these countries.

Literatures on soybean curds were recorded about 2,100 years ago at the era of Hoenam King, Han dynasty, China, and showed the propagation of soybean curds to Korean Peninsula before the end of the era of Goryeo Dynasty (Kim, 2005).

Then, the production technology of soybean curds was propagated to other neighbor countries since the era.

The reason that the soybean curds have been used in recent times even though it has such a long history is considered as its unique taste and particular texture (Jang, 1993 [b])

The chemical composition of soybean curds are about 80~85% of water, 8.5% of protein, 5.5% of fat, and 1.5% of ash in which protein covers a high portion of about 42~52% in its solid part. The nutrients included in soybean curds are essential amino and fatty acids required by the human body. Also, soybean curds include some active minerals such calcium, iron, and so on. Fig. 4 shows the process of producing soybean curds (Jang et al., 2008).

water   water   antiformer   coagulant
  ↓   ↓   ↓   ↓

soybean → screening → steeping → crushing → Filter → boiling → soy milk → coagulation →

press, mouliding → cutting → soaking → soybean curd

  ↓

waste water
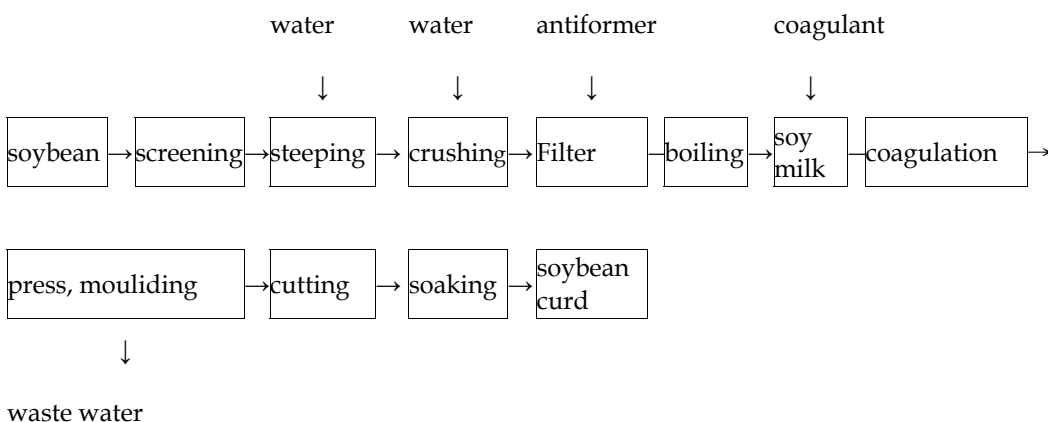
Fig. 4. Manufacturing process of common soybean curd

The major soybean curd products produced in Korea are regular soybean curds, coagulated bean curds, and soft soybean curds. The most largely produced regular soybean curds represent soft texture and rectangular shapes, and have the characteristics of simple taste and flexible resilience. The soft soybean curds represent satiny surfaces and very soft textures. The coagulated bean curds partially include some solid parts in a liquid phase.

Although such soybean curds are directly taken, soybean curds are usually processed as stews, soups, soybean curds pancakes, and soybean curds dumplings. The daily intake of soybean curds in Korea has been recorded as 24.5g per person (National Health and Nutrition Examination Survey, 2005). Thus, a lot of people in Korean take soybean curds every day as a large sum.

2.3 Creamy soybeans (Soy milk)

Soybean curds are produced using coagulants after grinding and filtering soybeans, and the phase before applying the gelling agent is called creamy soybeans or soybean juice (Kim, 2005). As the creamy soybeans were mentioned in the Hyangyakgugeupbang, which was published in King Gojong (1236), Goryeo Dynasty, it is estimated that the creamy soybeans had already produced by average people in the era of the Unified Shilla(an old country in Korean peninsula) .

Although creamy soybeans are produced by grinding steamed soybeans or by grinding just after applying water immersion, it can be regarded that the grinding and filtering of soybeans are used in the early stage of producing such creamy soybeans (Jang, 1993a). The creamy soybeans include several functional substances and then considered as good nutritious foods for preventing chronic diseases (Lee et al., 1997). Creamy soybeans are directly taken as a type of beverage and have been largely known as Korean exquisite foods that are usually prepared with other foods including noodles, such as creamy soybean noodles. Fig. 5 illustrates a simple process for producing such creamy soybeans (Jang et al., 2008).
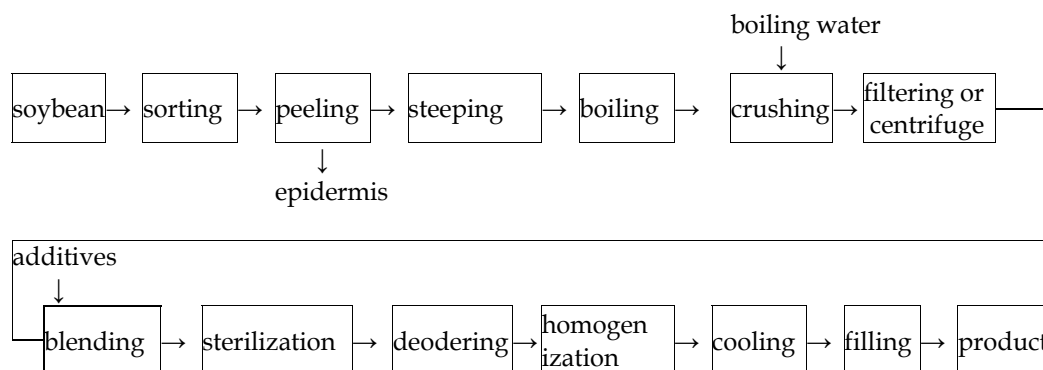
Fig. 5. Processing procedure of soybean milk

2.4 Other soybean products

There are many soybean used as foods in Korea. Some foods use soybeans directly such as soybean Gangjeong(a kind fried glutinous rice or soybeans), Jorim(boiled soybean with seasonings) and roasted soybean powder for making rice cake. Also, soybeans have been used as several purposes for increasing tastes by grinding and applying it to other rice

products such as soybean rice cakes. In addition, there are lots of homemade soybean used foods (Lee, 2005).

Furthermore, soybeans have been used to compensate the nutrition of rice by mixing it with other different foods including boiled rice and boiled rice with different grains.

Soybean Gangjeong mixed soybean that has been taken as one of the most popular soybean foods is produced by mixing some puffed grains or soybeans with a concentrate malt syrup. Then, the mixtures of soybeans, sesames, walnuts, pine nuts, peanuts, and so on are cut by proper sizes. The concentrate malt syrup mixed with soybean is taken as a snack because they show nutty, sweet, and other particular tastes.

Jorim(boiled soybean with seasonings) are made using black soybeans and boiled in soybean sauces with some starch syrup after immersing soybeans into water. The properly Jorim are taken as a side dish of boiled rice. Soybeans applied to rice cakes contribute to softening its texture and to improving taste.

Roasted soybean flours are used to produce rice cakes, such as glutinous rice cakes, using the dough of glutinous rice by mashing it using a wooden hammer. Then, the rice cakes are coated with roasted soybean flours and cut by proper sizes. Rice cakes have been contributed to all types of ceremonies including some happy events and sacred rites and then the cakes are distributed to families and relatives. In addition, rice cakes have been recently taken as good snacks.

Roasted soybean flours are produced using a fine mesh in order to obtain fine flours after roasting it with high temperature and grinding it.

Also, oil extraction using soybeans is performed applying a modern method. The soybean oil products have been produced and distributed using imported soybeans, which are about one million ton annually. The remains of soybeans after extraction it are used as feeds or soybean sauces and pastes.

3. Fermented soybean products

Korean traditional foods use agricultural products from a longstanding heritage of agrarian society, and such agricultural products provided by nature have been largely used in practical life due to distinctive four seasons. In particular, the dietary life was determined based on the principle food of rice and then cultures with boiled rice were already settled in B.C. Also, some side dishes were also introduced as the major elements in the dietary life. In addition, some subsidiary food materials that provide specific flavors to boiled rice, which has no particular taste, were required and then subsidiary foods were also developed. However, vegetables were not properly used as such side dishes for boiled rice because these vegetables showed no flavors. Thus, certain brining methods for these vegetables were introduced and developed. By introducing brining methods in Korea, it was an occasion that the dietary life in Korea was changed to fermentation based diets.

Fermented foods in Korea have been formed as the principle foods in Korean dietary life where there are no foods that do not use fermentation methods in diets directly or indirectly. Also, fermented products play important roles in determining the tastes of foods. In particular, such brining and fermentation methods have been used as a way for spending a winter season, which has no vegetables, and are the chances to form the characteristics of dietary cultures in Korean people. In addition, for presenting tastes, savory, sour, and sweet tastes are created based on the fermentation by salts and that contributed to represent the originality of Korean foods (Shin, 2008).

Therefore, the food cultures in Korea cannot be considered by excluding the fermented foods, and Korean traditional foods should be discussed on the basis of the characteristics of fermented foods. That is, the dietary culture of fermentation becomes the basic fabric of Korean foods and takes a large part of life.

Table 4 shows the products that can be produced using fermentation methods.

Variety	Sub division	Typical products
Fermented foods	Fermented food	Agro-products(Fermented soybean products, Kimchi, Pickles), Marine products(Jeotgal), Live stock and dairy products
	Alcoholic liquor	Grain liquor, fruit liquor, Beer
Fermented products	Primary products	Amino acids(MSG, lysine), nucleic acids(IMP< GMP), organic acids(citrate, succinate, lactate, acetate, gluconate)
Fermented products	Secondary products	Antibiotics, pigments, toxin, alkaloids
	Health materials	Probiotics, vitamin, oligosaccharides, polysaccharides
	Derivatives	Amino acids(>3000), nucleic acids, organic acids(PLA, PSA)
Enzymes	Physiological	edible, medicine, industrial, restriction endonucleace

Table 4. Various products from fermentation technology

As shown in Table 4, lots of products can be produced using such fermentation methods. This section describes fermented products produced by using soybean in Korea.

3.1 Traditional Ganjang (soybean sauces) and Doenjang (fermented soybean pastes)

Soybean sauces are classified into traditional soybean sauces and improved soybean sauces. These two soybean sauces show very different production processes. For producing traditional soybean sauces, it is necessary to produce Meju (fermented soybean lump). Traditional Mejus are made by housewives in average households and handed down to the present time.

3.1.1 Production process of traditional Meju

1. Preparation: Soybeans are boiled with water for 3~4 hours after washing it. The soybeans should be fully cooked and that becomes an important factor for deciding the quality of Mejus.
2. Meju shaping and molding: The cooked soybeans are shaped as a proper size (usually 15x15x20cm) by hand after mashing it. The shaped soybeans lump, Mejus, are to be fermented for 4~5 days at room temperature after drying its surface. The fermentation is to be carried out at 25~30℃ for 1~2 months.
3. Maturation: In the fermentation process, drying of Mejus is continued and then bacteria and molds are naturally generated. Then, fermentation and maturation are proceeded at the same time. During the drying of Mejus, there are some apertures on Mejus. The fermentation and maturation are continued through winter.
4. Storage: Finished Mejus are dried in sunlight and stored. Finished Mejus are used as the materials of soybean sauces and pastes.

3.1.2 Production of Ganjang (soybean sauce)

1. After preparing Mejus followed by the mentioned process, Mejus are to be washed using water and dried again. Then, dried Mejus are soaked in brine (18-19Be'). The ratio of Mejus:salt: water is determined as 1:1:3. For maintaining a uniform quality, the concentration of brine and the ratio of Mejus to brine are to be specifically determined. Also, Mejus should be immersed under the brine because it floats on the surface of brine.

2. After soaking Mejus in brine, some red peppers and charcoals are applied to the brine in order to prevent bad smells and germs.

3. Mejus soaked in brine are to be matured for about 60 days at a sunny place and occasionally open the cover of the jar for scorching sun light.

4. After soaking Mejus in brine for 70~80 days, Mejus are to be separated from brine using a filtering patch or mesh. The separated solution is used as raw Ganjang, and the separated solid matter is used to produce Doenjang.

3.1.3 Production of Doenjang (fermented soybean paste)

1. Separated Meju masses are to be mashed and mixed with some soybean sauces, Meju flours, and steamed rice or barley according to needs and preferences.

2. Well mixed paste type raw soybean pastes are stored in a jar or pottery and pushed. Then, the post maturation of the stored Doenjang are processed for 3~6 months before using it on the table. Fig. 6 shows the production process of Doenjang.

Fig. 6. Process of traditional Doenjang(fermented soybean paste) & Ganjang(soybean sauce)

3.1.4 Improved Ganjang and Doenjang

Korean Ganjang and Doenjang are classified into traditional and improved products. In producing improved products, bacteria and fermentation conditions are fully controlled. Fermentation methods are simply presented in Fig. 7 and Fig. 8.

```
┌──────────┐    ┌──────────┐    ┌──────────┐
│ defatted │ →  │ Watering │ →  │ steaming │────┐
│ soybean  │    └──────────┘    └──────────┘    │
└──────────┘                                    │
               16h          130℃ 2min  →  ┌─────────┐ → ┌──────────┐ → ┌──────────────┐
┌──────────┐    ┌──────────┐              │ mixing  │   │  making  │   │ adding brine │
│  wheat   │ →  │ roasting │──────────────┘         │   │   koji   │   └──────────────┘
└──────────┘    └──────────┘              └─────────┘   └──────────┘
                                                          35℃ 48h
┌──────────────┐ → ┌──────────┐ → ┌──────────────┐ → ┌─────────┐ → ┌──────────┐ → ┌──────────┐
│ fermentation │   │ pressing │   │  blending &  │   │ cooling │   │ filtering│   │ products │
└──────────────┘   └──────────┘   │ sterilization│   └─────────┘   │    &     │   └──────────┘
   180days                        └──────────────┘                 │ packing  │
                                                                    └──────────┘
```
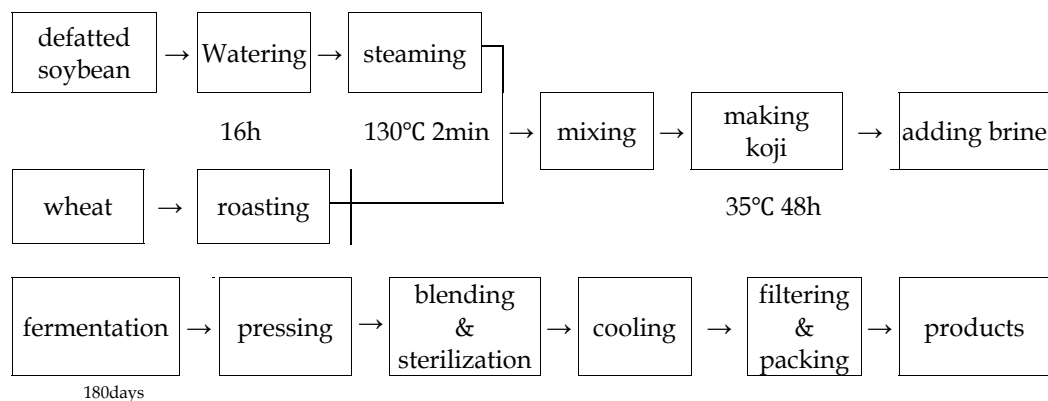
Fig. 7. Common process of Ganjang making

```
┌──────────┐ → ┌──────────┐ → ┌──────────┐ → ┌─────────┐ → ┌──────────┐
│ soybean  │   │ steeping │   │ steamiing│   │ cooling │   │  making  │────┐
└──────────┘   └──────────┘   └──────────┘   └─────────┘   │   koji   │    │
                                                            └──────────┘    │  ┌──────────┐
                  16h          125℃          35℃ 48h                        ├──│ blending │
                               20min                                        │  └──────────┘
┌──────────────┐ → ┌──────────┐ → ┌─────────┐ → ┌──────────┐                │
│  wheet flour │   │ steaming │   │ cooling │   │   koji   │────────────────┘
└──────────────┘   └──────────┘   └─────────┘   │  making  │
                                                 └──────────┘
                   110℃ 3min                      35℃ 48h
→ ┌──────────────┐ → ┌──────────────────┐ → ┌──────────┐
  │ fermentation │   │ adding alcohol & │   │ product  │
  └──────────────┘   │     packing      │   └──────────┘
   30-60 days        └──────────────────┘
```
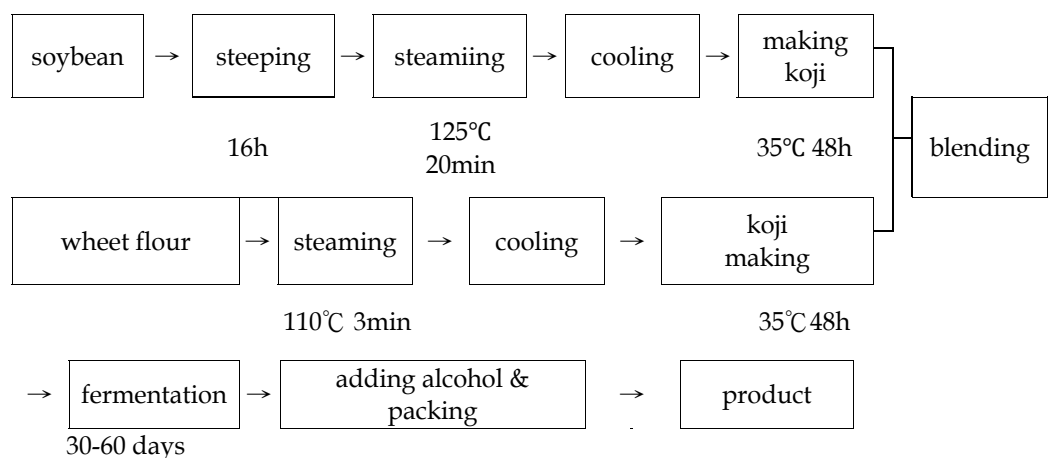
Fig. 8. Common process of Doenjang making

3.2 Gochujang (Fermented hot pepper soybean paste)

For producing fermented hot pepper pastes (Gochujang), the mixture of soybeans and grains (rice and others) are cooked and mashed. Then, the mashed mixture is shaped and fermented by molds and bacteria introduced to make Gochujang Mejus. Then, the mixture of Gochujang Mejus, hot pepper flours, and a digested rice syrup by malt, which is produced using some barley malts, is to be fermented for a long time (Shin et al., 2001; Oh et al., 2001).

Gochujang products are divided into two categories such as traditional Gochujangs produced by using traditional fermentation methods and improved Gochujangs produced by improved methods for mass production. Traditional Gochujangs are usually produced by natural fermentation, and improved Gochujangs represent different ways in managing microbesa and fermentation conditions.

Recently, Gochujang products are made by housewives in average households and produced by middle and large factories based on traditional methods. Improved Gochujangs are produced in a mass production system and cover almost 80~90% of Gochujang markets in Korea. Traditional Gochujangs are usually sold to middle aged consumers, and improved Gochujangs are largely consumed because its major markets are young people and food service businesses.

3.2.1 Production of traditional Gochujangs
1.   Production of Gochujang Meju

The mixture with the mixing ratio of soybeans to rice as 6:4 is to be cooked and mashed. Then, the mashed mixture is formed as a donut shape and to be matured and fermented at room temperature for 2~3 months. The fermented Gochujang Mejus are to be dried and powdered for storing it. It has been known that Gochujang Mejus are processed and fermented by *Aspergillus* species and *Bacillus subtilis* (Oh et al., 2001)

3.2.2 Production process of Gochujang
1.   Traditional Gochujang

Fig. 9 shows a general process for producing traditional Gochujangs.
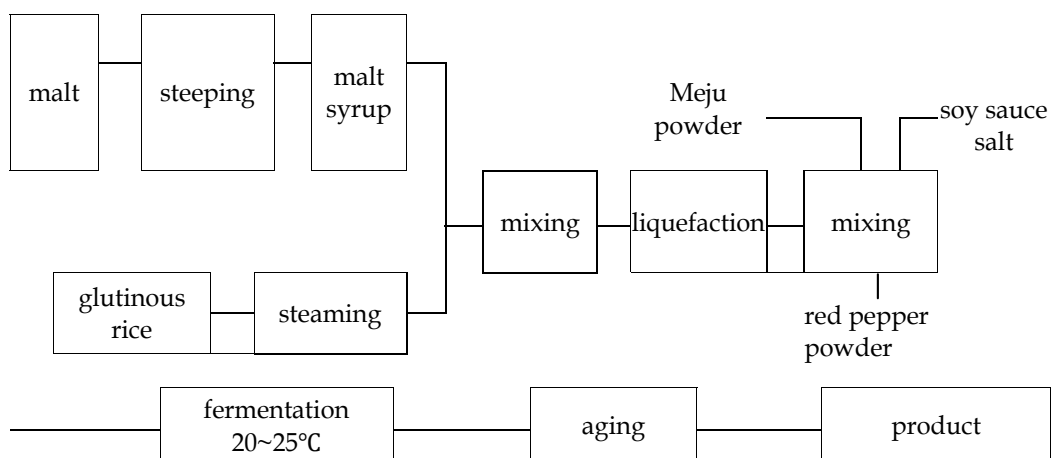
Fig. 9. Process of traditional Gochujang making

2.   Improved Gochujang

A basis for Gochujang is to be prepared and mixed with some flours, rice, and starch sources. Then, the mixture is to be fermented.

Pure microorganisms are used to produce the basis of Gochujang, and *Aspergillus oryzae* and *Bacillus subtilis* are used to improve its taste.

Fig. 10 simply shows the production process of improved Gochujangs.

3.   Quality characteristics of Gochujang

The taste of Gochujang represents a spicy taste caused by hot peppers, a sweet taste presented by sugars from starch hydrolysis, and a savory taste due to amino acids, peptides and nucleic acid related substances generated by the dissolution of soybeans. Then, these tastes become a fermented spice mixed with a salty taste generated by salts.

Several molds, bacteria, and yeasts are contributed to the fermentation of traditional Gochujangs, and taste facts are generated by protease and amylase (Kim et al., 1998; Shin et al., 1996).
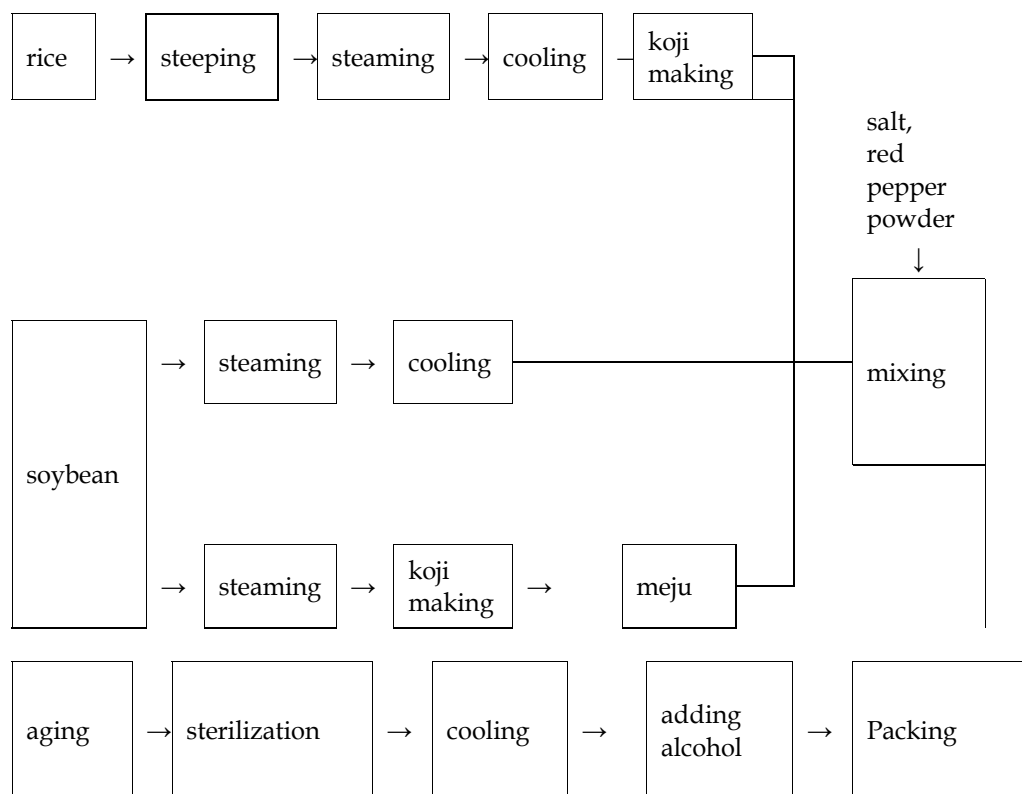
Fig. 10. Production process of improved Gochujang

Therefore, the quality characteristics of Gochujang represent a complex taste with various tastes and are very different according to microorganisms related, fermentation conditions, and materials used.

In addition, various odors of Gochujang including 3-methyl butanol are generated during its fermentation process and that plays an important factor for presenting flavors according to products (Oh et al., 2001).

3.3 Cheonggukjang (Fermented soybean paste by *Bacillus*)

Cheonggukjang is a traditional soybean fermented food that is fully fermented within 2~3 days. Cheonggukjang represent a particular quality characteristic caused by some microorganisms that shows a palatable taste and a unique smell due to the protein generated by protease, which is produced from the proliferation of *Bacillus sp.* In addition, it produces a sticky viscous material. Although there is a similar product in Japan, Natto, to the Cheonggukjang, it shows different fermentation bacteria and dietary usages. Korean Cheonggukjang are usually used in stews and mixed with other foods instead of taking it directly. However, Natto are directly taken.

Various physiological activities in Cheonggukjang have been largely known, and Cheonggukjang have been produced using traditional methods in average households. Also, it has been taken as a side dish in boiled rice. Also, it can easily be produced in households due to its easy production.

3.3.1 Production process of Cheonggukjang

Although fermentation conditions in Cheonggukjang are almost same for traditional and improved products, the differences in these products are whether they use natural fermentation or manage bacteria using a specific way.

The production process of Cheonggukjang is simply presented as follows.

1. Soaking soybean in water

Soybeans are soaked in water for 24~30 hours in winter (0~5℃), 16~24 hours in spring and fall (10~16℃), and 10~16 hours in summer (18~25℃).

2. Cooking soybeans

Soybeans are to be cooked using a pot in households for about 5~6 hours. Also, the steaming can be performed using high pressure steam (1.5~2kg) for about 30 minutes. If soybeans are not fully cooked, the quality of Cheonggukjang is not guaranteed. Also, over cooking shows bad colors and physical properties.

3. Fermentation

Cooked soybeans are to be fermented in a jar. Traditionally some rice straws are inserted to soybeans or used at the bottom and upper sections of the jar for inoculating bacteria. It has been known that rice straws have bacteria, which have high protease activities (Lee et al., 1971).

The use of rice straws represents a certain way of natural inoculation in which *Bacillus sp.*, which has been known as *B. subtilis*, contributes to the process (Lee et al., 2008; Ju et al., 2009). Also, the fermentation takes 30~35 hours at 40~43℃. The fermentation time are largely varied according to temperature and applied bacteria. Also, a humidity condition is important to the fermentation. Tastes and odors are different according to its fermentation period. Recently a two-stage fermentation method that uses two fermentation stages at 42~43℃ and 50~53℃ is used to remove a disgusting smells.

After completing the fermentation of cooked soybeans, some sticky and viscous materials are presented at the surface of soybeans and a unique smell is also generated.

4. Maturation

Fermented Cheonggukjang masses are to be roughly mashed and mixed with some salts and garlic according to needs and preferences. Then, finished products are matured and stored. The distribution of finished products is distributed under refrigeration. The taste of Cheonggukjang is not changed as it is frozen.

5. Quality characteristics of Cheonggukjang

Thermophilic bacteria are usually proliferated in Cheonggukjang according to the fermentation of soybeans at high temperature (higher than 40℃) in which *Bacillus sp.* is participated. These bacteria represent very particular and unique smells by dissolving soybean proteins. The smells are due to the trimethyl pyrazin and tetra methyl pyrazin newly generated from the fermentation with the 3-methyl-1-butanol in soybeans itself (Choi et al., 1989). It is considered that a large amount of free amino acids are generated in its fermentation process and that affects the taste of Cheonggukjang (Joo, 1971). In addition, soluble proteins are largely increased as much as 40~80kDa and differed from that of Natto (Santos et al., 2007).

The viscous substance that is a particular material in Cheonggukjang represents 61% of crude proteins. Also, the glutamic acid in amino acids shows the highest content, 32%, and a fibrinolytic activity is presented (Lee et al., 1991). In the fermentation process of Cheonggukjang, the viscous substance are different according to applied bacteria (Baek et al., 2008), and the molecular weight of the substance is about 15,000~65,000. The viscous substance usually consists of glutamate and fructose, and the composition of polymers is varied according to their proliferation conditions (Lee et al., 1992). In addition, there are some reports that the odors, compositions, and threshold values in Cheonggukjang are changed according to bacteria applied to the fermentation of Cheonggukjang (Kim et al., 2003; Seo et al., 1983).

3.4 Other fermented products
In addition to the fermented soybean products mentioned above, other various soybeans fermented products have been used in Korean dietary life. For instance, Doenjang dipping that are produced by mixing some Gochujang, garlic, ginger, hot pepper, and etc. to traditional Doenjang are taken with some wrapping vegetables including lettuces and cabbages. The Doenjang dipping are different types of improved and mixed Doenjang and enjoyed in Korea.

In addition, there are some soybean pastes, such as Eoyukjang made of dried beefs and fishes and Jeupjang made of Meju and dried vegetables. The Jeupjang show a fast fermentation process and can be taken as a side dish of boiled rice during summer season.

Such soybean fermented pastes produced by using various sub-materials based on Meju represent different tastes and preservations.

4. Functionalities of fermented soybean products

Various functional ingredients are already included in soybeans. In particular, a large amount of isoflavones (genistin, genestein, daidzin, and daidzein), which are a type of phytochemical, represent some functionalities in itself (Liu, 1997b). In addition, phenolic compounds (syringic, vanillic, chlorogenic, ferulic, and cinnamic acid), lignan, and carotenoids (lutein, and α- and β-carotene) including linoleic acid, linolenic acid, licithin, choline, and saponin are included in soybean (Park, 2009). As these ingredients in soybeans represent their own particular physiological activities, the value of soybean is reevaluated by the people that have no concerns to take soybean as foods.

As mentioned above, there are many types of physiological active ingredients in soybean. In addition, according to some related studies on such issues, it has been known that fermented soybean products create new functional substances through various microbes.

This section proposes the summary of the functionalities of fermented soybean products in Korea, such as Doenjang, Gochujang, and Cheonggukjang.

4.1 Functionalities of Doenjang (Fermented soybean paste)
Doenjang are one of the traditional fermented soybean foods in Korea and produced with some other sub-materials including salts based on Meju produced by using soybeans as a principal material through its fermentation process. Meju represent its own physiological activities by generating new functional ingredients during its fermentation process in addition to the functional substances presented in soybeans.

The functionalities of Doenjang are deeply related to the isoflavones of soybeans, and anti-oxidant, anti-mutagenicity, and anti-cancer effects and ACE functions caused by fermented products have also been known.

4.1.1 Changes in the isoflavone content in Doenjang

It has been known that isoflavones are the glycoside of phenolic compounds and represent effects of preventing breast cancer and prostate diseases (Pagliacci, 1994; Severson, 1989). Also, various functionalities of soybeans presented in Korean Doenjang have been recognized (Kim, et al 1999). Based on this study, it can be seen that the ingredients of isoflavones are newly generated during the fermentation process of soybean (Table 5).

	Soybeans	Meju	Doenjang
Daidzein, free	106±7[a1]	269±38[b]	578±70[d]
Daidzein, total	406±29[a]	433±41[bc]	538±59[c]
Daidzein, aglycones(%)	26.03±0.96[a]	61.96±3.34[b]	107.68±10.26[a]
Genistein, free	95±20[a]	137±16[b]	455±10[d]
Genistein, total	486±86[a]	200±7[b]	538±57[b]
Genistein, aglycones(%)	19.49±1.1[a]3	68.52±6.62[b]	85.26±8.72[d]
D/G ratio[2]	0.85±0.09[ab]	2.16±0.17[c]	1.00±0.10[b]

[1]In each column, different alphabets in superscript show statistically significant difference($p < 0.05$)
[2]Total daidzin contents/total genistein contents ratio

Table 5. Daidzein and genistein contents of soybean, Meju and Doenjang (mg/kg dry basis)

As shown in Table 5, the fermented Meju and Doenjang represent increases in the ingredients of isoflavones. In particular, the Doenjang show a high increase rate in these ingredients. In addition, the change of these ingredients to aglycone, which has a high absorption rate in the body, during its fermentation process by β-glucosidase is also presented (Kim et al., 1999).

The overall isoflavone contents in traditional and improved Doenjang are 370~723μg/g and 170~537μg/g, respectively, where the traditional Doenjang show higher level in the contents (Lee et al., 2010). The isoflavones of Doenjangs are presented as a type of aglycone.

4.1.2 Anti-oxidant effects

In general, anti-oxidant activities are related to anti-cancer and anti-mutagenicity characteristics. It is verified that lots of anti-oxidant substances exist in Doenjang. Doenjang extracts show an increase in oxidation delaying effects according to increases in the addition of the extracts as noted in Table 6 based on the results of the measurement of peroxide values by adding the extracts to linoleic mixtures (Cheigh et al., 1990).

Treatments	Peroxide value(meq/kg)	
Treatments	24 hrs	48 hrs
LA(Control)	790±55*	1113±40
LA+0.1%DP	707±64	1098±12
LA+0.2% DP	630±10	1056±60
LA+0.5% DP	393±28	1031±99

* Mean ± standard deviation

Table 6. Peroxide value of linoleic acid mixture(LA) with the addition of Doenjang powder(DP) during oxidation reaction at 50℃ for 24 and 48hrs.

Anti-oxidant effects in Meju and Doengjang show that it has relation to the amount of browning substances generated from phenolic compounds and its fermentation process (Lee et al., 1991).

In the results of the comparison of the anti-oxidant capabilities in browning chromatic substances, which are divided into fat and water soluble substances, significant anti-oxidant effects are presented and the structure of the primary amine is also verified (Kim, 1994[a]). Also, a strong anti-oxidant activity is presented in water soluble browning substances in which the activity is varied according to its fermentation period (Lee et al., 1994). Peroxide values are largely decreased according to the fermentation period of Meju and Doengjang (Table 7) (Kim, 1994[b]).

Sample	Days of storage	
sample	3	5
Control	230.4	770.1
Raw soybean	34.1	36.6
Meju(fermentation)		
30days	33.6	39.1
50days	32.4	38.3
80days	31.1	34.1
Doenjang(fermentation)		
0days	32.7	37.1
30days	23.9	32.0
60days	22.0	30.6

Table 7. Changes in peroxide values of linoleic acid after addition of isoflavone fraction at a 1% level (meq/kg)

In other studies, fermentation processes of Doenjang show increases in phenolic compounds and improve anti-oxidant capabilities in the comparison of peroxide values. Thus, it is concluded that the results are obtained by phenolic compounds and browning substances (Oh et al., 2007).

4.1.3 Lowering angiotensin converting enzyme (ACE)

The angiotensin II generated by ACE increases blood pressure and that makes possible to constrain hypertension by interrupting the effect.

In the fraction obtained by dissolving Doenjang, the fractions that show ACE lowering effects of 90% and 70% and their structures are determined as arginin and proline as a type of dipeptide (Kim et al., 1999). In other studies, physiological activity peptides are separated from Doenjang and their activities are compared in which the histidine content in amino acids show the highest level (Shin et al., 1995).

In addition, in the results of the comparison of the ACE lowering activity using methanol extracted from traditional Doenjang, the lowering effects are about 23.6~74.5% where the activity of α-glucosidase is also compared (Hwang et al., 2009). Also, in the results of the identification of the strains separated the microorganisms, which contribute to the fermentation of Doenjang, that show excellent ACE activities, the strains are verified as *Bacillus pumilus*. In other studies, by extracting *B. subtilis* SCB-3, which represents an effect of lowering ACE, the ACE lowering rate of the bacterium is 61% by applying it to the fermentation of Doenjang, and IC$_{50}$ shows a high value of 0.02mg/mL (Hwang et al., 1997).

4.1.4 Anti-cancer and anti-mutagenicity of Doenjang

In several studies on Doenjang, it has been known that Doenjang have anti-cancer and anti-tumer effects. Table 8 shows the results of the comparison of anti-cancer effects using SRB assays with respect to cancer cells through extracting the raw materials of Doenjang and methanol extracts (Lim et al., 1999).

Samples	OD510	Survival rate[5](%)	Inhibition rate(%)
Control	0.99±0.02[a4]		
Doenjang(SF)	0.45±0.01[d]	45	55
SF(Soybean+flour)[1]	0.64±0.03[b]	65	35
Doenjang(S)	0.47±0.01[d]	47	53
S(soybean)[2]	0.51±0.00[c]	52	48
Miso[3]	0.54±0.02[c]	55	45
Chongkukjang	0.51±0.01[c]	52	48

Soybean : flour = 7:3, Soybean(100%), Light yellow Miso

Means with the different letters beside symbols are significantly different at the 0.01 level of significance as determined by Duncan's multiple range test.

$$\text{Survival rate(\%)} = \frac{\text{OD510 of treated cells}}{\text{OD510 of control cells}} \times 100$$

Table 8. Inhibitory effect of methanol extracts(2mg/assay) of doenjang, other soybean products and soybean on the growth of AGS human gastric adenocarcinoma cells in sulforhodamine B(SRB) assay that determined after 2 days of incubation at 37℃

As presented in Table 8, Doenjang products represent higher anti-cancer effects than that of the raw materials of Doenjang. In the similar experiments of anti-cancer effects for liver and

large intestine cancer cells, Doenjang products show higher anti-cancer effects than other raw materials. (Lim et al., 2004).

In the results of the comparison of anti-cancer effects for liver and large intestine cancer cells using the Doenjang produced from Sunchang, which is a representative region of producing Korean traditional Doenjang, it shows higher lowering effects in the fractions of acetate and hexane more than 75% (Choi et al., 1999).

In addition, in the results of the studies (Hwang, 2007; Lee, 2009), it is considered that the peptides and other substances generated during the fermentation of Doenjang contribute to increase such anti-cancer effects.

Moreover, as the water used in producing Doenjang is replaced by germanium contained water or maple sap, it show high anti-mutagenicity effects. Also, nine times burned bamboo salts represent an increase in the effects (Lee et al., 2008). In the case of the Doenjangs produced by applying mushroom mycelium hyphaes, it shows higher anti-tumor and anti-cancer effects than that of traditional Doenjang (Lee et al., 2003).

4.1.5 Other functions

In traditional Doenjang, other various functions in addition to the above mentioned functions are verified by experiments. The excellent bacteria isolated from Doenjang are used to produce the Doenjang that can prevent thromboses and have high β-glucosidase contents (Ra et al., 2004), In addition, Doenjang show the lowering effects of hyaluronidase activities, which have anti-inflammation and anti-allergic effects, about 56~70% (Ahn et al., 2005). Also, Doenjang represent anti-bacterial effects for *B. cereus, E. coil, L. monocytognese, and S .aureus* (Yi et al., 1999).

4.2 Functionalities of Gochujang

Gochujang are a type of fermented seasoning by mixing some rice products dissolved by using hot pepper powders and barley malts based on Gochujang Meju. The functionality of this seasoning is deeply related to the ingredients of capsaicin and fermented products in red peppers.

In the classification of the studies on the functionalities of Gochujang, studies are focused on anti-obesity, anti-cancer, anti-tumor, and cardiovascular improvement effects. Also, some studies are focused on the anti-cancer effects on the basis of anti-oxidant effects.

4.2.1 Anti-obesity effects

The capsaicinoid included in Gochujang shows anti-obesity effects by promoting fat metabolism in the body. Based on the effects, a study on the anti-obesity effects using Gochujang is performed through animal and clinical tests (Park, 2009).

In the results (a 9.5% of the diet weight) of the measurement of anti-obesity effects in rats, which intake high fat diet, their weights and fat accumulation decrease as 13% and 30%, respectively (Fig. 11). The results show increases in energy consumption (Choo, 2000). In the results of the comparison of anti-obesity effects with that of hot pepper powders, the effects are caused by not only hot pepper powders but the fermentation of Gochujang (Fig. 12).

In the results of the comparison of the experiments performed by using various Gochujang, which show different production methods, for rats with high fat diet and Gochujang diet, the fermented rice Gochujang diet shows the highest effects of reducing fat tissues (Lee, 2002). Also, in the results of the comparison of the changes in weight for SD rats with high

fat diet and Gochujang diet in order to verify the differences before and after the fermentation of Gochujang, the rats with fermented Gochujang represent high weight-loss effects. In particular, the rats with traditional Gochujangs show large decreases in their weights. It shows that the fermentation process of Gochujangs increases weight-loss effects and decreases fats in epididymis and kidney (Lee et al., 2003). In similar studies, in the results of the animal tests by applying traditional and improved Gochujang and hot pepper
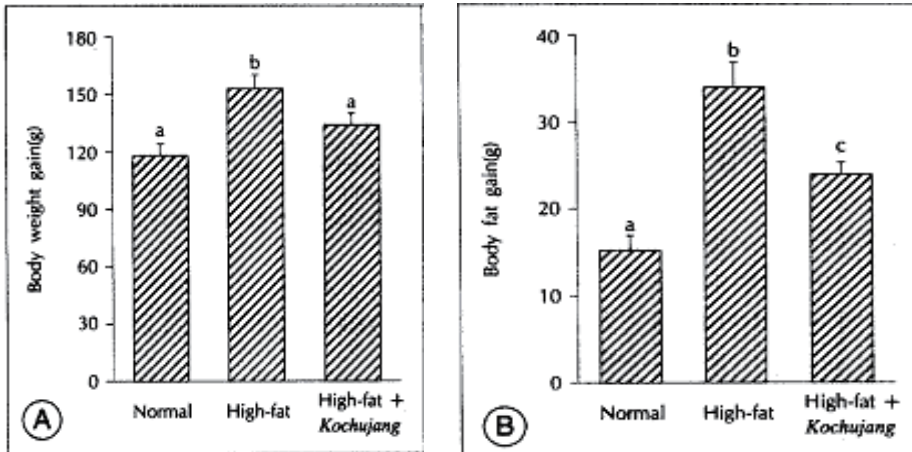
Fig. 11. Effect of Kochujang on body weight(A), body fat(B) in rats fed on a high-fat diet over 21days; normal diet(Normal), hight-fat diet(High-fat). Values are means for eight rats, with their standard errors indicated by vertical bars. Bars not sharing a common letter were significantly different, P<0.05.

Fig 12. Effect of red pepper on body weight(A), and body fat(B) in rats fed on a high-fat diet over 21days; normal diet(Normal), high-fat diet(High-fat). Values are means for eight rats, with their standard errors indicated by vertical bars. Bars not sharing a common letter were significantly different, P<0.05.

powders, the fermented Gochujang represent significantly lower increases in their weights and improve the effects of its fermentation based on low intestine weights, low triglyceride contents, and low cholesterol contents (Kim, 2004).

The results of the actual clinical tests using Gochujang are presented (Kim, 2009). Gochujang are made as pills and applied to 54 subjects by 32g per day over 12 weeks. In the results, visceral areas and the ratio of visceral/subcutaneous fats are improved as a significant level. Also, the Gochujang intake group shows decreases in triglycerides and improves in serum lipids, fat proteins, altherogenic indexes, and coronary calcium indexes. Thus, it is estimated that the intake of Gochujang for a long period in obese people can prevent obesities and coronary diseases by improving serum lipids and fat proteins.

4.2.2 Anti-cancer and anti-tumor effects

The largest numbers of studies have been conducted are on the functionalities of Gochujang for anti-cancer and anti-tumor effects. In particular, there are many studies on the anti-cancer functions of Gochujang in which the results of positive effects are verified. In addition, traditional Gochujang products represent higher effects than other products (Park, 2002).

The substances for the anti-cancer effects are not determined as a single substance but a complex substance. In the results of the anti-cancer tests for the cells of stomach cancer using the samples extracted from methanol extracts, which are obtained by a freeze drying method from commercially distributed Gochujang with wheat koji, and other materials, the Gochujang fully fermented by using wheat koji represent the most excellent effects (Kim et al., 2005). For measuring anti-cancer and immune activities, onion added Gochujang are produced and processed using a freeze drying method in order to extract methanol extracts (Kim et al., 2005). The results show the effects of anti-mutagenicity as a concentration dependent way of *Salmonella typhimurium*, and onion added Gochujang represent higher effects than others. Also, in the case of MCF-7 cancer cell lines, the onion added Gochujang represent higher anti-cancer effects than others. The generation of NO in macrophages shows increases as a concentration dependent way and that also represents higher level than regular Gochujang.

In the results of the comparison of the weight of tumors since 32 days after transplanting Sacroma-180 tumor cells using the samples obtained from freeze dried traditional Sunchang Gochujang and factory made Gochujang by extracting them using methanol, the control group shows 6.0 g and the fermented traditional Gochujang shows 3.3 g. Thus, the traditional Gochujang represents an anti-tumor effect of 45%. However, unfermented Gochujang shows an anti-tumor effect of 17% only (Table 9). Although factory made Gochujang represents an anti-tumor effect of 23%, it represents lower effects than traditional Gochujang (Park et al., 2001).

Using the ethanol extracts of traditional Gochujang, the anti-mutagenicity characteristic is verified. Also, in Meju, it is verified that the extracts constrain the mutagenicity caused by aflatoxin B_1 (Kim et al., 1999). By obtaining the ethanol extracts of sea tangle powder added Gochujang, the tests of mutagenicity and cell toxicity are performed (Cui et al., 2002).

The results show that fermented Gochujang represent significant anti-cancer effects and increase such effects as the fermented Gochujang are mixed with other anti-cancer materials.

Sample	Tumor wt.(g)	Inhibition rate(%)
S-180+PBS	6.0±0.1[a6)]	-
S-180+CK[2)]	4.5±0.1[bc]	23
S-180+TK Ⅰ[3)]	5.0±0.9[ab]	17
S-180+TK Ⅱ[4)]	3.3±0.3[c]	45
S-180+RPP[5)]	4.7±0.3[b]	22

Table 9. Antitumor activities of methanol extracts from various kinds of Gochujang and hot pepper powder(RPP) in tumor bearing Balb/c mouse with sarcoma-180 cell[1)]

1. 7-days sarcoma-180 ascites cells were s.c. transplanted into the left groin of inbredstran. 1.0 mg/kg of methanol extract from various kinds of Gochujang, red pepper powder or the equal volume of phosphate buffered saline (control) was i.p. injected once a day for 20 days from 24 hr following transplantation. All mice were sacrificed at 5 weeks following transplantation, and tumor, spleen and liver weight were measured.
2. Commercial Gochujang: Daesang Co.
3. Traditional Gochujang I: 0 day fermented Gochujang, Moonokrye Co.
4. Traditional Gochujang Ⅱ: 6 month fermented Gochujang, Moonokrye Co.
5. RPP: the same as added in TK1 and TKⅡ
6. Means with the different letters are significantly different (p<0.05) by Duncan's multiple range test.

4.2.3 Other functions

In the case of the extracts of green teas or natural plants that are added to Gochujang, the extracts added Gochujang show increases in anti-oxidant capabilities and anti-bacterial effects (Kim et al., 2005). Also, garlic porridge added Gochujang show anti-oxidant and anti-cancer effects (Song et al., 2008).

In other experiments, as applying orotic acids, which have been known as a cause substance in accumulating triglycerides to the liver, the adding of capsaicin prevents the generation of such triglycerides and largely decreases the activity of phosphatidate phosphohydrolase, which contributes to the composition of triglycerides, and that leads to constrain triglycerides to the liver (Cha et al., 2004). Studies on the bacteria, which are separated from Gochujang, represent various functionalities in which the strains that produce a polymer of poly-γ-glutamate are *Bacillus* similar species. This substance has been largely used to control viscosity, to constrain bitter tastes, and to produce various medicines and show a possibility of economic mass productions (Kang et al., 2005). In addition, by isolating the bacteria that have the effects of thromboses dissolution, immunity activation, and cell toxicity from Gochujang, the possibility of the bacteria contributed to the fermentation of Gochujang is considered as a different way through identifying it into *Bacillus stearothermophilus* and *B. amyloliquefacience* (Seo et al., 2007).

4.3 Functionalities of Cheonggukjang

Cheonggukjang are a pure soybean fermented product that can be used in the table within a short period of time after fermenting cooked soybeans at high temperature and require a small amount of salts and seasonings as in the table. Cheonggukjang have a particular viscous substance and represent a unique small. Also, it shows specific functionalities due to the substances created during its fermentation including the functionalities of isoflavones presented in soybeans itself.

Effects of preventing hypertension, improving fat metabolism, thromboses dissolution, anti-oxidant, and preventing osteoporosis including physiological effects have been known (Kim et al., 1999; Lee et al., 2005).

4.3.1 Anti-cancer effects of Cheonggukjang

In the results of the tests of the lowering effects for cancer cells using the Cheonggukjang methanol extracts, the lowering rate is 65% and that is a higher level than that of Miso. Also, it shows the lowering effects for liver cancer cells in which the lowering rate is 60% (Lim et al., 2004). In addition, it shows an effect of constraining other cancer cells (Lim et al., 1999). In particular, by adding the porphyran extracted from lavers to Cheonggukjang, the prophyran added Cheonggukjang represent high constraint effects for cancer cells such as bowel cancer (Min et al., 2008). The results represent that although regular Cheonggukjang show the constraint effects of proliferation as 19~27%, the prophyran added Cheonggukjang represent increases in the constraint effects of proliferation about 27~32%.

Thus, it is verified that Cheonggukjang show significant constraint effects on cancer cells.

4.3.2 Lowering Angiotensin converting enzyme (ACE)

Studies on the constraint of blood pressure increases by lowering ACE activities are performed. Cheonggukjang show specific ACE lowering effects (Kil et al., 1998) and peptides are known as its cause factor (Cho et al., 2000).

The peptide contents are the order of alanine (30.84%), phenylalanine (30.3%), and histidine (20.24%). In the results of the calculation of the lowering rate of ACE, a 1mg of peptides represents 74.74%. Although traditional Cheonggukjang use straws as a starter (Kim et al., 1982), some superior bacteria are separated from Cheonggukjang and used to the fermentation of Cheonggukjang in order to manage the fermentation (Kil et al., 1998; Hwang, 2010). In these results, the fibrinolytic activity generated by such superior bacteria represents a high level (Table 10). Also, it is verified that the threshold value of enzyme shows increases as Meju are only used as a matrix.

In addition, although the microorganisms that contribute to the fermentation of Cheonggukjang are usually *Bacillus sp.*, some strains that strongly secret fibirinolytic enzymes employed in the *Bacillus* sp. (Kim et al., 1995).

Temperature (℃)	Strain	Fibrinolytic activity (cm)		
		Fermentation time (hr)		
		24	48	72
40	CJJN-4	2.30±0.31	2.50±0.24	2.40±0.45
	CJJN-5	2.20±0.18	2.30±0.37	2.30±0.26
45	CJJN-4	1.40±0.37	1.50±0.44	1.80±0.12
	CJJN-5	2.10±0.25	2.00±0.27	1.90±0.38

Values are mean±SD.

Table 10. Comparison of fibrinolytic activities of Cheonggukjang fermented with CJJN-4 or CJJN-5 on fermentation temperature

4.3.3 Other functions

In the results of the animal tests, white rats, by inoculating the extracted superior bacteria with respect to high cholesterol and Cheonggukjang intake groups, the effects that constrain

the generation of peroxide fats in liver tissues and protects liver damages are verified (Kim et al., 2009). Regarding other effects, it removes sodium nitrides including anti-oxidant effects and verifies the effects on the constraint of inflammations(Ahn et al., 2005). In addition, the application of Cheonggukjang effectively constrains increases in blood pressures from animal tests (Yang et al., 2003). Also, the sticky viscous substance shows decreases in blood sugars and serum lipids (Kim et al., 2008).

5. Conclusion

The origin of soybeans has been known as Manchuria, Far East Asia. Then, soybeans were propagated to various countries of the world. In some Southeast Asian countries, in particular Korea, China, and Japan, soybeans have been variously used in their dietary life and contributed to improve nutrients as protein sources. The history of using soybeans goes back to B.C. On the other hand, in Western countries, soybeans have been largely used as feeds and oil source. However, recently these countries also use soybeans as food materials and produce various soybean processing products.

Soybeans are an important position in Korean dietary life as one of the five major grains and have been taken by soybeans itself with boiled rice. Also, soybeans have been variously used by processing it or by growing it as vegetables. Then, soybeans have been taken as vegetables, powders, and other various types of foods differed from other countries. In particular, fermentation methods using molds, bacteria, and yeasts are developed using cooked soybeans and that leads to produce Ganjang(soybean sauces,) Doenjang (fermented soybean pastes), Cheonggukjang, and Gochujang(fermented hot pepper soybean paste). These products have been used in Korean dietary life as the primary seasonings and side dishes. Also, these soybean products play important roles in differentiating Korean foods from other foods.

Recently phytochemicals including isoflavones in soybeans have been known as functional ingredients. Also, it is verified that soybean proteins have physiological activities from the results of various studies. Therefore, people around the world represent interests on the use of soybeans as foods. In addition, lots of soybean processing products have been produced and distributed due to their effects on preventing various chronic diseases through simple pharmacological treatments only.

In Korea, regarding the characteristics of using soybeans, various soybean products have been developed using specific fermentation processes. It has been scientifically proven that various functionalities are newly created during several fermentation processes. Also, studies on this issue have been continuously conducted. In the specific functionalities of soybean products, it contributes to present effects on anti-cancer, anti-tumor, improving blood circulation, and preventing chronic diseases. In the case of Gochujang, it has been known that Gochujangs represent significant effects on constraining obesities.

6. References

Ahn, S & Hong, K. (2005). Hyaluronidase inhibitory activity of extract from Doenjang, Chungkookjang and Miso. *Journal of Korean Society Food Science and Nutrition*, Vol.38, No. 8, pp. 1119-1123, ISSN 1226-3311

Baek , L.; Park, L.; Park, K. & Lee, S. (2008). Effect of starter cultures on the fermentative characteristics of Cheonggukjang. *Korean Journal of Food Science & Technology*, Vol.40, No.4, pp. 400-405, ISSN 0367-6293

Cha, J.; Jun, B. & Cho, Y. (2004). Prevention of orotic acid induced fatty liver in rats by capsaicin. *Food Science and Biotechnology*, Vol.13, No.5, pp. 597-602 ISSN 1226-7708

Cheigh, H.; Park, K.; Moon, G. & Park , K. (1990). Antioxidative characteristics of fermented soybean paste and its extracts on the lipid oxidation. *Journal of Korean Society Food science & Nutrition*, Vol.19, No.21, pp. 63-167, ISSN 1226-3311

Cho, Y.; Cha, W.; Bok, SK..; Kim, MU.; Chun, SS.; Choi, UK.; Kim, SH. & Park, KS. (2000). Production and separation of angiotensin converting enzyme inhibitor during natto fermentation. *Journal of Korean Society Food Science & Nutrition*, Vol. 29, No.4, pp. 737-742 ISSN 1226-3311

Choi, S. & Ji, Y (1989). Changes in flavor of Chungkookjang during fermentation. *Korean Journal of Food Science & Technology*, Vol.21, No.2, pp. 229-234 ISSN 0367-6293

Choi, S; Cheigh, M; Lee, J; Kim, L; Hong, S; Chung, K.& Lee, B. (1999). Growth suppression effect of traditional fermented soybean paste(Doenjang) on the various tumor cells. *Journal of Korean Society Food Science & Nutrition*, Vol.28, No.2, pp. 458-463, ISSN 1226-3311

Choo, J. (2000). Anti-obesity Effects of Kochujang in Rats Fed on a High- fat Diet. *Korean Journal Nutrition*. Vol.33, No.8, pp. 787~793 ISSN 0367-6453

Cui, C.; Oh, S.; Lee, D & Ham, S. (2002). Effect of the biological activities of ethanol extract form Korean traditional Kochujang added with sea tangle (*Laminaria longissima*). *Korean Journal of Food Preservation*. Vol. 9, No.1, pp. 1~7 ISSN 1225-8636

Hwang, J.; Oh. U.; Lim, J.; Park, J.; Kim, M.; Yoon, H. & Lim, S. (2009). Physiological properties of Jeju traditional Doenjang. *Journal of the Korean Society Food Science & Nutrition*. Vol.38, No.12, pp. 1656-1663 ISSN 1226-3311

Hwang, J. (1997). Angiotensin I converting enzyme inhibitory effect of Doenjang fermented by B. subtilis SCB-3 isolated from Meju. Korean traditional food. *Journal of the Korean Society Food Science & Nutrition,* ,Vol.26, No.5, pp. 776-783 ISSN 1226-3311

Hwang, J. (2010). The fermentation characteristics of Cheonggukjang prepared by starter culture *of Bacillus* spp. with fibrinolytic activity. *Journal of the Korean Society Food Science & Nutrition*, Vol.39, No.12, pp. 1832-1838 ISSN 1226-3311

Hwang, K.; Oh, S. & Park, K. (2007). Increased antimutagenic and in vitro anticancer effects by adding green tea extract and bamboo salt during Doenjang fermentation. *Journal of the Korean Society Food Science & Nutrition*. Vol.38, No.1, pp. 1-7 ISSN 1226-3311

Jang H. & Ryu, B. (2008). The technology of Food Processing and Preservation. In: Soybean Processing, (2nd ed.), 143-144, Life Science Publishing, ISBN 97-8896-154-0216, Seoul, Korea

Jang, J[a]. (1993). Food and Cooking of Korea using Traditional Soybean Processing Historical Research. ISBN 2003821001339, Suhak Press, Seoul, Korea

Jang, J[b]: (1993). Study for Legumes Cultivation in Korea. ISBN 2003821001353, Suhak Press, Seoul, Korea

Joo, H. (1971). Studies on the manufacturing of Cheongkukjang. *Korean Journal of Food Science & Technology*. Vol. 3, No.1, pp. 64-67 ISSN 0367-6293

Ju, K. & Oh, N. (2009). Effect of the mixed culture of *Bacillus subtilis* and *Lactobacillus plantarum* on the quality of Choenggukjang. *Korean Journal of Food Science & Technology*, Vol.41, No.4, pp. 399-404 ISSN ISSN 0367-6293

Kang, S.; Rhee, J.; Park, C.; Sung, M. & Lee, I. (2005). Distribution of poly-γ-glutamate (γ-PGA) producers in Korean fermented foods, Chungkukjang, Doenjang, and Kochujang. *Food Science & Biotechnology*, Vol.14, No.5, pp. 704~708 ISSN 1226-7708

Kil, J.; Kim, G. & Park, I. (1998). Production and charterization of fibrinolytic enzyme: optimal condition for production of the enzyme from Bacillus SP. KP-6408 isolated from cheonggukjang. *Journal of the Korean Society Food Science & Nutrition*, Vol.27, No.1, pp. 51-56 ISSN 1226--3311

Kim, A.; Lee, J.; Chang, H. & Lee, M. (2009). Antioxidative effects of cheonggukjang fermented using *Bacillus subtilis* DJI in rats fed a high cholesterol diet. *Journal of the Korean Society Food Science & Nutrition*, Vol.38, No.12, pp. 1699-1706 ISSN 1226-3311

Kim, H.; Lee, T.; Moh, B. & Park, J. (1998). Characteristics of Sarmjang prepared with different Doenjang as a main material. *Korean Journal of Food Science & Technology*, Vol.30, No.1, pp. 54-61 ISSN 0367-6293

Kim, J.; Kim, M.; Jung, S.; Seo, H.; Kim, E. & Lee SY. (2005). Development of Gochujang for controlling *V. parahaemolyticus* with green tea and natural products. *Journal of Life Science*, Vol.15, No.15, pp. 783-789 ISSN 1225-9918

Kim, J.; Oh, H. & Ha, J. (2003). Volatile flavor components of Kochujang prepared with *Aspergillus oryzae, Bacillus licheniformis* and *Saccharomyces rouxii*. *Food Science & Biotechnology*, Vol.12, No.1, pp. 18-22 ISSN 1226-7708

Kim, J. & Yoon, S. (1999). Isoflavone contents and β-glucosidase activities of soybean, meju, and Doenjang. *Korean Journal of Food Science & Technology*, Vol.31, No. 6, pp. 1405-1409 ISSN 0367-6293

Kim, J.; Kim, J. & Kim, W. (2004). Changes in isoflavone and oligosaccharides of soybeans during germination. *Korean Journal of Food Science & Technology*, Vol.36, No.2, pp. 294-298 ISSN 0367-6293

Kim, J.; Park, K.; Yang, H.; Cho, Y.; Jeong, C.; Shin, K.; Yee, S. & Seo, K. (2005). Anticancer and Immuno-activity of Methanol Extract from Onion Kochujang. *Korean Journal of Food Preservation*, Vol.12, No.2, pp. 173~178 ISSN 1225-8636

Kim, J. (2004). Antiobestic and cancer preventive effects of Kochujang. Busan University. MA thesis

Kim, K.; Ryu, M. & Kim, S. (1982). Cheonggukjang Koji fermentation with rice straw. Korean *Journal of Food Science & Technology*, Vol.14, No.4, pp. 301-308 ISSN 0367-6293

Kim, M.; Kim, I.; Oh, J. & Shin, D. (1998). Quality changes of traditional Kochujang prepared with different Meju of red pepper during fermentation. *Korean Journal of Food Science & Technology*, Vol.30, No.4, pp. 924-933 ISSN 0367-6293

Kim, S.; Kim, S. & Hong, E. (1993). Composition of soybean sprout and its nutritional value. *Journal Korea Soybean Digest*, Vol.10, No.1, pp. 1-9 ISSN 1225-0686

Kim, S.; Jung, S.; kim, I.; Lee, Y.; Lee, J.; Kim, J.; Lee, M.; Choi, M. & Kim, D. (2008). The effects of Cheonggukjang biopolymer on blood glucose and serum lipid lowering in streptozotocin-induced diabetic rats. *Journal of the Korean Society Food Science & Nutrition*, Vol.37, No.1, pp. 35-41 ISSN 1226-3311

Kim, S.; Yang, J. & Song, Y. (1999). Physiological function of Cheonggukjang. *Food Industry and Nutrition* Vol.4, No.2, pp. 40-46 ISSN 1226-3338

Kim, S.; Jung, K. & Park, K. (1999). Inhibitory effect of Kochujang extracts on chemically induced mutagenesis. *Journal of Food Science & Nutrition*, Vol.4. No.1, pp. 38-42 ISSN 1226-332X

Kim, S.; Park, C.; Park, S.; Kim, Y.; Cho. H.; Lim, D.; Kim,J.; Lee, J. & HA, Y. (2003). Enhanced antitumorigenicity and antimutagenicity of Doenjang prepared from mushroom mycelia-cultured traditional Meju. *Journal of the Korean Society Food Science & Nutrition*, Vol. 32, No.1, pp. 143-148 ISSN 1226-3311

Kim, S.; Kong, C.; Kil, J.; Kim, J.; Han, M.& Park KY. (2005). Fermented wheat grain products and Kochujang inhibit the growth of AGS human gastric adenocarcinoma cells. *Journal of Food Science & Nutrition*, Vol.10, No.4, pp. 349~352 ISSN 1226-332X

Kim, S. (2009). Effects of Kochujang supplementation on obesity and arterosclerosis index in obese subjects. MS thesis, Chonbuk National University, Korea

Kim, W. (2005). Soybean, In: Processing Characteristics of Soybean, 260-268, Korea University Press, ISBN 89-7641-562-0, Seoul, Korea

Kim, Y.; Jung, H.; Park, Y. & Yu, T. (2003). Characteristics of flavor and functionality *of Bacillus subtillis* K-20 Chunggukjang. Korean *Journal of Food Science & Technology*, Vol.35, No.3, pp. 475-478 ISSN 0367-6293

Kim, Y.; Rhee, C. & Park, H. (2001). Isolation and characterization of Bacterium from korean soy paste Doenjang producing inhibition of angiotensin converting enzyme. *Korean Journal of Food Science & Technology*, Vol. 33, No.1, pp. 84-88 478 ISSN 0367-6293

Kim, Y.; Kim, W. & Oh, H. (1995). Screening and identification of the fibinolytic bacterial strain from Cheonggukjang. *Korean Journal of Microbiology & Biotechnology*, Vol.23, No.1, pp. 1-5 ISSN 1598-642X

Kim,[a] M.; Lim, S.; Kim, S.; Kim, G. & Lee, J. (1994). Antioxidative materials in domestic Meju and Doenjang(2). *Journal of the Korean Society Food Science & Nutrition*, Vol.23, No.2 pp. 251-260 ISSN 1226-3311

Kim,[b] M.; Im, S.; Yoo, Y.; Kim, G. & Lee. J. (1994). Antioxidative materials in domestic Meju and Doenjang(4). *Journal of the Korean Society Food Science & Nutrition*, Vol.23, No.5, pp. 792-798 ISSN 1226-3311

Kwon[a], T.; Kim, S.; Kim, W.; Park, K.; Son, H.; Seung, J.; Shin, D.; Shin, S.; Lee, K.; Lee, Y.; Lee, C.; Lee, H.; Cho, S.; Cho, J.; Cho, H.; Ji, K.& Hong, E. (2005). Soybean, In; Utilization History of Soybean, 3-12, Korea University Press, ISBN 89-764-156-20, Seoul, Korea

Kwon[b], T.; Kim, S.; Kim, W.; Park, K.; Son, H.; Seung, J.; Shin, D.; Shin, S.; Lee, K.; Lee, Y.; Lee, C.; Lee, H.; Cho, S.; Cho, J.; Cho, H.; Ji. K.& Hong, E. (2005). Soybean, In: Second Miracle of Soybean, 294-312, Korea University Press, ISBN 89-764-156-20, Seoul, Korea

Lee, B.; Kim, D. & Kim, K. (1991). Physico-chemical properties of viscous substance extracted from Chungkookjang. *Korean Journal of Food Science & Technology*, Vol.23, No.5, pp. 599-604 ISSN 0367-6293

Lee, H (2005) Soybean Cousine of Korea Soy Foods of Korea. Soybean. 455-528, Korea University Press, Seoul, Korea

Lee, H. (2002). Preparation and fermentation characteristics of traditional Kochujangs and their cancer preventive and antiobesity effects. MA thesis, Busan University, Korea

Lee, J. & Lee, S. (1997). Effects of the types and concentrations of sugars on the physicochemical and sensory characteristics of soy milks during storage. *Journal of the Korean Society Food Science & Nutrition*, Vol.13, No.2, pp. 251-260 ISSN 1226-3311

Lee, J.; Kim, M. & Im, S. (1991). Antioxidative materials in domestic Meju and Doenjang(1). *Journal of the Korean Society Food Science & Nutrition*, Vol.20, No. 2, pp. 148-155 ISSN 1226-3311

Lee, J.; Kim, M.; Im, S.; Kim, S. & Kim, G. (1994). Antioxidative materials in domestic Meju and Doenjang(3). *Journal of the Korean Society Food Science & Nutrition*, Vol. 23, No.4, pp. 604-613 ISSN 1226-3311

Lee, J.; Ha, S.; Kim. A.; Yuh, C.; Bang, I. & Park, S. (2005). Industrial application and physiological functions of Cheonggukjang. *Food Science & Industry*, Vol.38, No.2, pp. 69-78 ISSN 0257-2397

Lee, K.; Lee, H. & Chung, M. (1971). Studies on Chung-kook-jang(part I). *Journal of Applied Biological Chemistry*. Vol.14, No.3, pp. 191-200 ISSN 1229-2737

Lee, S.; Baek, L. & Park, L. (2008). Physiological characteristics of Bacillus spp. isolated from rice straw as Cheonggukjang starter. *Korean Journal of Food Science & Technology*, Vol. 40, No.5, pp. 562-567 ISSN 0367-6293

Lee S.; Lee, K.; Moon, S. & Park, K. (2008). Antimutagenic effects on methanol extracts of Doenjang made with various Kinds of water or salt. *Journal of the Korean Society Food Science & Nutrition*, Vol.37, No.6, pp. 691-695 ISSN 1226-3311

Lee, S. & Chang, H. (2009). Growth-inhibitory effect of the solar salt-Doenjang on cancer cell, AGS and HT-29. *Journal of the Korean Society Food Science & Nutrition*, Vol. 38, No.12, pp. 1664-1671 ISSN 1226-3311

Lee, S.; Park, Y. Chang, P. & Lee, J. (2010). Isoflavone distribution and β-glucosidase activity in home-made and factory-produced Doenjang. *Korean Journal of Food Science & Technology*, Vol.42, No.2, pp. 125-129 ISSN 0367-6293

Lee, S. (1988). The historical review of traditional Korean fermented foods. *Journal of the Korean Society of Dietary Culture*. Vol.3, No.4, pp. 331-339 ISSN 1225-7060

Lee, Y.; Kim, S.; Chong, N. & Yim, M. (1992). Studies on the production of viscous substance during the Choengkookjang fermentation. *Journal of Applied Biological Chemistry*, Vol.35, No.3, pp. 202-209 ISSN 1229-2737

Lim, S.; Park, K. & Rhee, S. (1999). Anticancer effect of Doenjang in vitro sulforhodamine B(SRR) assay. Journal *Journal of the Korean Society Food Science & Nutrition*, Vol.28, No.1, pp. 240-245 ISSN 1226-3311

Lim, S.; Rhee, S. & Park, K. (2004). Inhibitory effect of methanol extract of Doenjang on growth and DNA synthesis of human cancer cell. *Journal of the Korean Society Food Science & Nutrition*, Vol.33, No.6, pp. 936-940 ISSN 1226-3311

Liu, K[a]. (1997) Soybeans, Chemistry, Technology and Utilization, 83-93, An Aspen Publisher, ISBN 0834212994, Newyork, USA

Liu, K[b]. (1997). Soybeans, Chemistry, Technology and Utilization, 442-446, An Aspen Publisher, ISBN 0834212994, Newyork, USA

Min, H.; Kim, H. & Chang. H. (2008). Growth-inhibitory effect of the extract of porphyran - Cheonggukjang on cancer cell. *Journal of the Korean Society Food Science & Nutrition*, Vol.37, No.7, pp. 826-833 ISSN 1226-3311

National Health & Nutrition examination Survey. (2005) Ministry of Health & Welfare, Korea

Oh, B.; Kim, Y.; Jeong, P. & Shin, D. (2006). Quality characteristics of Kochujang Meju prepared with *Aspergillus* species and *Bacillus subtilis*. *Food Science & Biotechnology*, Vol.15, No.4 pp. 549-554 ISSN 1226-7708

Oh, H. & Kim, C. (2007). Antioxdant and nitrite scavenging ability of fermented soybean foods(Chungkukjang, Doenjang). *Journal of the Korean Society Food Science & Nutrition*, Vol.36, No.12, pp. 1503-1510 ISSN 1226-3311

Oh, J.; Kim, Y.; Kim, Y. & Shin, D. (2001). Volatile flavor compounds of Kochujang prepared with different Kojis. *Food Science & Biotechnology*, Vol.10, No.1, pp. 45-53 ISSN 1226-7708

Paglicacci, M.; Smacchia, M. & Migliprati, G. (1994). Growth inhibitory effects of the natural phytoestrongen genistein in MCF-7 human breast cancer cells. *European Journal of Cancer*, Vol.30, pp. 1675-1679 ISSN 1359-6359

Park, K. (2009). Science and Health Functionality of Korean Fermented Soybean Foods. Korea Jang Cooperative. pp.146-175

Park, K.; Kong, K.; Jung, K. & Rhee, S. (2001). Inhibitory effects of Kochujang extract on the tumor formation and lung metastasis in mice. *Journal of Food Science & Nutrition*, Vol. 6, No.3, pp. 187-191 ISSN 1226-332X

Park, K. (2002). Korean traditional foods and their anticancer effects. *Journal of Korean Society People, Plants & Environment*, Vol.5, No.1, pp. 41-45 ISSN 1226-9778

Park, K. (2009). Science and Health Functionality of Fermented Soybean Products. Korea Jang Cooperative. pp 8-9

Park, W. (1991). Encyclopedia of Foods and Food Science. Shin Kwang, ISBN 8970695117, Seoul, Korea

Ra, K.; Oh, S.; Kim, J. & Suh, H. (2004). Isolation of fibrinolytic enzyme and β-glucosidase producing strains from Doenjang and optium conditions of enzyme production. *Journal of the Korean Society Food Science & Nutrition*, Vol.33, No.2, pp. 439-442 ISSN 1226-3311

Rhee, S.; Kong, K.; Jung, K. & Park, K. (2003). Decreasing Effect of Kochujang on Body Weight and Lipid Levels of Adipose Tissues and Serum in Rats Fed a High - Fat Diet. *Journal of the Korean Society Food Science & Nutrition*, Vol.32, No.6 pp. 882-886 ISSN 1226-3311

Santos I.; Sohn, I.; Choi, H.; Park, S.; Ryu, S.; Kwon, D.; Park, C.; Kim, J.; Kim, J. & Lim, J. (2007). Changes of protein profile in Cheonggukjang during the fermentation preriod. *Korean Journal of Food Science & Technology*, Vol.39, No.4 pp. 438-446 ISSN 0367-6293

Seo, J.; Ryu, M. & Hur, Y. (1983). Effect of *Bacillus* strains on the Chungkookjang processing, *Korean Journal of Food Science & Technology*, Vol.15, No.4, pp. 385-391 ISSN 0367-6293

Seo, M.; Kim, S.; Lee, C. & Cha, S. (2007). Fibrinolytic, immuno-stimulating, and cytotoxic activities of microbial strains isolated from Kochujang. *Korean Journal of Food Science & Technology*, Vol.39, No.3, pp. 315-322 ISSN 0367-6293

Severson, R.; Nomura, A.; Grove, J. & Stemmerman, G. (1989). A prospective study of demographics, diet and prostate cancer among men of Japanese ancestry in Hawaii. *Cancer Research*, Vol.49, No.7, pp 1857-1860 ISSN 0008-5472

Shin, D.; Ahn, E.; Kim, Y. & Oh, J. (2001) Changes in physicochemical characteristics of Gochujang Prepared with different Koji during fermentation. *Korean Journal of Food Science & Technology*, Vol.33, No.2, pp. 256-263 ISSN 0367-6293

Shin, D.; Kim, D.; Choi, U.; Lim, D. & Lim, M. (1996) Studies on taste components of traditional Kochujang. *Korean Journal of Food Science & Technology*, Vol.28, No.1, pp. 152-156 ISSN 0367-6293

Shin, D. (2008). Fermented food and Korean dietary culture, Proceedings of Korean Food Science Society, ISSN 0367-6293, Jeju National University, June, Korea

Shin, J.; Ahn, C.; Nam. H.; Lee, H. & Moon, T. (1995). Fractionation of angiotensin converting enzyme(ACE) inhibitory peptide from soybean paste. *Korean Journal of Food Science & Technology*, Vol.27, No.2, pp. 230-234 ISSN 0367-6293

Snyder[a] , H. & Kwon, T. (1987) *Soybean utilization*. 1-3, Van Nostrand Reinhold Company, ISBN 0442282168, Newyork, USA

Snyder[b] , H. & Kwon, T. (1987) *Soybean utilization*. 31-70, Van Nostrand Reinhold Company, ISBN 0442282168, Newyork, USA

Song, H.; Kim, Y. & Lee, K. (2008). Antioxidant and anti cancer activities of traditional Kochujang added with garlic porridge. *Journal of Life Science*, Vol.18, No.8, pp. 1140-1146 ISSN 1225-9918

Song, H. (2005*). Soybean*, In: Processing Characteristics of Soybean. 313-312, Korea University Press, ISBN 89-764-156-20, Seoul, Korea

Yang, C. & Kim, J. (1977). Asparagine biosynthesis in soybean sprouts. *Journal of Applicd Biological Chemistry*, Vol.20, No.1, pp 33-42 ISSN 1229-2737

Yang, J.; Lee, S. & Song, Y. (2003). Improving effect of powders of cooked soybean and Cheonggukjang on blood pressure and lipid metabolism in spontaneously hypertensive rats. *Journal of the Korean Society Food Science & Nutrition*, Vol.32, No.6, pp. 899-905 ISSN 1226-3311

Yi, S.; Yang, J.; Jeong, J.; Sung, C. & Oh, M. (1999). Antimicrobial activities of soybean paste extracts. *Journal of the Korean Society Food Science & Nutrition*, Vol.28, No.6, pp. 1230-1238 ISSN 1226-3311

Soybean Meal Quality and Analytical Techniques

W. A. Dozier, III and J. B. Hess
Auburn University, Auburn, AL
USA

1. Introduction

Soybean meal is considered the "gold standard" among intact protein sources used in the feed industry (Cromwell, 1999). It has an excellent amino acid profile that complements cereal grains in diet formulation, as methionine is typically the only limiting amino acid for poultry. Soybean meal is characterized as either from dehulled beans or beans having hulls (NRC, 1994). Dehulled soybean meal has a higher composition of crude protein, amino acids and metabolizable energy than soybean meal produced from soybeans having hulls (NRC, 1994); Soybean meal is known to vary in amino acid composition among samples. Geographical location of soybean production, soybean variety, and processing methods are factors known to influence variability of crude protein and amino acid composition of soybean meal (Parsons et al., 1991, 2000; de Coca-Sinova, 2008, 2010; Baker et al., 2011). de Coca-Sinova (2008) evaluated amino acid composition of soybean meal samples obtained from Argentina, Brazil, Spain, and the United States. Crude protein content varied from 45.2 to 50.6% with lysine expressed as a percent of crude protein ranging from 5.51 to 6.26%. Samples from Spain had the highest crude protein content, whereas lysine expressed as a percentage of crude protein was the highest for samples obtained in the United States.

Moreover, soybean varieties are being selected to contain higher amino acid concentrations than conventional soybean varieties resulting in soybean meals having more balanced amino acid content for swine and poultry diets (Baker and Stein, 2009; Baker et al., 2011). Baker et al. (2011) reported high protein soybean meal having a crude protein and lysine composition of 54.86 and 3.56% compared with conventional soybean meal containing crude protein and lysine contents of 47.47 and 3.14%. Soybean meal is known to vary in crude protein and amino acid content among soybean production years and using current amino acid data bases of soybean meal composition are important to avoid variability in diet formulation with swine and poultry (Table 1).

Amino acids originating from intact protein sources are not digested and absorbed with 100% efficiency. Formulating diets on a digestible amino acid basis is increasing around the globe and this formulation strategy allows for the use of lower cost feed ingredients that may contain amino acids that are less available to the animal while minimizing nitrogen excretion. Digestible amino acid composition is calculated by multiplying a digestible coefficient by amino acid total composition. Digestible coefficient is the digestibility percentage of an amino acid in a specific feed ingredient or a complete diet. In poultry,

amino acid digestibility coefficients for feed ingredients are typically determined using a true digestibility assay with cecectomized roosters (Parsons, 1986) or standardized amino acid assay using broilers (Lemme et al., 2004). Amino acid digestibility coefficients have been reported to be higher with cecectomized roosters compared with using broilers (Garcia et al., 2007; Adedokun et al., 2007). Amino acid digestibility assays are highly variable and a large number of assays are needed for specific feedstuffs to generate accurate digestibility coefficients. Amino acid digestibility coefficients for soybean meal have been found to range from 82 to 93% (Table 2).

	Sriperm et al., 2011[1]	Evonik[2]	Novus[3]
Lysine	3.23±0.11	2.95±0.07	2.96±0.15
Methionine	0.77±0.04	0.64±0.01	0.64±0.04
Cysteine	0.69±0.04	0.70±0.02	0.64±0.05
Arginine	3.73±0.19	3.48±0.11	3.41±0.17
Tryptophan	0.68±0.68	0.64±0.01	0.66±0.04
Isoleucine	2.31±0.09	2.15±0.06	2.20±0.13
Leucine	3.90±0.15	3.61±0.09	3.63±0.17
Valine	2.41±0.09	2.25±0.05	2.33±0.13
Histidine	1.35±0.07	1.26±0.03	1.25±0.06
Phenylalanine	2.65±0.02	2.40±0.06	2.37±0.12
Sample size	225	457	75

[1]Values are expressed on a dry matter basis as average ± SD and were determined from samples analyzed in 2009 at Ajinomoto Heartland LLC's amino acid laboratory.
[2]Values are expressed on a "as-is basis" as average ± SD and were determined from samples analyzed in 2010 at Evonik's amino acid laboratory.
[3]Values are expressed on a "as-is basis" as average ± SD and were determined from samples analyzed in 2010 at Novus International Inc., amino acid laboratory.

Table 1. Essential amino acid composition (%) of soybean meal obtained from various regions of the United States

	True Digestibility[1]	Standardized Assay[2]
Lysine	91±2.8	90
Methionine	92±2.5	91
Cysteine	84±5.1	82
Threonine	88±3.4	85
Arginine	93±2.8	93
Tryptophan	89±4.8	89
Isoleucine	92±3.3	89
Leucine	92±2.2	89
Valine	91±2.5	88
Histidine	90±6.5	92
Phenylalanine	92±3.7	88
Sample size	88	37

[1]Values are expressed as average ± SD of 88 samples from Ajinomoto Heartland.
[2]Values are expressed as average of 35 samples from Evonik.

Table 2. Digestible amino acid coefficients (%) of soybean meal

2. Soybean meal in poultry and swine feeds

Soybean meal is the most commonly-used source of protein for poultry and swine feeds in the world, with 67% of the animal feed market (Pettigrew et al., 2002). In order for a feed ingredient to be considered an important component of an industry feeding program, it must have several fundamental qualities. First, it must provide one or more important nutrients. Second, it must be available in amounts that allow it to be used regularly and on a large scale. Third, it must be cost effective to use. Soybean meal abundantly fits into this category as a high-protein product with good amino acid balance that is highly digestible. It is available in large quantities year round and has had most of the associated antinutritional compounds inactivated. Interestingly, antinutritional factors in soybeans are relatively easy to inactivate and are reduced substantially by normal soybean processing. This is in contrast to many of the other commonly-used plant proteins that have non-labile antinutritional factors (Pettigrew et al., 2002).

In the early years of compound feed production, grain products were paired with animal protein meals that provided a natural balance of vitamins and minerals in addition to protein. As animal protein products such as fishmeal became more expensive, and synthetic sources of vitamins (particularly vitamin B_{12}) were developed, soybean meal captured a larger portion of the animal feed protein market. Modern feed formulation programs further increased the demand for soybean meal as the principle protein source as least cost diet formulation became more common.

Worldwide, nearly 2/3 of the protein sources used in animal feeds come from soybean meal, with canola meal, cottonseed meal and sunflower meal providing additional plant protein sources. In the United States, plant protein source usage in animal feeds is primarily (92%) soybean meal. Over half of the soybean meal produced in the United States is fed to poultry (Waldroup and Smith, 1999). Approximately 66% of protein in broiler feeds comes from soybean meal. With the development of reasonably-priced synthetic methionine sources, feed manufacturers are now able to produce relatively simple feeds based on a combination of corn and soybean meal with supplementation of minerals, vitamins and methionine. Swine account for 27% of the soybean meal used in animal feeds in the United States. Soy protein's digestibility, combined with a relative abundance of lysine, which is the first limiting amino acid in swine feeds, make soybean meal an excellent protein source for swine.

Most areas of swine and poultry production have economical access to soybean meal for compounding animal feeds. In some places, however, local access to soybeans has led to interest in the processing of full fat soybeans meals for local usage. Full fat soybean meal, often an extruded product, has the advantage of higher energy values due to the full complement of oil in the native seeds as compared to commercial soybean meal, which has had most of the oil extracted for sale (Reese and Bitney, 2000). Other advantages include: 1) the addition of fat to a feed in a more easily-handled granular form and 2) the addition of fat to a feed in a form that is less likely to reduce pellet quality (Waldroup, 1985).

Performance results indicated that there was significant variation in the nutrient content from various batches of extruded soybean meals (Reese and Bitney, 2000). The authors concluded that it would be difficult to compare extruded soybean meal to regularly-processed soybean meal for this reason. It would be wise if considering these products to do extra nutrient analysis. Numerous research groups have explored the use of full fat soybean meals in poultry feeds as well (Waldroup, 1985). Extruded full fat soybean meals have seen limited use, although dry roasting, followed by grinding, has also been tested. Waldroup

and Cotton (1974) determined the levels of full fat soybean meal that could be included in mash broiler feeds before performance suffered (less than 25%). Higher levels could be utilized in pelleted broiler feeds because the pelleting process causes more cell wall disruption and increases the digestibility of full fat soybean meal products (Waldroup and Cotton, 1974).

Soybean geneticists are continually improving productivity characteristics of soybeans for crop production. Additionally, efforts have been underway for some time to enhance the quality of soybeans in relation to animal feeding of soybean meal (Bajjalieh, 2002). Areas of interest include increasing levels of sulfur containing amino acids, increasing the proportion of soybean meal phosphorus that is available for digestion (reducing phytate-bound phosphorus) and increasing energy availability through selection away from carbohydrate fractions of low availability to monogastrics.

3. Protein digestion

Dietary protein consists of complex polypeptides, which must be cleaved into dipeptides and amino acids to facilitate absorption. In poultry, the crop, proventriculus, gizzard, pancreas, and small intestine have an active role in protein digestion (Moran, 1982). Proteolysis is the first stage of digestion and it occurs in the proventriculus and gizzard (Hill, 1971). The contents found in the proventriculus and gizzard have a pH of 1.80 and 2.50, respectively, which is relatively lower than the crop, small intestine, cecum, and cloaca (Figure 1). This low pH is central to gastric digestion. The Proventriculus is the site for pepsin and HCl production and contains gastric glands located in the mucosa (Toner, 1963). At low pH, protein denaturation occurs through unfolding of proteins and cleavage of peptide bonds by pepsin, which is an endopeptidase.
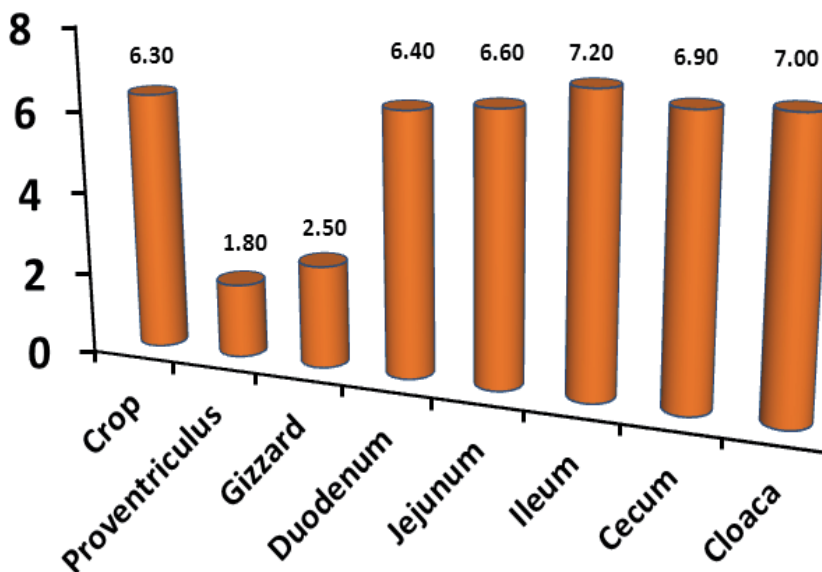
Fig. 1. pH of the contents in the digestive tract of poultry (Herpol and Van Grembergen, 1967)

One of the functions of the pancreas is to supply digestive enzymes for protein digestion (Brody, 1994). Trypsin, chymotrypsin A, chymotrypsin B, proelastase, and carboxypeptidase are produced by the pancreas and these enzymes are endopeptidases with the exception of carboxypeptidase (Brody, 1994). Pancreatic enzymes play a central role in protein digestion in the small intestine by breaking down polypeptides into oligopeptides (Alpers, 1994; Lowe, 1994). Approximately 13 peptidases are present in the brush border membrane or the cytoplasm of the small intestine that breakdown oligopeptides into dipeptides and amino acids (Alpers, 1994). The resulting dipeptides and amino acids are absorbed in the small intestine for the synthesis of body proteins.

Soybean meal that has been underprocessed contains trypsin inhibitors, which are antinutritional factors. These proteins bind to trypsinogen and chymotrypsinogen preventing the conversion into their active forms limiting protein digestion. A detailed description of trypsin inhibitors will be discussed in the following section.

4. Trypsin inhibtor in soybean meal and protein digestion

Growth depression effects due to antinutritional factors present in soybeans have been well-documented for more than half a century (Ham et al., 1945; Chernick et al., 1948; Liener, 1953; Lyman and Lepkovsky, 1957; Gestetner et al., 1966). Trypsin inhibitor is the primary antinutritional factor in soybean meal (Araba and Dale, 1990a,b; Anderson-Hafermann et al., 1992; Mian and Garlich et al., 1995), which is a globulin-type protein having a molecular weight of 24,000 and isoelectric point of 4.5 (Kunitz, 1945). Trypsin inhibitor inhibits the conversion of zymogens to active proteases of trypsin and chymotrypsin. The mechanism of action differs for trypsin and chymotrypsin (Kunitz, 1947). Trypsin inhibitor binds with trypsinogen to form an irreversible compound preventing the formation of an active protease. Conversely, trypsin inhibitor action of chymotrypsin is less pronounced forming a reversible dissociated compound (Northrop, 1922).

In addition to its detrimental effects on proteolytic action, trypsin inhibitor dramatically affects the size of the pancreas and amount of trypsinogen produced. Chernick et al. (1948) reported that pancreas weight as a percent of body weight was increased by 56% and had 43% higher trypsinogen content per gram of pancreas nitrogen content with chicks fed diets containing raw soybean meal compared with diets containing heat-treated soybean meal. Moreover, Lyman and Lepkovsky (1957) reported low trypsin content in the small intestine of rats immediately after feeding a diet containing raw soybean meal, but increased 3 fold the normal concentration 6 hours postfeeding. This provides evidence the pancreas produced trypsinogen in excess to compensate for the trypsin inhibitor. Hence, the justification for the trypsin content observed several hours after feeding. The inhibitory action is reduced by subjecting soybeans or soybean meal to heat by deactivating anti-nutritional toxins (Hayward et al., 1936; Kunitz, 1947). Broiler growth has been shown to be increased by approximately 140 to 150% with autoclaving raw hexane-extracted soybeans or soybean meal compared with chicks fed diets containing raw hexane-extracted soybeans or soybean meal not subjected to heat (Araba and Dale, 1990b; Anderson-Hafermann, 1992). If adequate heat is not applied during soybean processing, soybean meal will be produced containing active toxins compromising its nutritional value.

5. Overheating of soybean meal

Overheating of soybean meal reduces its nutritional value for poultry (Renner et al., 1953; Warnick and Anderson, 1968; Araba and Dale, 1990a). It has been shown that overcooking

of soybean meal decreases digestibility of amino acids (Lee and Garlich, 1992; Parsons et al., 1992). The explanation for the decreased amino acid digestibility and reduced growth responses appear to be related to the Maillard reaction with cross-linking involved to a lesser extent. Parsons et al. (1992) examined the effects of overprocessing dehulled, solvent-extracted soybean meal by autoclaving at 121°C and 105 kPa for 0, 20, 40, and 60 min. Increasing the time of autoclaving reduced total concentration of lysine, arginine and cysteine, but other amino acids were not influenced by overprocessing. The largest decrease in true amino acid digestibility occurred with lysine, cystine, histidine, and aspartic acid, whereas digestibility of threonine, serine, alanine, and leucine was decreased to a lesser extent. Moreover, a growth assay using broiler chicks determined that autoclaving at 121°C for 40 min reduced lysine bioavailability by 15% compared with birds fed soybean meal not subjected to autoclaving. The destruction of lysine and arginine content of soybean meal and reduced lysine digestibility due to autoclaving indicates the presence of the Maillard reaction. In addition to chemical composition, color differences are apparent with soybean meal subjected to overprocessing indicating a browning during the latter stage of Maillard reaction (Figure 2).

Maillard reaction is a series of complex reactions occurring when feed ingredients, food, and animal tissues are subjected to overprocessing (Iqbal et al., 1999; Fayle and Gerrard, 2002). The series of reactions involve early, advanced, and final stages (Mauron, 1981). In the early reactions, amino groups react with aldehyde groups of free sugars producing a schiff base, which cyclizes to form a glycosylamine (Mauron, 1981; Dillis, 1993). The glycosylamine undergoes a rearrangement to form either Amadori products (1-amino-1-deoxy-2-ketose) if produced from glucose or Heyns products if derived from fructose. In this series of reactions, ε-amino group of lysine is affected the most and ε-amino groups located at the terminal end of proteins are also involved but to a lesser extent. With lysine, an aldose is changed to a ketose creating a fructosyl-lysine. In the advanced reactions, Amadori or Heyns products are decomposed to form deoxydicarbonyl sugars and these resulting sugar derivatives can react with other amino acids producing aldehydes, ketones, and/or deoxydicarbonyl compounds (Dillis, 1993). Heterocylic compounds (pyrazines, pyrroles, pyridines, and thiazoles) are formed during the latter stages of these reactions, which are known to provide aromas and flavor to food (Mauron, 1981; Dillis, 1993). In the final reactions, food or feed ingredients are characterized by exhibiting a dark color associated with brown melanoidin pigments produced by this set of reactions, hence the name of browning well known for the Maillard reaction (Hurrell and Carpenter, 1981). Proteins are modified through cross-linking reactions as deoxydicarbonyl sugars or carbonyl compounds react with amino acids (Mauron, 1981; Dillis, 1993).

Poor digestibility of intact protein sources subjected to overprocessing (Maillard reaction) may be due to the formation of Amadori or Heyns products, reduced absorption of lysine, and the formation of cross-links (Mauron, 1981; Sherr et al., 1989; Dillis, 1993). Sherr et al. (1989) determined that, in the presence of Maillard products derived from lysine (glycosylated lysine derivatives), absorption of lysine was inhibited. The glycosylated lysine derivatives compete with lysine for absorption carriers, but the majority of these derivatives have poor utilization with excretion being 72 and 96% of the amounts absorbed. The cross-links are not very digestible as endogenous proteases are not able to cleave this complex during digestion resulting in poor utilization to the animal. Soybean meal contains sugar complexes in the form of raffinose and stachyose and overprocessing may contribute to Maillard reactions (Hancock et al., 1990). Cysteine content has been shown to be reduced in

soybean meal with overprocessing (Parsons et al., 1992). Cysteine is not thought to be involved with Maillard reactions, but rather forming lanthionine during overprocessing (Miller et al., 1965; Hurrell et al., 1976). With the formation of lanthionine, cysteine would probably be expected to decrease when soybean meal is subjected to overprocessing.

Fig. 2. Soybean meal samples exposed to varying temperatures with samples on the bottom row subjected to excessive heating as noted by the darker color (Courtesy of Dr. N. Dale, Department of Poultry Science, University of Georgia).

6. Analytical assays to estimate soybean meal quality

Based on the popularity of soybean meal as a protein source in poultry and swine feeds, it is not surprising that quite a lot of time and effort are expended in measuring soybean meal protein quality. Over the years, a number of techniques have been examined to measure the protein quality of plant protein products. Those most used in practice have changed as research-based comparisons of the various techniques have shed light into the relative merits of each. Currently, the analytical technique most commonly used to measure soybean meal quality is protein solubility, perhaps combined with the urease test. Protein solubility has been a tool to test soybean meal solubility for many decades (Smith and Circle, 1938, Lund and Sandstrom, 1943). These early attempts examined protein solubility in water. Later, a range of acid and alkaline chemicals were compared for their utility in measuring soybean meal protein solubility. More recently, Araba and Dale (1990a) and Parsons et al. (1991) examined the use of a 0.2% potassium hydroxide (**KOH**) solution. Protein (nitrogen) concentration is then quantified using the kjeldahl method. In general, KOH solubility decreases as the degree of heat treatment associated with soybean processing increases. While raw soybean products would be 100% soluble, they obviously have a full complement of antinutritional factors that have not been deactivated. Research comparing protein solubility to other measures of protein quality indicate that KOH solubilities between 78 to 84% are optimal for animal performance. Values ranging from 84 to 89% are slightly under-processed and may be acceptable for older animals, while values under 74% are over-processed and will have reduced lysine digestibility. Araba and Dale (1990b) compared protein solubility to Orange G binding and trypsin inhibitor activity. They found that protein solubility compared favorably to measurements of broiler growth and trypsin inhibitor activity while the Orange G binding technique was not sensitive to processing

changes in autoclaved soybean meals (Figure 3). The combined works of Araba and Dale (1990a,b) concluded that the KOH solubility test is useful for detecting both over-processed and under-processed soybean meals.

The urease test has been used for some time as a measure of soybean meal processing. Urease is an enzyme in soybean meal that is of little interest in animal nutrition. It is, however, easier to measure than many of the antinutritional factors of interest. Because trypsin inhibitors and lectins are denatured by heat processing of soybeans at a similar rate to the urease enzyme, testing for urease is a useful marker for degree of soybean meal underprocessing (Caskey and Knapp, 1944; Wright, 1981). Unfortunately, the urease test does not do an adequate job of measuring overprocessed meals. Over time, meals ranging from 0.05 to 0.15 change in pH were considered properly processed for poultry. Recently, meals higher than a 0.15 pH change have been deemed usable by older chickens. Also, changes in soybean processing methods have raised questions regarding the lower range of this test (i.e. levels under 0.05 pH may not cause problems).
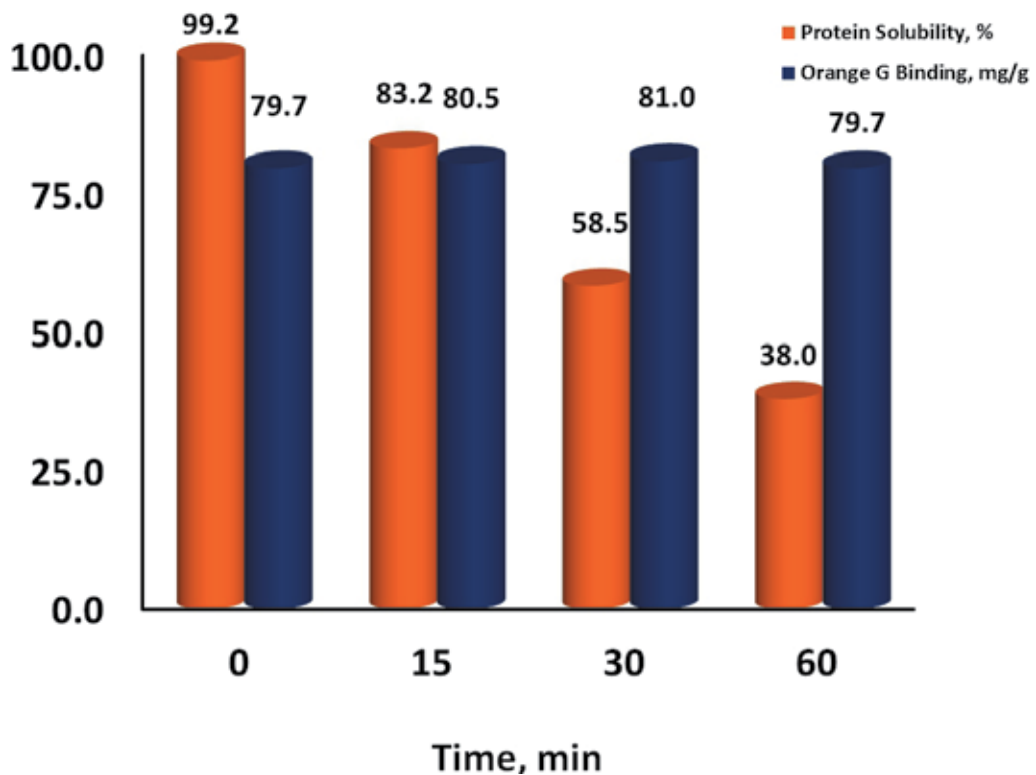
Fig. 3. Effects on protein solubility and Orange G binding of overprocessed soybean meal (Araba and Dale, 1990b).

Despite the ease of measuring the urease enzyme as opposed to more complicated assays, it is possible to routinely measure trypsin inhibitors in soybean meals. Directly measuring trypsin inhibitors in soybean meals is obviously a desirable assay and trypsin inhibitors are one of the major antinutritional factors of note. Kakade et al. (1974) described the most commonly-used method for determining trypsin inhibitors in soybean products for animal

feeds. Work by McNaughton et al. (1981) indicated that direct measurement of trypsin inhibitor levels was an accurate indicator of animal performance for undercooked soybean products. For practical applications, the easier-to-complete urease test still predominates as a marker for under-processed soybean meals.

The use of Orange G dye to determine the amount of heat processing a soybean meal sample has been subjected to is based on the dye's ability to bind the free ε-amino group of lysine under acidic conditions. As lysine progressively becomes less available during extended heat processing, less of the Orange G dye can bind. Moran et al. (1963) correlated Orange G dye binding with broiler chick growth and found agreement across a range of heat treatments (autoclaving in this case). Araba and Dale (1990b) found protein solubility more sensitive to soybean meal processing variation than the Orange G binding technique.

There are other dye binding tests that have been suggested as methods to monitor soybean meal quality, including the cresol red test (Olomucki and Bornstein, 1960; Vorha and Kratzer, 1991) and coomassie blue staining (Vorha and Kratzer, 1991). A coomassie blue dye solution can be used to titrate protein solubility after KOH treatment in place of the kjeldahl protein test (Kratzer et al., 1990). The optical density of the stained proteins is then measured against a set of lysozyme standards at 595 nm. Coomassie blue staining may be more accurate than the kjeldahl procedure at measuring protein solubility because coomassie blue binds with intact proteins and not free amino acids (Vorha and Kratzer, 1991), also, the coomassie blue dye test would be faster in producing results than using the Kjeldahl portion of the KOH solubility test. Because this is, in essence, a KOH solubility test, it is particularly useful in detecting overprocessed soybean meals.

Protein dispersibility index refers to the amount of soybean meal protein dispersed in water after blending a soybean meal sample in water with a high speed blender. Research by Batal et al. (2000) correlated chick growth with several methods of soybean meal quality assessment in meals that had been heat treated. Their results indicated that protein dispersibility index was a sensitive measure of soybean meal quality and gave better results than either the urease or protein solubility assays. Protein dispersibility indexes of 40 to 45% indicate a soybean meal that is neither over- or under-processed. These authors suggested that the protein dispersibility index will give an accurate picture of soybean processing if paired with another test such as the urease test. A number of other tests have been proposed to measure soybean meal quality, including formaldehyde titration (Almquist and Maurer, 1953) and a fluorescence test (Hsu et al., 1949).

In conclusion, nutritional quality of soybean meal is of utmost importance to optimize the rate and efficiency of growth of poultry. It is necessary for ingredient quality control programs to understand the appropriate assays to determine if soybean meal has been subjected to under- or over-processing (Table 3). Protein solubility assay is easily conducted and provides more reproducible results than trypsin inhibitor activity assay. A value greater than 85% denotes underprocessing, whereas a protein solubility index less than 74% infers overheating. Protein dispersibility index is also a useful tool to measure protein quality with values ranging from 40 to 45% denoting acceptable quality. Conversely, urease activity is useful only for detecting underprocessing because its activity falls to zero as soybean meal has been exposed to overprocessing. Moreover, Orange G binding capacity exhibits small change with soybean meal subjected to overprocessing, hence this assay may not be appropriate to detect overheated soybean meal.

Technique	Underprocessing	Overprocessing	Comments
KOH Solubility	Acceptable Assay	Acceptable Assay	Commonly used
Orange G Binding	Not useful	Very little change	Low sensitivity
Trypsin Inhibitor	Acceptable Assay	Not useful	Complicated, time consuming, and expensive
Urease	Acceptable Assay	Activity falls to zero	Commonly used
Protein dispersibility	Acceptable Assay	Acceptable Assay	Has potential but is not commonly used

Table 3. Comparison of analytical techniques for under- and over-processed soybean meal

7. References

Adedokun, S. A., C. M. Parsons, M. S., Lilburn, O. Adeola, and T. J. Applegate. 2007. Comparison of ileal endogenous amino acid flows in broiler chicks and turkey poults. Poult. Sci. 86:1682-1689.

Alpers, D. H. 1994. Digestion and absorption of carbohydrates and proteins. Pages 1723-1749 in Physiology of the Gastrointestinal Tract. L. R. Johnson, D. H. Alpers, J. Christensen, E. D. Jacobson, and J. H. Walsh, ed. Raven Press, New York, NY.

Almquist, H.J., and S. Maurer. 1953. A method for study of the effects of heat on nutritive value of soybean oil meal. Poult. Sci. 32:549-550.

Anderson-Hafermann, J. C., Y. Zhang, and C. M. Parsons. 1992. Effect of heating on nutritional quality of conventional and kunitz trypsin inhibitor-free soybeans. Poult. Sci. 71:1700-1709.

Araba, M., and N. M. Dale. 1990a. Evaluation of KOH solubility as an indicator of overprocessing soybean meal. Poult. Sci. 69:76-83.

Araba, M., and N. M. Dale. 1990b. Evaluation of protein solubility as an indicator of underprocessing of soybean meal. Poult. Sci. 69:76-83.

Bajjaleih, N. 2002. The United Soybean board's Better Bean Initiative and the Poultry Industry. The Soybean Meal Information Center, United Soybean Board. www.soymeal.org Accessed on March 15, 2011.

Baker, K. M., and H. H. Stein. 2009. Amino acid digestibility and concentration of digestible and metabolizable energy in soybean meal produced from high protein or low oligosaccharide varieties of soybeans and fed to growing pigs. J. Anim. Sci. 87:2282–2290.

Baker, K. M., P. L. Utterback, C. M. Parsons, and H. H. Stein. 2011. Nutritional value of soybean meal produced from conventional, high-protein, or low-oligosaccharide varieties of soybeans and fed to broiler chicks. Poult. Sci. 90:390-395.

Batal, A. B., M. W. Douglas, A. E. Engram, and C. M. Parsons. 2000. Protein Dispersibility index as an indicator of adequately processed soybean meal. Poult. Sci. 79:1592-1596.

Brody, T. 1994. Digestion and Absorption. Pages 41-106 in Nutritional Biochemistry. Academic Press, San Diego, California.

Caskey, C. D., and F. Knapp. 1944. Method for determining inadequately heated soybean meal. Ind. Eng. Chem. (Anal. Ed.) 16:640-641.

Chernick, S. S., S. Lepkovsky, and I. L. Chikoff. 1948. A dietary factor regulating the enzyme content of the pancreas: Changes induced in size and proteolytic activity of the chick pancreas by the ingestion of raw soybean meal. Am. J. Physiol. 155:33-41.

Cromwell, G. L. 1999. Soybean Meal – The "Gold Standard". The Farmer's Pride, KPPA News, Vol. 11, no. 20.

de Coca-Sinova, D. G. Valencia, E. Jiménez-Moreno, R. Lázaro, and G. G. Mateos. 2008. Apparent illeal digestibility of energy, nitrogen, and amino acids of soybean meals of different origin in broilers. Poult. Sci. 87:2613-2623.

deCoca-Sinova, E. Jiménez-Moreno, J. M. González-Alvarado, M. Frikha, R. Lázaro, and G. G. Mateos. 2010. Influence of source of soybean meal and lysine content of the diet on performance and total tract apparent retention of nutrients in broilers from 1 to 36 days of age. Poult. Sci. 89:1440-1450.

Dillis, W. L., Jr. 1993. Protein fructosylation: fructose and the Maillard reaction. Am. J. Clin. Nutr. 58(suppl):779S-97.

Fayle, S. E., and J. A. Gerrard. 2002. What is the Maillard reaction? Pages 1-8 in The Maillard Reaction. P. S. Belton, ed. The Royal Society of Chemistry, Cambridge, England.

Garcia, A. R., A. B. Batal, and N. M. Dale. 2007. A comparsion of methods to determine amino acid digestibility of feed ingredients for chickens. Poult. Sci. 86:94-101.

Gestetner, B., Y. Birk, A. Bondi, and Y. Tencer. 1966. Soybeans saponins. VII. A method for the determination of sapogenin and saponin contents in soybeans. Phytochemistry 5:803-806.

Ham, W. E., R. M. Sandstedt, and F. E. Mussehl. 1945. The proteolytic inhibiting substance in the extract from unheated soy bean meal and its effect upon growth in chicks. J. Biol. Chem. 161: 635-642.

Hancock, J. D., E. R. Peo, Jr., A. J. Lewis, and J. D. Crenshaw. 1990. Effects of ethanol extraction and duration of heat treatment of soybean flakes on the utilization of soybean protein by growing rats and pigs. J. Anim. Sci. 68:3233-3243.

Hayward, J. W., H. Steenbock, and G. Bohstedt. 1936. The effect of heat as used in the extraction of soy bean oil upon the nutritive value of the protein of soy bean oil meal. J. Nutr. 11:219-234.

Herpol, C. and G. Van. Grembergen. 1967. La significance du pH dans le tube digestif de gallus domesticus. Ann. Biol. Anim. Biochem. Biophys. 7:33-38.

Hill, K. J. 1971. The physiology of digestion. In Physiology and Biochemistry of the Domestic Fowl. Vol. I. Ed. D. J. Bell and B. M. Freeman. Academic press, London.

Hurrell, R. F., K. J. Carpenter, W. J. Sinclair, M. S. Otterburn, and R. S. Asquith. 1976. Mechanisms of heat damage in proteins 7. The significance of lysine-containing isopeptides and of lanthionine in heated proteins. Br. J. Nutr. 35:383-395.

Hurrell, R. F., and K. J. Carpenter. 1981. The estimation of available lysine in foodstuffs after maillard reactions. Progr. Food Nutr. Sci. 5:159-176.

Hsu, P.T., W. D. Graham, J. S. Carver, and J. McGinnis. 1949. Correlation of nutritive value for chicks of autoclaved soybean meal with fluorescence, browning and sticky droppings. Poult. Sci. 28:780.

Iqbal, M., L. L. Probert, N. H. Al-Humadi, and H. Klandorf. 1999. Protein glycosylation and advanced glycosylation end products (AGEs): An avian solution. J. Gerontol. Bio. Sci. 54:B171-B176.

Kakade, M. L., J. J. Rackis, J. J. McGee, and G. Puski. 1974. Determination of trypsin inhibitor activity in soy products: A collaborative analysis of an improved procedure. Cereal Chem. 51:376-382.

Kratzer, F. H., S. Bersch, and P. Vorha. 1990. Evaluation of heat damage to protein by Coomassie Blue G dye-binding. J. Food Sci. 55:805-807.

Kunitz, M. 1945. Crystallization of a trypsin inhibitor from soybean. Science 101:668-669.

Kunitz, M. 1947. Crystallization of a trypsin inhibitor. II. General properties. J. Gen. Physiol. 30:291-310.

Lee, H., and J. D. Garlich. 1992. Effect of overcooked soybean meal on chicken performance and amino acid availability. Poult. Sci. 71:499-508.

Lemme, A., V. Ravindran, and W. L. Bryden. 2004. Ileal digestibility of amino acid in feed ingredients for broilers. World's Poult. Sci. J. 60:423-427.

Liener, I. E. 1953. Soyin, a toxic protein from the soybean. I. Inhibition of rat growth. J. Nutr. 49:527-539.

Lowe, M. E. 1994. The structure and function of pancreatic enzymes. Pages 1531-1541 in Physiology of the Gastrointestinal Tract. L. R. Johnson, D. H. Alpers, J. Christensen, E. D. Jacobson, and J. H. Walsh, ed. Raven Press, New York, NY.

Lund, A. P., and W. M. Sandstrum. 1943. The proteins of various tree seeds. J. Agric. Res. 66:349-356.

Lyman, R. L., and S. Lepkovsky. 1957. The effect of raw soybean meal and trypsin inhibitor diets on pancreatic enzyme secretion in the rat. J. Nutr. 62:269-284.

Mauron, J. 1981. The maillard reaction in food; A critical review from the nutritional standpoint. Progr. Food Nutr. Sci. 5:5-35.

McNaughton, J. L., F. N. Reece, and J. W. Deaton. 1981. Relationship between color, trypsin inhibitor contents and urease index of soybean meal and effects on broiler performance. Poult. Sci. 60:393-400.

Mian, M. A., and J. D. Garlich. 1995. Tolerance of turkeys to diets high in trypsin inhibitor activity from undertoasted soybean meals. Poult. Sci. 74:1126-1133.

Miller, E. L., A. W. Hartley, and D. C. Thomas. 1965. Availability of sulphur amino acids in protein foods. 4. Effect of heat treatment upon the total amino acid content of cod muscle. Br. J. Nutr. 19:565-573.

Moran, E. T., L.S. Jensen, and J. McGinnis. 1963. Dye binding by soybean and fish meal as an index of quality. J. Nutr. 79:239-244.

Moran, E. T., Jr. 1982. Gastric Digestion. Pages 48-86 in Comparative Nutrition of Fowl and Swine The Gastrointestinal Systems. University of Guelph, Guelph, Ontario N1G 2W1, Canada.

NRC. 1994. Nutrient Requirements of Poultry. 9th Rev. Edition. Natl. Acad. Press, Washington, D.C.

Northrop, J. H. 1922. The mechanism of influence of acids and alkalies on the digestion of proteins by pepsin or trypsin. J. Gen. Physiol. 5:263-274.

Olomucki, E., and S. Bornstein. 1960. The dye absorption test for the evaluation of soybean meal quality. J. AOAC. 43:440-441.

Parsons, C. M., K. Hashimoto, K. J. Wedekind, and D. H. Baker. 1991. Soybean KOH solubility in potassium hydroxide: An in vitro test of in vivo protein quality. J. Anim. Sci. 69:2918-2924.

Parsons, C. M., K. Hashimoto, K. J. Wedekind, Y. Han, and D. H. Baker. 1992. Effect of overprocessing on availability of amino acids and energy in soybean meal. Poult. Sci. 71:133-140.

Parsons, C. M., Y. Zhang, and M. Araba. 2000. Nutritional evaluation of soybean meals varying in oligosaccharide content. Poult. Sci. 79:1127-1131.

Parsons, C. M.1986. Determination of digestible and available amino acids in meat meal using conventional and cecectomized cockerels or chick growth assays. Br. J. Nutr. 56:227-240.

Pettigrew, J. E., K. T. Soltwedel, J. C. Miguel, and M. F. Palacios. Soybean Use – Swine. Soybean Meal Information Center Fact Sheet. 2002. The Soybean Meal Information Center, United Soybean Board. www.soymeal.org Accessed on March 15, 2011.

Reese, D., and L. L. Bitney. 2000. Extruded-expelled soybean meal for pigs. Nebraska Swine Reports. University of Nebraska – Lincoln, NE, Pages 17-19.

Renner, R., D. R. Clandinin, and A. R. Robblee. 1953. Action of moisture on damage done during over-heating of soybean oil meal. Poult. Sci. 32:582-585.

Sherr, B., C. M. Lee, and C. Jelesciewicz. 1989. Absorption and metabolism of lysine maillard products in relation to utilization of L-lysine. J. Agric. Food Chem. 37:119-122.

Smith, A. K., and S. J. Cirlce. 1938. Peptization of soybean proteins. Ind. Eng. Chem. 30:1414-1418.

Sriperm, N., G. M. Pesti, and P. B. Tillman. 2011. Evaluation of the fixed nitrogen-to-protein (N:P) conversion factor (6.25) versus ingredient specific N:P conversion factors in feedstuffs. J. Sci. Food Agric. n/a. doi: 10.1002/jsfa.4292

Toner, P. G. 1963. The fine structure of resting and active cells in the submucosal glands in fowl proventriculus. J. Anat. 97:575-583.

Vorha, P., and F. H. Kratzer. 1991. Evaluation of soybean meal determines adequacy of heat treatment. Feedstuffs Vol 63 (8):22-28.

Waldroup, P. W. 1985. Whole soybeans for poultry feeds. Animal Nutrition Research Highlights – Special Addition. The Soybean Meal Information Center, United Soybean Board. www.soymeal.org Accessed on March 15, 2011.

Waldroup, P. W., and T. L. Cotton. 1974. Maximum usage levels of cooked, full-fat soybeans in all-mash broiler diets. Poult. Sci. 53:677-680.

Waldroup, P. W., and K. Smith. 1999. Soybean Use – Poultry. Soybean Meal Information Center Fact Sheet. The Soybean Meal Information Center, United Soybean Board. www.soymeal.org Accessed on March 15, 2011.

Warnick, R. E., and J. O. Anderson. 1968. Limiting essential amino acids in soybean meal for growing chickens and the effects of heat upon availability of the essential amino acids. Poult. Sci. 47:281-287.

Wright, K. N. 1981. Soybean meal processing and quality control. J. Am. Oil Chem. Soc. 58:296-300.

Advances in Soybean and Soybean By-Products in Monogastric Nutrition and Health

Samuel N. Nahashon[1] and Agnes K. Kilonzo-Nthenge[2]
[1]Department of Agricultural Sciences
[2]Department of Family and Consumer Sciences,
Tennessee State University, Nashville,
USA

1. Introduction

Soybean (*Glycine max*) is a leguminous oilseed and one of the world's largest and most efficient sources of plant protein. United States holds the largest share of soybean production (32%) followed by Brazil (28%), Argentina (21%), China (7%) and India (4%). Although there are variations based on geographical location, the average crude protein (CP) content of soybean is 38% with a rich and balanced amino acid profile. It is therefore a rich source of protein for humans and food animals besides being a rich source of vegetable oil. Soybean meal is the simplest form of soybean protein and a by-product of the oil milling which by National Research Council standards contains 44-48% CP. It contains higher energy [2,460 metabolizable energy (ME) kcal/kg] and protein than other plant protein sources and has an excellent balance of highly digestible amino acids with the exception of methionine which tends to be low. Soybean meal is however rich in the amino acids lysine, tryptophan, threonine, isoleucine, and valine which are deficient in cereal grains such as corn and sorghum most utilized in poultry and swine diets. These are essential amino acids for monogastric animals such as poultry and swine.

Soybeans and soybean meal are also a source of isoflavones which are known to improve growth, promote tissue growth in pigs, and prevent diseases. However, soybean meal possesses anti-nutritional properties which must be overcome to increase its nutritional value. These include antitrypsin inhibitors, oligosaccharides, such as rafinose and stachyose, which are poorly utilized by most food animals. Phytic acid and antigenic factors found in certain soybean proteins cause inflammatory response in the gastrointestinal tract of monogastric animals. Soybeans also contain lectins, compounds that bind with intestinal cells and interfere with nutrient absorption and other compounds such as saponins, lipoxidase, phytoestrogens and goitrogens whose anti-nutritional effects are not known. Soybeans and soybean meal may also be contaminated in the field as a result of using contaminated irrigation water or application of contaminated manure to the growing crop. Since many animal producers use soybean meal as a major constituent of animal feeds, contamination of these feeds with zoonotic foodborne pathogens such as salmonella has increasingly become a global concern.

When properly processed for specific purposes, the soybean and soybean by-products can be utilized by all classes of animals ranging from companion animals, monogastric food

animals such as poultry and swine to aquatic life. Heat processing is required to inactivate trypsin inhibitors. In addition, low trypsin inhibitor soybeans have been developed through classical breeding and genetic engineering of soybeans. The use of microbial phytase enzymes in soy-based diets of swine and poultry increases phosphorus bioavailability and minimizes excess phosphorus excretion. Excess phosphorus in animal manure contributes to environmental pollution in addition to added cost of supplementing soy-based diets with inorganic forms of phosphorus. Soybeans have also been engineered to contain low levels of phytate. Mutant genes which significantly reduce oligosaccharides in soybean have also been identified. Supplementation of soy-based diets with direct-fed microbials has also enhanced the utilization of oligosaccharides. The oligosaccharides serve as prebiotics for these beneficial microorganisms which confer synergistic contributions to the host. Further, implementation of food safety plans on the growing, harvesting, and packing of soybean has the potential to minimize contamination of Soybean as a primary feed ingredient. Rapid and reliable methods for the detection of foodborne pathogens in soybean meal, monitoring of soybean as a raw feed ingredient, and generally good manufacturing practices have been crucial in mitigation efforts in prevention of zoonotic pathogens entering the animal feed processing.

While soybean and soybean meal are readily available in many parts of the world especially where soybean is grown, certain climatic regions are not conducive for soybean production. In these areas alternative protein sources must be sought because soybean becomes expensive attributed to the cost of importation. Under these circumstances animal source proteins or other plant source proteins are sought. Animal protein products such as blood meal have a higher tendency to harbor pathogenic microorganisms such as *Salmonella* when compared to plant protein sources. Therefore, inclusion of feedstuffs that minimize the presence of these pathogenic microorganisms and maintain a healthy gut can increase Monogastric animal production efficiency. Also constraints such as cost, anti-nutritional factors and sometimes low nutritional value of these protein sources dictate substitution, in part, of these feed ingredients with plant source proteins such as soybean.

Blood meal, a by-product of animal rendering, is a potential protein source for poultry. However, full growth and productive performance cannot be achieved without the supplementation of other protein sources, such as soybean meal. Recent studies have shown that substitution of blood meal in diets of laying single comb white leghorn chickens with up to 50% soybean meal in corn-soy based poultry rations did not adversely affect their overall growth and egg production performance when these diets were supplemented with isoleucine. Isoleucine is the primary limiting amino acid in blood meal (less than 1% on a dry-matter basis) and the fourth limiting amino acid after methionine, lysine and tryptophan in corn-soybean based poultry rations. Blood meal contains about 80-88% CP compared to about 44-48% CP in soybean meal. It has a minimum biological availability of about 80% based on the species studied, feeding regimen, housing conditions, and other environmental factors. The methionine and lysine digestibility coefficients are about 90% while those of cysteine and isoleucine are below 80% in blood meal. On the other hand the bioavailability of the amino acids lysine, threonine, and methionine from soybean meal are 88, 81, and 90%, respectively. These factors favor the substitution of other protein sources for soybean meal in diets of monogastric animals.

Soybean meal is also a suitable partial substitute for fishmeal in efforts to reduce cost of feeding and environmental pollution resulting from nutrient (phosphorus and nitrogen) overload in aquaculture. Fish meal which is traditionally the protein source of choice in

aquaculture is expensive. There are reports indicating that soybean meal can replace up to 60% fish meal in fish diets without adversely affecting performance. Soybean meal can also replace 25% fish meal in diets of red snapper without adversely affecting performance. However, higher substitutions require phosphorus supplementation.

In summary, although soybean meal is deficient in methionine and to some extent lysine, it has a rich nutritional value as a protein source in monogastric nutrition. Its value can be enhanced further by its ability to complement other ingredients to overcome key deficiencies. Advancement in processing technology, bioengineering and the use of feed supplements such as enzyme and direct-fed microbials have further added value to soybean meal by increasing the core of its nutrient bioavailability. Nevertheless, there remain limitless opportunities for enhancing the nutritive value and bioavailability of soybean meal protein in monogastric animal nutrition.

2. Nutritional value of soybeans and soybean by-products

Soybean (*Glycine max*) is one of the world's largest sources of plant protein and oil. Soybean protein has high crude protein and a balanced amino acid profile most of which tend to be deficient in cereal grains which constitute large portions of diets of monogastric animals. When compared to other protein sources, soybean boasts being the standard by which other protein sources are compared. Soybean meal, a byproduct of the oil milling industry also has rich nutritive value when compared to other protein sources. Chang et al. (2003) reported relatively high crude protein content of soybean ranging from 44-48 percent. Soybean meal also contains considerably higher energy and lower fiber content than other oilseed meals. The high concentration of protein and energy, and the low fiber content make soybean meal an ideal feed ingredient in formulating balanced rations that provide optimum growth, production and reproductive performance of monogastric animals. Comparisons of the nutritive value of soybean meal with other protein sources are presented in Table 1.

Earlier reports of Holle, (1995) indicate that soybean meal provides the best balance for amino acids which are deficient in most cereal grains when compared with other oilseed meals. Later studies (Zhou et al., 2005) have also shown that soybean meal has a balanced amino acid profile when compared with other oilseed meals, although it is deficient in methionine and lysine (Zhou et al., 2005). Comparisons of the amino acid composition of soybean meal with other protein sources are presented in Table 2.

Among the major oilseed meal sources of protein, soybean ranks highest in value based on quality of protein which is reflective of its balance of amino acids and their digestibility. For instance, the digestibility coefficients of lysine in soybean (Heartland Lysine, 1996; NRC 1994), canola, cotton seed and sunflower meals is estimated at 91, 80, 67, 84%, respectively (NRC, 1994). It has, however, been reported that processing conditions of these meals have a significant effects in reducing the biological value of feed ingredients such as soybean (Papadopoulos et al., 1986). Recent reports (Bandegan et al., 2010) also demonstrated that among the oilseed feed ingredients; soybean meal is the most digestible with its amino acid digestibility values ranging from 83 to 93% for Cysteine and Phenylalanine, respectively.

Other factors that have favored the use of soybean in animal production include (1) consumer food safety concern of the inclusion of animal source protein in animal feeds, especially after the mad cow disease or bovine spongiform encephalopathy and (2) limited production of animal source proteins such as fish meal and (3) the high cost of the animal source proteins such as fish meal and meat and bone meal.

Nutrient	Soybean Meal[2]	Soybean Meal[3]	Cottonseed Meal[4]	Canola[a] Meal[4]	Safflower Meal[3]	Peanut Meal[5]	Sunflower Meal[2]
IFN[6]	5-04-604	5-04-612	5-07-872	5-06-145	5-07-959	5-03-650	5-09-340
Crude Protein, %	44	49	41	38	43	51	32
Energy, kcal/kg	2,230	2,440	2,400	2,000	1,921	2,200	1543
Crude fat, %	0.8	1.0	0.5	3.8	1.3	1.2	1.1
Crude fiber, %	7.0	3.9	13.6	12	13.5	10	24
Calcium, %	0.29	0.27	0.15	0.68	0.35	0.20	0.21
Phosphorus[7], %	0.65	0.62	0.97	1.17	1.29	0.63	0.93
Phosphorus[8], %	0.27	0.24	0.22	0.30	0.39	0.36	0.14
Potassium, %	2.00	1.98	1.22	1.29	1.10	115	0.96
Iron, mg/kg	120	170	110	159	484	142	140
Zinc, mg/kg	40	55	70	71	33	20	100

[1] National Research Council 1994.

[2] seeds, meal solvent extracted.

[3] seeds without hulls, meal solvent extracted.

[4] Seeds, meal pressed solvent extracted.

[5] Kernels, meal solvent extracted.

[6] International feed numbers.

[7] Total phosphorus.

[8] Non-phytate or available phosphorus.

[a] Low erucic acid and low glucosinolates rapeseed cultivars.

Table 1. Comparison of selected nutrient composition of soybean meal and other oilseed meals[1]

According to Hardy (2006) soybean meal is less expensive than fishmeal and is readily available for constitution of animal feeds. However, the price of soybean meal is higher than that of other plant source protein such as cotton seed, canola and sunflower meals. This may be attributed to the higher percent crude protein, better quality protein and highly digestible amino acids in soybean meal when compared with other plant source proteins. A recent survey of commodity prices by the University of Missouri (Table 3) revealed a direct correlation between protein content of feedstuffs and their corresponding prices.

There are many personal observations that soybean meal is in fact beneficial as a good source of amino acids (Green et al., 1987; Angkanaporn et al., 1996) given correct processing procedures. Previous reports have shown that soybean composition and processing conditions affect the nutritional quality of soybean meal (Grieshop and Fahey, 2001). On the other hand, Dudley (1999) emphasized the importance of accurate information on soybean meal composition and the availability of key nutrients in formulating balanced animal feeds. These include the quality, balance, and availability of amino acids and the processing conditions that are used in soybean processing to soybean meal or other byproducts.

Methods of processing soybean and variations in processing also contribute to the overall quality of the soybean products. These include extrusion and expelling, solvent extraction (Woodworth et al., 2001; Nelson et al., 1987), roasting and Jet-sploding (Marty et al., 1994; Subuh et al., 2002), and micronization (Marty et al., 1994; Subuh et al., 2002). These methods lead to variations in nutrient composition of the final product (s). In addition to the various methods used in the production of soybean products, there are also variations in the

parameters used in the production of soybean meal and soybean protein concentrates, which is reflected in the nutrient composition of the final products. These include the combinations of heat, timing, moisture and the quality of the soybean. These variations can be minimized through implementation of good quality control mechanisms during processing. A schematic presentation of the commercial production of the soybean products, soybean meal and soy protein concentrate is presented in Fig. 1.

Nutrient	Soybean Meal[2]	Soybean Meal[3]	Cottonseed Meal[4]	Canola[a] Meal[4]	Safflower Meal[3]	Peanut Meal[5]	Sunflower Meal[2]
IFN[6]	5-04-604	5-04-612	5-07-872	5-06-145	5-07-959	5-03-650	5-09-340
	--(%)--						
Arginine	3.14	3.48	4.59	2.08	3.65	5.33	2.30
Lysine	2.69	2.96	1.71	1.94	1.27	1.54	1.00
Methionine	0.62	0.67	0.52	0.71	0.68	0.54	0.50
Cystine	0.66	0.72	0.64	0.87	0.70	0.64	0.50
Tryptophan	0.74	0.74	0.47	0.44	0.59	0.48	0.41
Histidine	1.17	1.28	1.10	0.93	1.07	1.07	0.55
Leucine	3.39	3.74	2.43	2.47	2.46	2.97	1.60
Isoleucine	1.96	2.12	1.33	1.37	1.56	1.55	1.00
Phenylalanine	2.16	2.34	2.22	1.44	1.75	2.41	1.15
Threonine	1.72	1.87	1.32	1.53	1.30	1.24	1.29
Valine	2.07	2.22	1.88	1.76	2.33	1.87	1.74
Glycine	1.90	2.05	1.70	1.82	2.32	2.67	2.03
Serine	2.29	2.48	1.74	1.53	-	2.25	1.00
Tyrosine	1.91	1.95	1.13	1.09	1.07	1.80	0.91

[1]National Research Council, 1994.

[2] Seeds, meal solvent extracted.

[3] Seeds without hulls, meal solvent extracted.

[4] Seeds, meal pressed solvent extracted.

[5] Kernels, meal solvent extracted.

[6] International feed number.

[a] Low erucic acid and low glucosinolates rapeseed cultivars.

Table 2. Comparison of selected amino acid composition of soybean and other oilseed meals[1]

The two major products of soybean processing are soybean meal which is used extensively in livestock feeding and the soy protein concentrate which is used in production of specialty soy proteins after removal of soluble carbohydrates (oligosaccharides) from solvent extracted soybean flakes using aqueous alcohol leaching. The alcohol treatment of soybean flakes also removes other anti-nutritional factors which include estrogens and antigenic factors such as glycinin and β-conglycinin (Peisker, 2001). Hence, the soy protein concentrate differs from soybean meal in that it contains less oligosaccharides and antigenic substances when compared to soybean meal. The composition of oligosaccharides, lectins, β-conglycinin and saponins in soy protein concentrate and soybean meal are 1%, <1%, <10% and 0%, and 15%, 10-200 ppm, 16 and 0.6%, respectively (Peisker, 2001). The soy protein concentrate is produced by extraction of soluble carbohydrates from alcohol leached solvent

extracted soybean meal. The soy protein concentrate is of lesser significance in animal feeding but a favorable protein source for monogastric animals. The estrogens can also be extracted from solubilized carbohydrates to produce isoflavones rich nutraceuticals for human consumption.

Feed ingredient	CP content (%)	Minimum price ($)/ton	maximum price ($)/ton
Soybean meal	48	348	388
Cotton seed meal	41	310	360
Canola meal	38	202	202
Sunflower meal	32	240	240
Linseed meal	34	265	360
Ruminant blood meal	81	800	850
Fish meal	61	1,395	1,455

[1]Dairy and beef nutrition extension program of The University of Missouri Division of Animal Sciences and Extension Commercial Agriculture, Columbia, Missouri (March 24, 2011) and Feedstuffs (March 28, 2011 | Issue 13 | Volume 83).

Table 3. Comparison of prices of soybean meal and other protein sources[1]
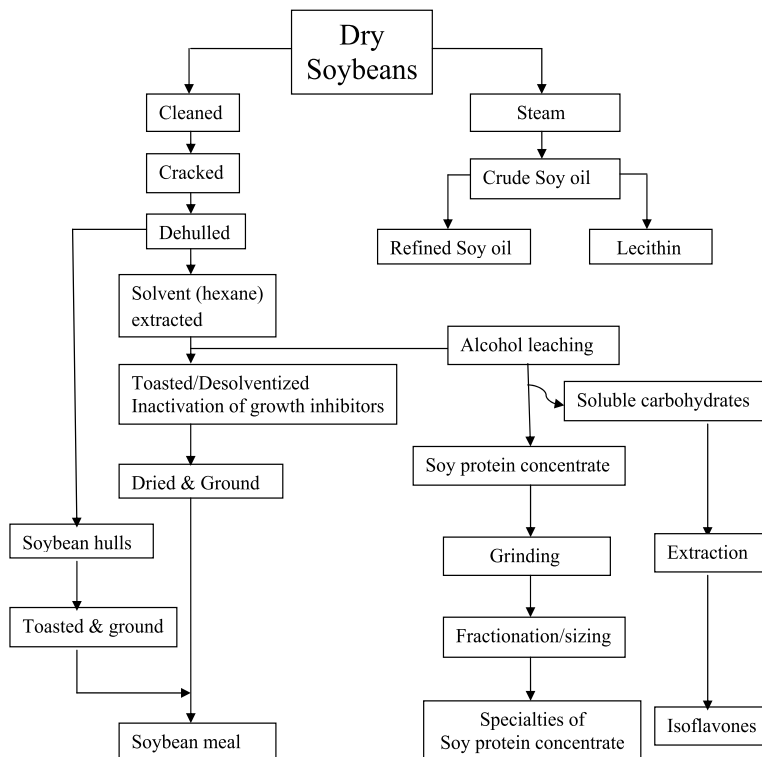
Fig. 1. Schematic presentations of the commercial production of the soybean products, soybean meal and soy protein concentrate.

3. Challenges and opportunities in enhancing quality and nutritive value of soybean

3.1 Challenges of soybean as a protein source
3.1.1 Variations in nutritional composition based on geographic locations

According to the American Soybean Association (2010), in 2009, soybean meal accounted for about 67% of the proteinaceous feed ingredients used in diets of all food-producing animals. The world soybean production totaled 80.7 million metric tons and the largest share of the crop was produced in the United States of America (38%). Other significant producers of soybean in 2009 were Brazil (27%), Argentina (15%), China (7%) and India (4%). Soybean meal is also widely consumed in animal production systems worldwide than any other meal ranging from cotton seed meal to fish meal (American Soybean Association, 2010). Soybean meal (SBM) remains the primary protein source in diets of poultry and swine.

Growing conditions in addition to location where soybean is grown can also affect the nutritional value and quality of soybean and soybean products. Karr-Lilienthal et al. (2004) imported soybean from Argentina, Brazil, China and India, other leading soybean producing countries after the United States and processed it to soybean meal. They reported that SBM produced in the U.S. exhibited a higher crude protein than the SBM produced in the other countries. There were also variations in amino acid and mineral concentration of the soybean meal from the various geographical regions. For instance, consistent with the crude protein levels, soybean from China had the highest levels of most amino acids including lysine, methionine, and arginine and the total essential and non-essential amino acids (Karr-Lilienthal et al. 2004). The content of iron and potassium seemed to be higher in soybean from Argentina when compared to the other countries (Karr-Lilienthal et al. 2004).

In more recent reports, evaluation and comparison of the quality of soybean and soybean meals (SBM) from US, Asia and South America, Thakur and Hurburgh (2007) reported that SBM from Brazil had the highest protein content whereas SBM originating from US and China had the highest percentage of total digestible amino acids. They also reported that US SBM had the highest content of total of five essential amino acids (threonine, methionine, tryptophan, cysteine and lysine) for poultry and swine nutrition. These amino acids also exhibited higher digestibility and overall amino acid balance. Many of these differences in nutrient composition and digestibility among SBM from various geographical regions may be due to variations in environmental conditions in which soybeans are grown. These include soils, water, climate etc. Differences in varieties of soybean and agricultural practices also contribute to the many of the variations in nutrient content of the SBM based on geographic location. In earlier studies, Grieshop and Fahey (2001) reported that soybeans from China had a higher crude protein and lower lipid content (42.14 and 17.25%, respectively) than those from Brazil (40.86% and 18.88%, respectively) and US (41.58 and 18.70%, respectively).

In the report of Thakur and Hurburgh (2007) the highest crude protein SBM was from Argentina, China and India. It has also been demonstrated that the quality of protein and digestibility of individual amino acids varies by geographical regions. Moizuddin (2003) reported that the lysine content of SBM from the US and EU were higher than those of other origins. The true digestibility of SMB from Argentina (87%) and Brazil (82%) were lower than that of the US SBM at 91% (Moizuddin, 2003). Other reports (Baize et al. 1997; Grieshop et al., 2003) have consistently shown differences in nutrient composition of soybean and SBM within and among geographical regions of the world.

Within individual countries there have been reported variations in nutrient concentrations of soybean and SBM from region to region. This is in most part attributed to variations in environmental conditions in which soybeans are grown. Different varieties of soybean thrive better in certain areas than others and since genetic differences are also associated with differences in various characteristics including nutrient composition, they also contribute to the variations in nutrient composition. Karr-Lilienthal et al. (2005) demonstrated that soybeans collected from seven different geographic regions within the US had variations in total amino acid and oligosaccharide concentrations. Therefore, it is essential to always quantify nutrient composition of soybean acquired from new sources and geographical locations outside of the common source to ensure formulation of balanced diets for monogastric animals or the development of proteinaceous products of soybean origin.

3.1.2 Nutrient deficiencies and anti-nutritional factors in soybean based diets

Soybean meal has an excellent balance of highly digestible amino acids with the exception of methionine which tends to be low. Soybean meal is however rich in the amino acids lysine, tryptophan, threonine, isoleucine, and valine which are deficient in cereal grains such as corn and sorghum most utilized in poultry and swine diets. However, similar to other oilseeds meals, soybean meal contains anti-nutritional factors (ANFs) which depress growth performance when fed to monogastric animals (Liener and Kakade, 1980). These ANFs, according Rackis et al. (1986), inhibit the proteolytic action of the pancreatic enzyme trypsin and they may limit the usage of soy products in feeds of young animals with undeveloped digestive tracts. Since the anti-nutritional factors of soybean are known, they are inactivated by optimized heat treatment without compromising the nutritional value of the meal. Herkelman et al. (1991) reported maximum performance when chicks were fed full-fat soybean heated at 120°C for 40 minutes and that sodium metabisulfite decreased the time required to inactivate the trypsin inhibitors by one-half. Therefore soybean meal has no ANFs when properly processed, has the highest nutrient content, excellent amino acid balance, low in fiber and highest in energy content when compared with other oilseed (NRC 1994). Earlier reports indicate that soybean genotype (Palacios et al., 2004) as well as the geographical location and environment in which the soybeans were grown were contributing factors to variations in the SBM nutrient content, digestibility and availability to animals of the SBM (van Kempen et al., 2002; Goldflus et al., 2006). These factors would also influence the level of anti-nutritional factors in soybeans.

There are other oil seed meals such as safflower (Table 1 and 2) which display richness of major nutrients and balanced amino acids almost comparable or better than soybean in some cases, but they also contain ANFs which have not been determined or characterized. The digestibility values of sufflower and its constituent amino acids have not been determined yet (Galacia-Gonzalez et al., 2010). Although soybean contains ANFs, these factors are known and can be reduced significantly during the meal processing to a level that will not interfere with animal performance. These include heat processing (Perilla et al. 1997) in order to denature inhibitory enzymes like urease and haemagglutinins.

Unlike heat pressed or processed soybean and soybean byproducts, raw soybean contain compounds that inhibit the activity of the proteolytic enzyme trypsin. Supplementation of the amino acids lysine, threonine and tryptophan in raw soybean diets improved pig performance (Southern et al., 1990).

The ANFs in soybean are either heat labile or heat stable. The heat labile ANFs are usually inactivated by heat treatment.

Heat labile ANFs

Soyin

The isolation and purification of a toxic protein "glicin" from defatted soybean flour were described by Liener and Pallansch (1952). The toxic protein was later identified as "Soyin" and Liener, (1953) reported that the protein was an albumin-like fraction derived from raw soy beans and was toxic when injected into guinea pigs. This preparation was also reported to possess hemagglutinating properties and was later reported to possess urease activity. Liener, (1953) also observed poor performance of rats fed raw soybean and suggested that the destruction of the heat-labile sub stance (soyin) was necessary in ensuring optimum performance.

Protease inhibitors

Protease inhibitors have been reported to hinder the activity of the proteolytic enzymes trypsin and chymotrypsin in monogastric animals which in turn lowers protein digestibility. The reports of Liener and Kakade, (1969) and Rackis, (1972) confirmed that trypsin inhibitors were key substances in soybean that affected its utilization by chicks, rats and mice. Earlier reports had shown that trypsin inhibitor which was isolated from raw soybeans (Kunitz, 1946) was for growth inhibition. The protease inhibitors were also reported to inhibit Vitamin B12 availability (Baliga et al., 1954). Later studies have also shown that the presence of dietary soybean trypsin inhibitors caused a significant increase in pancreatic proteases (Temler et al., 1984). Hwang et al. (1978) suggested that these plant source protease inhibitors may serve varius purposed which include storage of proteins in seeds, regulation of endogenous proteinases, and also as protective agents against insect and/or microbial proteinases. These protease inhibitors contain about 20% of S-containing amino acids, especially methionine, the most limiting essential amino acid in soybean seeds and cysteine (Hwang et al., 1978).

The effect of soybean trypsin inhibitor on monogastric animal performance has been evaluated extensively. Birth et al. (1993) cited evidence that ingestion of food containing trypsin inhibitor by pigs increased endogenous nitrogen losses hence the effect of the trypsin inhibitors affected nitrogen balance more by losses of amino acids of endogenous secretion than by losses of dietary amino acids. This may be due to compromised integrity of the gastrointestinal lining leading to reduction of absorptive surface. Gertler et al. (1967) attributed the depression of protein digestibility to reduced proteolysis and absorption of the exogenous or dietary protein which was caused by inhibition of pancreatic proteases.

More recent reports (Dilger et al., 2004; Opapeju et al., 2006; Coca-Sinova et al., 2008) indicate that the nutritional value of soybean meal for monogastric animals is limited by anti-nutritional factors which interfere with feed intake and nutrient metabolism. They reported that soybeans with high content protease inhibitors, especially trypsin inhibitors adversely affect protein digestibility and amino acid availability. However, heat processing inactivates these protease inhibitors, although there has to be a balance in conditions of heat inactivation since excessive heating could also destroy other essential nutrients. Qin et al. (1998) demonstrated that excess heat in the inactivation of protease inhibitors of soybean may increase Maillard reactions between the amino group of amino acids and reducing

sugars and as a result decrease the digestibility of energy and amino acids by monogastric animals.

Hemagglutinins or lectins

Hemaglutinins or lectins are a component of soybeans that were characterized as antinutritional factor by Schulze et al., (1995). Oliveira et al., (1989) reported that lectins are glycoproteins which bind to cellular surfaces via specific oligosaccharides or glycopeptides. They exhibit high binding affinity to small intestinal epithelium (Pusztai, 1991) which impairs the brush border and interfere with nutrient absorption. Hemaglutinins have also been implicated in producing structural changes in the intestinal epithelium and resisting gut proteolysis (Pusztai et al., 1990), changes which in most cases result in impairment of the brush border and ulceration of villi (Oliveira et al., 1989). This occurrence result in significant decrease in the absorptive surface and increased endogenous nitrogen losses as reported by Oliveira and Sgarbierri (1986) and Schulze et al. (1995). Pusztai et al. (1990) observed that hemagglutinins depressed growth rate in young animals.

Goitrogens

The possible goitrogenic effect of soybean in animals has not been researched. However, certain soy components may present some antithyroid actions, endocrine disruption, and carcinogenesis in animal and human. For example, Soybean contains flavonoids that may impair the enzymes thyroperoxidase activity (Messina, 2006). Reports have also shown that use of soy-based formula without added iodine can produce goiter and hypothyroidism in infants, but in healthy adults, soy-based products appear to have negligible adverse effects on thyroid function (Messina, 2006; Xiao, 2008; Zimmermann, 2009). In earlier reports (Fort, 1990) concentrations of soy isoflavones resulting from consumption of soy-based formulas were shown to inhibit thyroxine synthesis inducing goiter and hypothyroidism and autoimmune thyroid disease in infants. Still many questions linger on the full Impacts of soy products on thyroid function, reproduction and carcinogenesis, hence the need for further research in this context.

Heat stable ANFs

With the exception of oligosaccharides and antigenic factors, there is less likelihood that the other heat stable anti-nutritional factors would cause problems to monogastric animals consuming soybean-based feeds.

Cyanogens

Legumes such as soybean have long been recognized to contain cyanogenic compounds (Montgomery, 1980). Soybean is a major food ingredient in monogastric nutrition, therefore, any level of cyanogens is considered to be important. The content of cyanide in soybean meal protein was reported at 0.07-0.3 pg of hydrogen cyanide/g of sample in soy protein products and 1.24 pg/g in soybean hulls when browning was kept to a minimum. These values are relatively small when compared with the cyanide content of cassava which ranges from 1 to 3 mg/g (Honig et al. 1983). Cyanide is considered toxic even in small amounts, hence where soy is a major constituent of a diet, there are concerns of cyanide content from a toxicological point of view.

Saponins

Saponins are unabsorbable glucosides of steroids, steroid alkaloids or triterpenes found in many plants including soybeans. They form lather in aqueous solutions and impart a bitter

test or flavor in feed, resulting in reduction of feed consumption. In severe cases they cause haemolysis of red blood cells and diarrhea (Oakwndull, 1981). While raw soybeans have been reported to contain between 2 and 5 g saponins per 100 g, soy products, except those extracted with alcohol, contain high levels of saponins. Soy saponins are divided into groups A and B whereas group A saponins have undesirable astringent taste and are found in soybean germ. Group B saponins are found in both the soybean germ and cotyledons. Although soybean saponins possess anti-nutritional properties, some are edible and have been reported to possess some health benefits. They have been shown to stimulate the immune system, bind to cholesterol and make it unavailable for absorption and allowing its clearance into the colon and eventual excretion (Elias et al., 1990).

Estrogens

Environmental estrogens are classified into two main categories namely phytoestrogens which are of plant origin and xenoestrogens which are synthetic (Dubey et al., 2000). Soybeans contain phytoestrogens which can cause enlargement of the reproductive tract disrupting reproductive efficiency in various species, including humans (Rosselli et al., 2000), and rats (Medlock et al., 1995). In some cases these estrogens are hydrolyzed in the digestive tract to form poisonous compounds such as hydrogen cyanide. Woclawek-potocka et al. (2004) reported that phytoestrogens acting as endocrine disruptors may induce various pathologies in the female reproductive tract. Studies have shown that soy-derived phytoestrogens and their metabolites disrupt reproductive efficiency and uterus function by modulating the ratio of prostaglandins PGF2a to PGE2. Because of the structural and functional similarities of phytoestrogens and endogenous estrogens, there is the likelihood that the plant-derived substances modulate prostaglandin synthesis in the bovine endometrium, impairing reproduction. Previous research has shown that phytoestrogens may act like antagonists or agonists of endogenous estrogens (Rosselli et al., 2000; Nejaty and Lacey, 2001).

Antigens

Antigenic factors glycinin and β-conglycinin removal increases animal performance
A study was conducted to determine the relationship between adverse health outcomes and occupational risk factors among workers at a soy processing plant (Cummings et al., 2010). They reported that asthma and symptoms of asthma were associated with immune reactivity to soy dust. Further discussion of this topic is in the soybean and food safety issues.

Phytates

Phytic acid (inositol hexakisphosphate, IP6), which is considered an anti-nutritional factor, is the storage form of phosphorus in seeds such as those of soybean (Asada et al., 1969). The presence of phytic acid (Fig. 2) in seeds is even more critical in leguminous plants such as soybean which are commonly used in animal feeds because it not only makes phosphorus unavailable, but also reduces the bioavailability of other trace elements such as zinc. The composition of phytic acid in various by-products of oilseeds is presented in Table 4. Raboy and Dickinson (1984) timed the rate of accumulation of phytic acid in seeds of developing soybean and they reported a linear accumulation of phytic acid with the age of the plants. Studies have also shown that the accumulation of phytic acid is also associated with a decline in free phosphorus suggesting that phytic acid synthesis is involved in phosphorus homeostasis of growing soybean plants. This has an effect on the availability of phosphorus from soybean by monogastric animals.

Heaney et al. (1991) reported that the absorption of calcium from soybean-based diets was higher in low-phytate soybean when compared with high phytate-soybean. This supports the assertion that soybean has the potential to form phytate-mineral-complex which inhibits the availability of the minerals to monogastric animals. Phytate is usually a mixture of calcium/magnesium/potassium salts of inositol hexaphosphoric acid in soybean and is shown to adversely impact mineral bioavailability and protein solubility when present in animal feeds (Liener, 1994). Raboy and Dickinson (1984) observed that phytic acid levels and the available (free) phosphorus in mature soybean seeds are responsive to altered concentrations of nutrient phosphorus. However, they observed little or no significant change in content of protein and zinc in the soybean seeds.
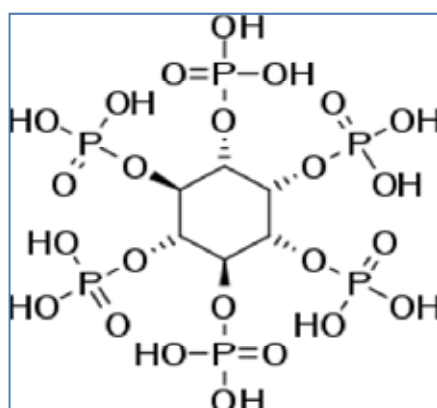
Fig. 2. Molecular structure of Phytic acid

According to Raboy et al. (1984) phytic acid accounts for 67-78% of the total phosphorus in mature soybean seeds and these seeds contain about 1.4-2.3% phytic acid which varies with soybean cultivars. In plants phytic acid is the principal store of phosphate and also serves as natural plant antioxidant. Reports of Vucenik and Shamsuddin (2003) point that inositol bears biological significance as antioxidant in mammalian cells. However, it interferes with mineral utilization and is the primary cause of low phosphorus utilization in soy-based poultry and swine diets. Phytin also chelates other minerals such as Calcium, Zinc, iron, Manganese and Copper, rendering them unavailable to the animals.

Foodstuffs	Minimum, %	Maximum, %
Soybeans	1	2.22
Soybean protein concentrate	1.24	2.17
Peanuts	1.05	1.76
Linseed	2.15	2.78
Sesame seed	5.36	5.36

Adopted from: Reddy, N. R. and Sathe, Shridhar K. (2001).

Table 4. Percent composition of phytic acid in various by-products of oilseeds

The anti-nutritional effects of phytate in soybean-based diets are primarily due to the chelation of calcium (Cheryan, 1980), amino acids (De Rham and Jost, 1979), and starch (Ravindran et al., 1999) by phytate. Ravindran et al., (2006) demonstrated this anti-

nutritional effect in broiler chickens where the digestibility of energy and amino acids declined with an increase in dietary phytate.

Non-starch polyssacharides and soy oligosaccharides

Soybean oligosaccharides (OS) such as raffinose and stachyose are carbohydrates consisting of relatively small number of monosaccharides and they have been reported to influence ileal nutrient digestibility and fecal consistency in monogastric animals (Smiricky et al. 2002). In soybean the OS raffinose and stachyose represent about 4 to 6% of soybean dry matter (Leske et al., 1993). The digestion of OS in the small intestine is limited because mammals lack *a*-galactosidase necessary to hydrolyze the *a* 1,6 linkages present in OS (Slominski, 1994). However, according to reports of Rackis, (1975), fermentation of OS occurs in the small intestine, to a limited extent, due the action of small intestinal microflora. The majority of digestion occurs in the large intestine, where OS function as selective growth factors for beneficial bacteria (Hayakawa et al., 1990). The OS in soybean, raffinose and stachyose, are not eliminated by heat treatment during processing (Leske et al., 1993). Coon et al. (1990) observed that removal of the OS from SBM in poultry diets increased the true metabolizable energy value of the diet by 20 percent. Previous research has demonstrated that soy OS are responsible for increasing intestinal viscosity of digesta and as a result interfere with digestion of nutrients by decreasing their interaction with digestive enzymes (Smits and Annison, 1996). Irish and Balnave (1993) demonstrated that stachyose derived from the oligosaccharides of soyabean meals exert anti-nutritive effects in broilers fed high concentrations soyabean meal as the sole protein concentrate. Certain oligosaccharides, however, are considered to be prebiotic compounds because they are not hydrolyzed in the upper gastrointestinal tract and are able to favorably alter the colonic microflora. Feeding a higher level of an oligosaccharide (8 g/kg) to chicks, however, may depress metabolizable energy and amino acid digestibility (Biggs et al., 2007). Smiricky-Tjardes et al. (2003) reported the presence of significant quantities of galactooligosaccharides in soy-based swine diets. These soy oligosaccharides are partially fermented by gut microflora functioning as prebiotics which promote selective growth of beneficial bacteria.

3.2 Enhancement of nutritive value of soybean in monogastric diets
3.2.1 Mechanisms of adding value to soybean

i. Direct-fed microbials and fructose oligosaccharides

In the recent past beneficial microorganisms (probiotics) and non-digestible ingredients (prebiotics) have been utilized to improve nutrient utilization in soybean-based diets and to enhance health and growth performance of monogastric animals. Probiotics, which is synonymous to direct-fed microbials, are defined as live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance (Fuller, 1989). They improve feed acceptance, feed efficiency, health and metabolism of the host animal (Cheeke, 1991). Other proposed modes of action of probiotics in monogastric animals are: (1) maintaining a beneficial microbial population by competitive exclusion and antagonism (Fuller, 1989), (2) improving feed intake and digestion and production performance (Nahashon et al., 1994a, 1994b, 1994c, 1996), and (3) altering bacterial metabolism (Cole et al., 1987; Jin et al., 1997).

Nahashon et al (1994a) evaluated the phytase activity in lactobacilli probiotics and the role in the retention of phosphorus and calcium as well as egg production performance of Single Comb White Leghorn laying chickens. They reported phytase activity in the direct-fed microbial and that supplementation of the corn-soy based diets with the probiotics (lactobacilli) to a 0.25% available phosphorus diet improved phosphorus retention and layer performance.

Prebiotics, on the other hand, are defined as non-digestible food ingredients that beneficially affect the host, selectively stimulating their growth or activity, or both of one or a limited number of bacteria in the colon and thus improve gut health (Gibson and Roberfroid, 1995). They are short-chain-fructo-oligosaccharides (sc-FOS) which consist of glucose linked to two, three or four fructose units. They are not absorbed in the small intestine but they undergo complete fermentation in the colon by colonic flora (Gibson and Roberfroid, 1995). Three events take place: (1) release of volatile fatty acids which are absorbed in the large intestine and contribute to the animal's energy supply; (2) although not conclusive, they have been reported to enhance intestinal absorption of nitrogen, calcium, magnesium, iron, zinc and copper in rats (Ducros et al., 2005); and (3) increase the number and/or activity of bifidobacteria and lactic acid bacteria (Hedin et al., 2007).

Many oligosaccharides are considered to be prebiotics compounds that can directly or indirectly improve intestinal health and as a result improve animal performance (Biggs et al. 2007), although the mode of action of several of these prebiotics are still obscure. It was reported that even low concentrations (4 g/kg) of an indigestible, prebiotic oligosaccharide can be fed with no deleterious effects on metabolizable energy and amino acid digestibility (Biggs et al., 2007). Fructooligosaccharides such as inulin, oligofructose,and other short-chain fructooligosaccharides can be fermented by beneficial bacteria such as bifidobacteria and lactobacilli (Bouhnik et al., 1994; Gibson and Roberfroid, 1995) which control or reduce the growth of harmful bacteria such as *Clostridium perfringens* through competitive exclusion. The bifidobacteria and lactobacilli are generally classified as beneficial bacteria (Gibson and Wang, 1994; Flickinger et al., 2003).

The benefit of utilizing oligosaccharides in soy-based diets of monogastric animals are due to the ability of these oligosaccharide to pass through to the hindgut of the monogastric animals intact and to be fermented by beneficial bacteria that are stimulated to grow and produce compounds that are beneficial to the host. These beneficial bacteria are also able to prevent the growth of bacteria such as *Escherichia coli* and *lostridiumperfringens* that can be harmful to the host through competitive exclusion (Gibson and Roberfroid, 1995). The digestibility of a few amino acids was increased by some oligosaccharides in cecectomized roosters (Biggs and Parsons, 2007).

ii. Enzymes-Phytases, carbohydrases and proteases

Phytase (myo-inositol-hexakisphosphate phosphohydrolase) is an enzyme that catalyzes the hydrolysis of phytic acid, an indigestible inorganic form of phosphorus in oil seeds and as a result increases the digestion of phosphorus, consequently increasing its utilization and reducing its excretion by monogastric animals. The phytase enzymes are derived from yeast or fungi and bacteria. Nahashon et al. (1994a) reported that P retention was improved in layers when the diet was supplemented with Lactobacillus bearing phytase activity. The use of phytase to hydrolyze phosphorus and possibly other mineral elements that may be bound onto phytate has been extensively researched (Selle and Ravindran, 2007; Powell et al. 2011). The ability of phytase to improve performance and the digestibility of Calcium and

phosphorus in layers fed a corn- and soybean-based diet is also well documented (Lim et al., 2003; Panda et al., 2005; Wu et al., 2006).

Recently, Liu et al. (2007) demonstrated that phytase supplementation in corn-soybean diets significantly improved the digestibility of phosphorus and calcium by 11.08 and 9.81%, respectively. A 2-8% improvement of the digestibility of amino acids was also noted. Phytase supplementation in corn-soybean layer diets also improved egg mass, the rate of lay and egg shell quality of laying birds. These findings suggest that phytase supplementation in soybean; corn-based diets of layers can improve the digestibility of calcium, phosphorus and amino acids.

These results demonstrate that high dietary levels of efficacious phytase enzymes can release most of the phosphorus from phytate, but they do not improve protein utilization (Augspurger and Baker, 2004). Supplemental phytase has also been reported to improving dietary phosphorus utilization by pigs (Sands et al., 2001; Traylor et al., 2001). Recent reports have suggested that the presence of calcium negatively affects the activity of phytase enzymes. Applegate et al. (2003) reported that 0.90% dietary calcium reduced intestinal phytase activity of turkey poults by 9% and phytate phosphorus hydrolysis by 11.9% compared with 0.40% calcium. However, recent report of Powell et al. (2011) indicate that dietary calcium level, within the ranges of 0.67-1.33% did not negatively affect the efficacy of phytase. Other reports (Pillai et al., 2006) demonstrated that addition of *E. coli* phytase to phosphorus deficient broiler diets improved growth, bone, and carcass performance.

Carbohydrases such as xylanase and amylase are enzymes that catalyze the hydrolysis of carbohydrates into sugars which are readily available or metabolizable by monogastric animals. Proteases on the other hand break down long protein chains into short peptides. Most enzyme complexes in monogastric feeding comprise carbohydrases, proteases and phytases. In the animal feed industry these enzymes are produced commercially and used to hydrolyze soluble nonstarch polysaccharides (NSP) of viscous cereals such as rye, triticale, wheat, barley, and oats. Soybean meal contains approximately 3% of soluble NSP and 16% of insoluble NSP (Irish and Balnave, 1993) whereas corn contains approximately 8% of insoluble NSP, mainly arabinoxylans (Choct, 2006). Both corn and soybean contains negligible amounts of soluble NSP, not yielding digesta viscosity problems. Therefore, corn-soy based diets of monogastric animals are considered highly digestible, hence requiring less use of carbohydrases. Previous reports have, however, pointed out that since these cereal grains contain some soluble NSP, there is need to supplement corn-soy based diets with these enzymes to further improve their nutritional value (Maisonnier-Grenier et al., 2004).

Studies to determine the effect of supplementing a corn-soybean meal-based diet with a combination of multicarbohydrase, a preparation containing nonstarch polysaccharide-degrading enzymes, phytases and proteases reveled that these enzymes improved nutrient utilization and growth performance of broiler Chickens (Woyengo et al., 2010). Feeding a combination of xylanase, protease, and amylase resulted in significant improvements in feed conversion and body weight gain of broilers (Cowieson, 2005). When these enzyme combinations were fed in broler diets with both adequate and reduced energy and amino acid content, a 3% and 11% increase in apparent metabolizable energy and nitrogen retention, respectively, were observed (Cowieson and Ravindran, 2008).

Although the enhancement of monogastric animal performance using enzyme supplements in feed have been extensively researched and documented, the benefits of phytases in soy-

based diets of monogastric animals have not been fully explored and require further research. There is still a great deal of uncertainty regarding the mode of action of phytases, carbohydrases and proteases and their combination thereof in corn-soy based diets of monogastric animals.

iii. Genetic modifications

Increasing demand for soybean has necessitated genetic modifications to improve yield, develop disease resistant varieties and varieties with enhanced nutritional value. Drought tolerant varieties of soybean have also been developed through genetic engineering. The Roundup Ready soybean, also known as soybean 40-3-2, is a transgenic soybean that has been immunized to the Roundup herbicide. Although soybean's natural trypsin inhibitors provide protection against pests, weeds still remain a major challenge in soy farming (Wenzel, 2008). A herbicide used to control weeds in soybean farming contains glyphosate which inhibits the expression of the soybean plant's enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene. According to Wenzel, (2008), the gene is involved in the maintenance of the "biosynthesis of aromatic metabolites," and would kill the plant along with the weeds for which the herbicide was meant. Consequently, the soybean was genetically engineered by transferring a plasmid which provided immunity to glyphosate-containing herbicides into the soybean cells through the cauliflower mosaic virus, perfecting the Roundup Ready soybean.

Since drought stress is a major constraint to the production and yield stability of soybean, integrated approaches using molecular breeding and genetic engineering have provided new opportunities for developing high yield and drought resistance in soybeans (Manavalan et al., 2009). Recently, Yang et al. (2010) pointed out that genetic engineering must be employed to exploit yield potential and maintaining yield stability of soybean production in water-limited environments in order to guarantee the supply of food for the growing human population and for food animals.

There are efforts to also develop new soybean varieties that are resistant to diseases and pests. Hoffman et al. (1999) observed that plants commonly respond to pathogen infection by increasing ethylene production. They suggested that ethylene production and/or responsiveness can be altered by genetic manipulation and as a result they used mutagenesis to identify soybean lines with reduced sensitivity to ethylene. Two new genetic loci were identified, Etr1 and Etr2 and plant lines with reduced ethylene sensitivity developed similar or less-severe disease symptoms in response to virulent Pseudomonas syringae. Other reports (Yi et al., 2004) indicate that *CaPF1*, a ERF/AP2 transcription factor in hot pepper plants may play dual roles in response to biotic and abiotic stress in plants and that through genetic engineering this factor could be modified to improve soybean disease resistance as well.

Enhancement of the nutritional value of soybean through genetic engineering has been reported. According to Wenzel (2008), the soybean is a crop with the best amino acid composition within all cultivated protein crops. He pointed out that since amino acids are directly used in the genetic formation of proteins and fatty acids, this makes the soybean invaluable in oil production. One of the main goals in genetic modification of the soybean have essentially been to improve its oxidative stability by changing the mass percentage of certain fatty acids, which would provide a more useful oil, and to increase the overall amount of oil produced. The enhancement of soybean oil content was achieved by the

introduction of a seed-specific transgene for diacylglycerol acyltransferase (DGAT2)-type enzyme from the oil-accumulating fungus Umbelopsis ramanniana (Clemente et al., 2009). Without disrupting the protein content, the oil content was increased from approximately 20% of the seed weight to approximately 21.5%.

Attempts were also made to increasing the oxidative stability of soybean oil. The primary objective was to increase the composition in soybean of the fatty acids oleic and stearic and decrease linoleic acid content of the soybean without creating *trans* or polyunsaturated fatty acids (Clemente, 2009). Recently, DuPont has announced the creation of a high oleic fatty acid soybean, with levels of oleic acid greater than 80%, (Clemente, 2009; Clemente and Cahoon, 2009). Soybean mutants with elevated and reduced palmitate have also been developed (Rahman et al. 1999). While the palmitate content of commercial soybean cultivars is approximately 11%, elevated palmitate content in soybean oil may be important for the production of some food and industrial products.

Low phosphorus (P) availability is also a major constraint to soybean production, therefore, developing soybean varieties that can efficiently utilize phosphorus in the soils would be a sustainable and economical approach to soybean production. Wang et al. (2010) demonstrated the needed to develop more soybean varieties with enhanced P efficiency through root modification, which might contribute to reduced use of P fertilizers, expanding agriculture on low-P soils, and achieving more sustainable agriculture.

Soybeans, like many plants have also been reported to possess intrinsic allergens that present problems for people with food allergies. However, genetically modified soybean has not been shown to add any additional allergenic risk beyond the intrinsic risks already present (Herman, 2003a). Through genetic engineering, major allergens in soybean have been removed providing a very rich protein to both humans and food animals. According to Herman (2003a), the sensitivity to soybean proteins in humans is estimated to occur in 5 ± 8% of children and 1 ± 2% of adults. These allergenic reactions are only rarely life-threatening with the primary adverse reactions to consumption being atopic (skin) reactions and gastric distress. After eliminating a dominant allergen in soybean seeds through genetic engineering, Herman et al. (2003b) reported that there were no significant differences in composition of transgenic and non-transgenic seeds. They pointed out that the lack of a collateral alteration of any other seed protein in the Gly m Bd 30 K-silenced seeds supports the presumption that the protein does not have a role in seed protein processing and maturation.

iv. Synergistic value of soybean and other protein sources-supplementation/substitution

Protein for poultry diets may be derived from both animal and plant sources, with those from animal sources being considered "good-quality". They receive this designation because of their relatively high level of crude protein and their good balance of essential amino acids but they are much more expensive than their plant source counter-parts. Specific animal sources of protein include blood meal (80-88% CP), meat and bone meal (45-50% CP), fish meal (60-70% CP), and poultry by-product (50-55% CP). Common plant sources used in poultry production include soybean meal (41-50% CP), cottonseed meal (41-50% CP), canola meal (45-50% CP), peanut meal (40-45% CP), and alfalfa meal (15-20% CP). It should be noted that, because of the relative low cost and high CP levels, soybean meal is used by nearly all US poultry producers (Kilburn and Edwards, 2004).

In a study performed by Odunsi (2003), bovine blood was evaluated for its efficacy in layer diets. Results from that experiment suggest that full productive performance could not be

achieved without the supplementation of another protein source, in this case fish meal. This conclusion is supported by earlier work done by Onwudike (1981), where birds fed diets containing blood meal as the sole protein source had average hen-day egg production percentages far less than those given other feed ingredients. In that experiment, the average amount of feed required to produce one dozen eggs was also significantly higher for birds on blood meal than in any other test group. It has been suggested that lowered production observed in birds given blood-type protein products could be a result of nutrient imbalance. Blood meal, as a feedstuff, is used primarily to supplement protein requirements of livestock. In general, it has a crude protein content of 80 to 88% (Knaus et al., 1998) with varying digestibility and bioavailability depending on factors that include species, breed, feeding regimen, and climate. The low palatability of blood meal has been an issue and concern for producers (Lim, 2004; DeRouchey, 2002). For this reason, it is recommended that the use of blood meal in rations is restricted to no more than about 5 to 10% of the total ration. The specific amino acid content is generally good, but unbalanced. Isoleucine, for example, is the primary limiting amino acid, and can be found in only trace amounts (often less than 1% of total volume). In one study, isoleucine availability was found to be only 39%, compared to 59% or better for all other essential amino acids (Gaylord and Rawles, 2003). Researchers have studied blood meal as a viable protein supplement in many species including beef cattle (Rangngang et al., 1997), dairy cattle (Schor and Gagliostro, 2001), nursing swine (DeRouchey et al., 2002), sheep (Hoaglund et al., 1992) and poultry (Tyus et al., 2009).

Blood meal contains about 80 to 88 percent CP compared to about 48 percent CP in soybean meal. It has a minimum biological availability of 80 percent based on the species studied, feeding regimen, housing conditions and other environmental factors. (Hoaglund et al., 1992; Sindt et al., 1993; and Kats et al., 1994). The National Research Council (1994) reports methionine and lysine digestibility coefficients of about 90 percent while cysteine and isoleucine figures were both below 80 percent. Blood meal is considered to be deficient in isoleucine, containing less than one percent on a dry-matter basis. When deficient, isoleucine, a limiting amino acid in blood meal, has been shown to cause fatal blood clots and reduced egg production in layers (Peganova and Eder, 2002).

The suitability of blood meal supplemented with isoleucine as protein source for Single Comb White Leghorn (SCWL) chicks was evaluated (Tyus et al. 2009). Based on this study, substitution of up to 50 percent of soybean meal with blood meal supplemented with isoleucine in corn-soy based diets did not adversely affect growth performance of SCWL chicks from day-old to 10 weeks of age. Laying performance of chicks fed diets containing blood meal supplemented with isoleucine from hatch to ten weeks of age was also evaluated (Tyus et al., 2008). They reported that feeding corn-soy diets containing blood meal and supplemented with isoleucine to SCWL chicks at 0-10 WOA significantly improved their subsequent egg production performance, but depressed their internal egg quality and egg shell thickness.

Soybean meal is also a suitable partial substitute for fishmeal in efforts to reduce cost of feeding and environmental pollution resulting from nutrient (phosphorus and nitrogen) overload in aquaculture. Fish meal which is traditionally the protein source of choice in aquaculture is expensive. There are reports indicating that soybean meal can replace up to 60% fish meal in fish diets without adversely affecting performance. Soybean meal can also replace 25% fish meal in diets of red snapper without adversely affecting performance. However, higher substitutions require phosphorus supplementation.

Soybean meal has also been used as partial substitute for groundnut meal in diets of broiler chickens. This has been attributed in part to the seasonal failure of the groundnut crop and the susceptibility of the groundnut cake to aflatoxins. Fishmeal could be a viable substitute but the variations in quality because of adulterations and the cost of the meal has led to the search for other potential protein sources as substitutes. Ghadge et al. (2009) suggested that soybean meal can adequately serve as economical substitute for groundnut cake at 75-100 percent substitution.

A recent study was also conducted to evaluate the replacement of rapeseed meal with soybean meal in diets of broilers because rapeseed meal contains anti-nutritional factors. These include goitrogens or progoitrogens and glucosinolates which reduce growth and egg production when fed to poultry at high concentrations (NRC 1994). Leeson et al. (1987) reported that inclusion of rapeseed as protein source in poultry feeds causes an imbalance between lysine and arginine. They also reported that leucine and isoleucine of rapeseed or canola would be limiting in poultry diets. The rapeseed contains about 42% of oil while its seed meal has an average of 38% crude protein (Montazer-Sadegh et al., 2008).

4. Soybean in monogastric animal nutrition and health

Soybean meal is the most widely used protein source in livestock diets around the globe and according to Kohl-Meier, (1990) it accounts for more than 50% of the world's protein meal. It is also a source of isoflavones which are known to improve growth, promote tissue growth in pigs, and prevent diseases. Isoflavones are a class of phytoestrogens, a group of nonsteroidal plant chemicals with estrogen-like activity. Recent report of Sherrill et al. (2010) indicated that perinatal exposures of male rats to isoflavones affected Leydig cell differentiation, and they imply that including soy products in the diets of neonates has potential implications for testis function. On the other hand, soy isoflavones supplements, which are phyto-oestrogens widely used as alternatives to alleviate menopausal syndromes or prevent chronic diseases, may exert oestrogenic and anti-oestrogenic activities. Hong et al. (2008) reported a significant increase in the oestrogenic activity of the methanol extracts of soy isoflavones for oestrogen receptor (ER) β, but not (ER) α, suggesting that soy isoflavones have a selective modulation of ER activation. The soy isoflavone supplementation did not aggravate murine lupus, but apparently ameliorated the disease.

Human health benefits of soy isoflavones have been reported and they are thought to be due, in part, to their estrogenic activity (Dixon, 2004; McCue and Shetty, 2004). Genistein and daidzein are the two principle isoflavones in soybeans and they are known to bind to estrogen receptors. As a result and as suggested by Wilhelms et al. (2006), isoflavones may exert modest endocrine disruptor-like effects on reproduction in male, but not female, quail. Studies were conducted to determine the effect of soy isoflavones on growth and carcass traits of commercial broilers (Payne et al., 2001). They observed a decrease in average daily weight gain and feed intake of broilers fed diets containing isoflavones. Isoflavones may also affect carcass traits in broilers. Earlier work (Cook, 1998) indicated that supplementation of broiler diets with isoflavones at 1,585 mg/kg diet significantly increased growth rate and carcass muscling in pigs from 6-32 kg body weight. Payne et al. (2001) also reported that addition of isoflavones to a corn-soy protein concentrate increases carcass leanness and decreases carcass fat in broiler chickens.

Processed soybean products which are of lesser significance in monogastric animal feeding have been cited as possessing functional properties to human health such as cancer

prevention (Linz et al., 2004) and liver disease (Gudbrandsen et al., 2006). Partial replacement of soybean meal with extruded soy protein concentrate improved pig performance significantly (Lenehan et al., 2007).

As demonstrated by the supplementation of diets of monogastric animals with isoflavones and soy protein concentrates, there are significant differences among the monogastric animals in response to the inclusion of soybean in their diets.

a.   Soybean in Poultry feeding

Soybean meal (SBM) is the primary protein source in corn-soy based poultry rations. It is fed to poultry as soybean meal and is primarily the by-product of soybean oil extraction; it's the ground defatted flakes. Various studies have been conducted to evaluate methods of enhancing the acceptability of soybean and the enhancement of its nutritional value in poultry feeding. For instance, a study was conducted to evaluate the effect of extruding or expander processing prior to solvent extraction on the nutritional value of soybean meal for broiler chicks. The results of this study indicate that pre-solvent processing method (expander or non-expander) had no significant effect on the nutritional value of SBM for Broiler chicks. However, both Methionine and Lysine supplementation increased feed efficiency (Douglas and Persons, 2000). Several other studies (Coca-Sinova et al., 2008; Dilger et al., 2004; Opapeju et al., 2006) have evaluated various methods of enhancing the digestibility of individual amino acids and protein of soybean meal.

b.   Soybean in Swine feeding

Soybean meal and soybean products have also been used extensively in swine production because of its relatively high concentration of protein (44 to 48%) and its excellent profile of highly digestible amino acids. Soy protein contains most amino acids that are deficient in most cereal grains commonly fed as energy sources in swine production. Due to the high cost of feeding, attempts to minimize the amount of soybean in swine rations and also to improve its digestibility have taken center stage. Bruce et al. (2006) evaluated the inclusion of soybean processing byproducts such as gums, oil, and soapstock into soybean meal. Addition of these processing by-products significantly reduced the nutritive value of the resultant meal. Several other approaches to enhance and expand the utilization of soybean in swine production include the use of oligosaccharides as reported by Smiricky-Tjardes et al. (2003). They evaluated the effect of galactooligosaccharides on ileal nutrient digestibility of nutrients in pigs fed soy-based diets. The digestibility of soy amino acids by swine have also been researched quite extensively (Smiricky-Tjardes et al. 2002; Sohn et al. 1994; Grala et al. 1998; Liener, 1981; NRC 1998; Sohn et al. 1994).

c.   Soybean in aquatic feeding

The feeding value of soybean as a rich protein source has also been extended to aquaculture. Soybean meal and genetically modified soybean products have also been employed in aquaculture (Hammond et al., 1995). Naylora et al. (2009) points to the importance of fish oils and fishmeal as a protein source in food animal production and also the extensive use of soybean and soybean products as protein supplements in aquaculture feeds.

d.   Soybean Food safety issues

This section is discussed in three parts: 1) bacterial contamination of soybean meal and its relation to human foodborne illness; 2) bacterial contamination of soy products; and 3) soy allergies.

Bacterial contamination of soybean meal and its relation to human foodborne illness

Soybean crop fertilized with animal manure has potential for higher yields when compared to soybeans fertilized with commercial fertilizer (McAndrews et al., 2006; Barbazan, 2004).

However, application of contaminated manure to the growing crop may contaminate soybeans with foodborne pathogens such as *Salmonella spp*, and *E. coli* O157:H7. Foodborne pathogens present in the intestinal tracts of animals may contaminate soybean crop via field application of animal manure. Since many animal producers use soybean meal as a major constituent of animal feeds, contamination of these feeds with zoonotic foodborne pathogens has increasingly become a global concern. Animal feeds are frequently tainted with vital human foodborne bacterial pathogens such as *Salmonella* spp.and *E. coli* O157:H7 (Crump et al., 2002; Davis et al., 2003). Use of contaminated soybean meal as ingredients in animal feeds affects the quality and safety of foods of animal origin. Food animals may get infected with foodborne pathogens via contaminated animal feed. Bacteria from the animal's gastrointestinal tract has the potential to contaminate raw meats during evisceration and processing stages (Madden et al., 2004). Raw retail meats have been reported as a major source of zoonotic foodborne pathogens (Foley et al., 2006; NARMS, 2006). *Salmonella* is the leading cause of foodborne illness in the United States and poultry has been identified as the primary source of infection (Braden, C.R. 2006).

Previously, Salmonella has been detected in poultry feed (Williams, J.E., 1981) and can be transmitted to human through animals infected by consuming the contaminated feed (Hinton, 1998). Contaminated feed is therefore a potential path for transmission of foodborne illnesses to humans.

Several environmental sources may be contributing to Salmonella contamination in monogastric animals, but feed is alleged to be the leading source. Implementation of food safety plans on the growing, harvesting, and packing of soybean has the potential to minimize contamination of soybean as a primary feed ingredient. Heat treatment (Stott et al.1975) and ionizing radiation should be applied to eliminate or limit microbial contamination in animal feed (Macirowski et al, 2004). Soybean crop growers should constantly practice Good Agricultural Practices (GAPs) in their farms. Implementation of food safety plans on the growing, harvesting, and packing of soybean has the potential to minimize contamination of soybean as a primary feed ingredient. Detecting *Salmonella* in feed can be difficult as low levels of the pathogen may not be recovered using traditional culturing methods. Rapid and reliable methods for the detection of foodborne pathogens in soybean meal, and monitoring of soybean as a raw feed ingredient have been crucial in mitigation efforts in prevention of zoonotic pathogens entering the animal feed processing. Since animal feed is the first step of the farm to fork continuum for food safety, it is crucial to test for foodborne pathogens in the feed ingredients such as soybean meal for control of *Salmonella* and other foodborne pathogens.

Bacterial contamination of soy products

Various products are derived from soybeans including milk, infant formula, meal, flour, tofu, cereals, meat analogs and meat products. Consumers should follow manufactures instructions for ideal storage and shelf life of soy products. Recently, consumption of soymilk products has been increasing for the reason that these foods contain proteins which are lactose and cholesterol free. According to Liu and Tser-KeShun (2008), Listeria monocytogenes has the ability to survive and multiply in soymilk products and cannot be prevented by refrigeration. Listeria monocytogenes has the ability to grow at low temperatures and therefore permits multiplication at refrigeration temperatures. Consumer's improper handling and storage, especially of soy milk or yogurt is a food safety threat in regard to post-production contamination with foodborne pathogens. As with

soymilk and soymilk based products, post-production contamination of soybean products is a potential health hazard. Ikuomola and Eniola (2010) found high bacterial counts in samples of a popular non-fermented Nigerian fried soybean snack, Beske collected from various markets and Hawkers in Ikeji-Arakeji, Nigeria. Staphylococcus aureus, Micrococcus luteus, Bacillus subtilis, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris along* with four fungi, Penicillium spp, Rhizopus stolonifer and Mucor mucedo were some of the microorganusms isolated and identified from the Beske.

Recently, consumption of fresh green sprouts has increased, all over the world, in part due to health benefits. Sprouts have a risk of being contaminated with pathogenic bacteria such as *Salmonella*, *Escherichia coli O157:H7*, and *Listeria monocytogenes*. The consumption of sprouts has resulted to a number of outbreaks of foodborne illness in several countries. Seed sprouts are regularly linked to foodborne illness, especially those caused by *enterobacteriaceae* (2002; Harris et al., 2003; DuPont, 2007). Water used for soy sprouts should be potable and free of foodborne pathogens. The most significant factor in germination and sprouting of soy is clean water supply. Food-borne pathogens in the water supply have the potential to proliferate in the warm, moist environment that trays of sprouts provide. Soy sprouts are grown from seeds placed in warm, moist, nutrient-rich conditions, which are perfect environments for bacteria growth.

Soy allergy

Food allergies have become a common serious health threat and food safety concern globally. Food allergies can often turn into a lifelong concern. Eight types of foods which include milk, eggs, peanuts, tree nuts, fish, shellfish, soy and wheat account for 90 % of food allergies (Sicherer and Simpson, 2010). Allergy to soy is major allergy and one of the more frequent food allergies. Soybean (Glycine max L. Merr.) is described as one of the main allergenic food crops in the labeling regulation in many continents. The severity of the soy allergy reaction ranges from mild rashes up to anaphylaxis. Allergic reactions to soybean can be systemic, but typically have more localized effects including the skin, the gastrointestinal tract, or the respiratory tract (Savage et al., 2010). The prevalence of soybean allergy is estimated at 0.4% in children and 0.3% of adults in North America (Sicherer and Sampson, 2010).

Soy products are widely used as a major ingredient in most manufactured products and fast food restaurants such as McDonalds and Wendy's (http://www.allergicchild.com/soy _allergies.htm). There have been recalls by the Food and Drug Administration (FDA Enforcement Reports) of several products containing soy proteins, paste, oils and flour due to improper labeling. Consumers who have allergies to soy are often at a risk of serious or life threatening allergic reaction if they consume these products. Unlisted soy protein on product labels is considered a potential hazard for people who may be allergic to soy. Recently, Pasta Mia Veal Ravioli Gastronomica and Mooney's Kentucky Bourbon Cheese by Nina Mia, Inc and Shuckman's Fish Co, respectively, were recalled due to undeclared soy labelling (http://www.foodsafety.gov/). Therefore, failing to list soy proteins on the label places consumers with allergies at risk. Companies often supply soy substrates to other food processors and use as fillers, consequently recalls due to soy ingredients may include a wide range of prepared or processed foods: frozen pizza, cereals, granola bars and meat products. Consumer, particularly with food allergy concerns, must take time and read food labels while purchasing their foods**.**

5. References

Asada, K., K. Tanaka, and Z. Kasai. 1969. Formation of phytic acid in cereal grains. Ann NY Acad Sci 165:801-814.

Applegate, TJ, DM Webel, and XG Lei. 2003. Efficacy of a phytase derived from Escherichia coli and expressed in yeast on phosphorus utilization and bone mineralization in turkey poults Poult. Sci. 82: 1726-1732.

Angkanaporn, K., Ravindran, V. and Bryden, W.L. 1996. Additivity of apparent and true ileal amino acid digestibilities in soybean meal, sunflower meal, and meat and bone meal for broilers. *Poultry Science* 75(9): 1098-1103.

Augspurger, N. R. and D. H. Baker. 2004. High dietary phytase levels maximize phytate-phosphorus utilization but do not affect protein utilization in chicks fed phosphorusor amino acid-deficient diets. J. Anim. Sci. 82:1100–1107.

Barbazan, M. 2004. Liquid swine manure as a phosphorous source for corn-soybean rotation. Thesis. Iowa State University, Ames, Iowa.

Biggs and Parsons, 2007.The Effects of Several Oligosaccharides on True Amino Acid Digestibility and True Metabolizable Energy in Cecectomized and Conventional Roosters. Poultry Science 86:1161–1165.

Bruce, K. J., L. K. Karr-Lilienthal, K. E. Zinn, L. L. Pope, D. C. Mahan, N. D. Fastinger, M. Watts, P. L. Utterback, C. M. Parsons, E. O. Castaneda, M. Ellis, and G. C. Fahey Jr. 2006.

Braden, C.R. 2006. *Salmonella enterica* serotype enteritidis and eggs: a national epidemic in the United States. *Clin. Infect. Dis. 43*, 512-517.

Barth, Christian A., Bruta Lãoehding, Martin Schmitz and Hans Hagemeister. 1993. soybean trypsin inhibitor(s) reduce absorption of exogenous and increase loss of endogenous protein. J. Nutr. 123:2195-2200 (1993).

Biggs, P., C. M. Parsons,1 and G. C. Fahey. 2007. The Effects of Several Oligosaccharides on Growth Performance, Nutrient Digestibilities, and Cecal Microbial Populations in Young Chicks. Poultry Science 86:2327–2336.

Bouhnik, Y., B. Flourie´, F. Ouarne, M. Riottot, N. Bisetti, F.Bornet, and J. Rambaud. 1994. Effects of prolonged ingestion of fructo-oligosaccharides on colonic bifidobacteria, fecal enzymes and bile acids in humans. Gastroenterology 106:A598. (Abstr.)

Balloun, S. L. 1980.Soybean meal processing. In: K.C. Lepley (Ed) Soybean Meal in Poultry. pp 6-12. The Ovid Bell press, Inc., Fulton, Missouri.

Clemente, T. E. and E. B. Cahoon. 2009. Soybean Oil: Genetic Approaches for Modification of Functionality and Total Content. Plant Physiology 151:1030-1040 .

Cook, D. R., 1998. The effect of dietary soybean isoflavones on the rate and efficiency of growth and carcass muscle content in pigs and rats. Ph.D. Dissertation. Iowa State Univ.,Ames, IA.

Cowieson, A. J. 2005. Factors that affect the nutritional value of maize for broilers. Anim. Feed Sci. Technol. 119:293–305.

Clawson GA "Protease inhibitors and carcinogenesis: a review" Cancer Invest 14:597-608, 1996.

Clemente, Tom E. and Edgar B. Cahoon. "Soybean Oil: Genetic Approaches for Modification of Functionality and Total Content." Plant Physiology. 151.3 (2009): 1030-40.

Coca-Sinova,A., D. G. Valencia,† E. Jimenez-Moreno,† R. Lazaro,† and G. G. Mateos. 2008. Apparent Ileal Digestibility of Energy, Nitrogen, and Amino Acids of Soybean Meals of Different Origin in Broilers. Poultry Science 87:2613–2623

Cheryan, M. 1980. Phytic acid interactions in food systems. CRC Crit. Rev. Food Sci. Nutr. 13:297–335.

Cowieson, A. J., and V. Ravindran. 2008. Effect of exogenous enzymes in maize-based diets varying in nutrient density for young broilers: Growth performance and digestibility of energy, minerals and amino acids. Br. Poult. Sci. 49:37–44.

Coon, C. N., K. L. Leske, O. Akavanichan, and T. K. Cheng. 1990. Effect of oligosaccharide-free soybean meal on true metabolizable energy and fiber digestion in adult roosters. Poult. Sci. 69:787–793.

Crump JA, Griffin PM, Angulo FJ. 2002. Bacterial contamination of animal feed and itsrelationship to human foodborne illness. Clin Infect Dis 35:859-865.

Cheeke, P. R. 1991. Applied Animal Nutrition. AVI Publishing Company, Inc., Wesport, CT.

Cole, C. B., R. Fuller, and M. J. Newport. 1987. The effect of diluted yogurt on the gut microbiology and growth of piglets. Food Microbiol. 4:83-85.

Choct, M. 2006. Enzymes for the feed industry: Past, present and future. World's Poult. Sci. J. 62:5–16.

Cummings, K.J., D.M. Gaughan, G.J. Kullman, D.H. Beezhold, B.J. Green, F.M. Blachere, T. Bledsoe, K. Kreiss and J. Cox-Ganser. 2010. Adverse respiratory outcomes associated with occupational exposures at a soy processing plant. ERJ 36:1007-1015.

Dilger, R. N., J. S. Sanders, D. Ragland and O. Adiola. 2004. Digestibility of nitrogen and amino acids in soybean meal with added soyhulls. J. Anim. Sci. 82:715-724.

Dixon, R. A. 2004. Phytoestrogens. Annu. Rev. Plant Biol. 55:225–261.

Douglas, M. W. and C. M. Parsons. 2000. Effect of Presolvent Extraction Processing Method on the Nutritional Value of Soybean Meal for Chicks. Poultry Science 79:1623–1626.

Davis MA, Hancock DD, Rice DH, Call DR, DiGiacomo R, Samadpour M, et al. 2003. Feedstuffs as a vehicle of cattle exposure to *Escherichia coli* O157:H7 and *Salmonella enterica.* Vet Microbiol 95:199-210.

Dilger, R. N., J. S. Sands, D. Ragland, and O. Adeola. 2004. Digestibility of nitrogen and amino acids in soybean meal with added soy hulls. J. Anim. Sci. 82:715-724.

De Rham, O., and T. Jost. 1979. Phytate-protein interactions in soybean extracts and low-phytate soy protein products. J. Food Sci. 44:596–600.

Dubey RK, Rosselli M, Imthurn B, Keller PJ, Jackson EK. Vascular effects of environmental oestrogens: implications for reproductive and vascular health. Hum Reprod Update 4:351–363, 2000.

DuPont, H.L., 2007. The growing threat of foodborne bacterial enteropathogens of animal origin. Clinical Infectious Diseases 45, 1353–1361.

Ducros, V., Arnaud, J., Tahiri, M., Coudray, C., Bornet, F., Bouteloup-Demange, C., Brouns, F., Rayssiguier, Y., and Roussel, A. M. 2005. Influence of short-chain fructo-oligosaccharides (sc-FOS) on absorption of Cu, Zn, and Se in healthy postmenopausal women. J. American College of Nutr. 24(1):30-37.

DeRouchey JM, MD Tokach, JL Nelssen, RD Goodband, SS Dritz, JC Woodworth and BW James. 2002. Comparison of spray-dried blood meal and blood cells in diets for nursery pigs. J. Anim. Sci. 80:2879.

Elias R, DeMeo M, Vidal-Ollivier E, et al. "Antimutagenic activity of some saponins isolated from Calendula officinalis L., C arvensis L., and Hedra helix L." Mutagenesis 5:327-331, 1990.

Evaluation of the inclusion of soybean oil and soybean processing by-products to soybean meal on nutrient composition and digestibility in swine and poultry J. Anim. Sci. 2006. 84:1403–1414.

Fort P, Moses N, Fasano M 1990 Breast and soy-formula feedings in early infancy and the prevalence of autoimmune thyroid disease in children. J Am Coll Nutr 9:164–167

Flickinger, E. A., E. M. W. C. Schreijen, A. R. Patil, H. S. Hussein,C. M. Grieshop, N. R. Merchen, and G. C. Fahey Jr. 2003. Nutrient digestibilities, microbial populations, and protein catabolites as affected by fructan supplementation of dog diets. J. Anim. Sci. 81:2008–2018.

Fuller, R. 1989. Probiotics in man and animals. A review. J. Appl. Bacteriol. 66:365-378.

Gudbrandsen, O. A., H. Wergedahl, S. Mork, B. Liaset, M. Espe, and R. K. Berge. 2006. Dietary soya protein concentrate enriched with isoflavones reduced fatty liver, increased hepatic fatty acid oxidation and decreased the hepatic mRNA level of VLDL receptor in obese Zucker rats. Br. J. Nutr. 96:249–257.

Green, S., Bertrand, S.L., Duron, M.J. and Maillard, R. 1987. Digestibility of amino acids in soyabean, sunflower and groundnut meals, determined with intact and caecectomised cockerels. *British Poultry Science* 28(4): 643-652.

Grala, W., M. A. Verstegen, A. J. M. Jansman, J. Huisman, and P. Van Leeusen. 1998. Ileal apparent protein and amino acid digestibilitiesand endogenous nitrogenlosses in pigs fedsoybean and rapeseed products. J. Anim. Sci. 76:557-568.

Gertler, A., Birk, Y. & Bondi, A. (1967) A comparative study of the nutritional and physiological significance of pure soybean trypsin inhibitors and of ethanol-extracted soybean meals in chicks and rats. J. Nutr. 91: 358-370.

Goldflus, F., M. Ceccantini, and W. Santos. 2006. Amino acid content of soybean samples collected in different brazilian states –Harvest 2003/2004. Braz. J. Poult. Sci. 8:105–111.

Gibson, G. R., and X. Wang. 1994. Inhibitory effects of bifidobacteria on other colonic bacteria. J. Appl. Bacteriol. 77:412–420.

Gibson, G. R., and M. B. Roberfroid. 1995. Dietary modulationof the human colonic microbiota—Introducing the concept of prebiotics. J. Nutr. 125:1401–1412.

Ghadge, V. N., B. T. Upase and P. V. Patil. 2009. Effect of replacing groundnut cake by soybean meal on performance of broilers. Veterinary World, 2(5):183-184.

Gaylord BS, AJ Heeger and GC Bazan. 2003. DNA hybridization detection with water-soluble conjugated polymers and chromophore-labeled single-stranded DNA. J. Am. Chem. Soc. 125:896.

Grieshop, C. M., C. T. Catzere, G. M. Clapper, E. A. Flickinger, L. L. Bauer, R. L. Frazier, and G. C. Fahey, Jr. 2003. Chemical and nutritional characteristics of United States soybeans and soybean meals. J. Agric. Food Chem. 51:7684-7691.

Grieshop, C. M., Fahey, G. R. 2001. Comparison of quality characteristics of soybeans from Brazil, China and the United States. J. Agric Food Chem 49:2669-2673.

Hong, Y.H., T.C Wang, C.J Huang, W.Y Cheng, and B.F Lin. 2008. Soy isoflavones supplementation alleviates disease severity in autoimmune-prone MRL-lpr/lpr mice. Lupus, 17: 814 - 821Heaney, R. P., C. M. Weaver, and M. L. Fitzsimmons. 1991. Soybean phytate content: effect on calciumabsorption. Am. J. Clin Nutr 53;745-747.

Herman, E. M., Ricki M. Helm, Rudolf Jung and Anthony J. Kinney. 2003. Genetic Modification Removes an Immunodominant Allergen from Soybean. Plant Physiology May 2003 vol. 132 no. 1 36-43

Harris, L.J., Farber, J.N., Beuchat, L.R., Parish, M.E., Suslow, T.V., Garrett, E.H., Busta, F.F., 2003. Outbreaks associated with fresh produce: incidence, growth, and survival of

pathogens in fresh and fresh-cut produce. Comprehensive Reviews in Food Science and Food Safety 2 (Supplement), 79–141

Herman, E. M. 2003. Genetically modi®ed soybeans and food allergies. Journal of Experimental Botany, Vol. 54, No. 386, pp. 1317-1319.

Hwang, David l., Wen-kuang Yang, and Donald E. Foard. 1978. Rapid Release of Protease Inhibitors from Soybeans. Plant Physiol. 61, 30-34

Hayakawa, K., J. Mitzutani, K. Wada, T. Masai, I. Yoshihara, and T.Mitsuoka. 1990. Effects of soybean oligosaccharides on human fecal flora. Microbiol. Ecol. Health Dis. 3:293–303.

Hedin C et al; *Evidence for the use of probiotics and prebiotics in inflammatory bowel disease: a review of clinical trials.* Proc Nutr Soc. 2007; 66(3): P-307-15.

Hoaglund CM, VM Thomas, MK Peterson, and RW Knott. 1992. Effects of supplemental protein source and metabolizable energy intake on nutritional status in pregnant ewes. J. Anim. Sci. 70:273.

Hammond, B. C. J.L Vicini, C. F. Hartnell, M. W. Naylor,C. D. Knight, E. H. Robinson, R. L. Fuchs and S. R. Padgette. 1995. The feeding value of soybeans fed to rats, chickens, catfish and dairy cattle is not altered by genetic incorporation of glyphosate tolerance. J. Nutr. 126: 3 717-727

Holle, D.G. 1995. Amino Acids. *Ratite Feeds and Feeding* 1-2: 58, 59.

Herkelman, K. L., G. L. Cromwell and T. S. Stahly. 1991. Effect of heating time and sodium metabisulfite on the nutritional value of full-fat soybean for chicks. J. Anim. Sci. 69:4477-4486.

Honig, D. H., M. Elaine Hockridge, Robert M. Gould, and Joseph J. Rackis. Determination of Cyanide in Soybeans and Soybean Products. Journal of Agricultural & Food Chemistry, March-April 1983, Pages 272-275

Irish, G.G. and D. Balnave. 1993. Non-starch polysaccharides and broiler performance on diets containing soyabean meal as the sole protein concentrate. Australian Journal of Agricultural Research 44(7) 1483 - 1499

Ikuomola, D.S., Eniola, K.I.T., 2010. Microbiological Quality and Public Health Risk Associated with Beske: Fried Soybean Snack, Retailed in Ikeji-Arakeji, Osun State, Nigeria. Nigeria Journal of Microbiology, 24(1): 2114-2118.

Irish, G. G., and D. Balnave. 1993. Non-starch polysaccharides and broiler performance on diets containing soyabean meal as the sole protein concentrate. Aust. J. Agric. Res. 44:1483–1499.

Izabelawoclawek-potocka, Mamadou M. Bah, Anna Korzekwa, Mariusz K. Piskula Wiesławwwiczkowski, Andrzej Depta, and Dariusz J. Skarzynski. 2005. Soybean-Derived Phytoestrogens Regulate Prostaglandin Secretion in Endometrium During Cattle Estrous Cycle and Early Pregnancy. Experimental biology and Med 230:189–199.

Irish, G.G. and D. Balnave. 1993. Non-starch polysaccharides and broiler performance on diets containing soyabean meal as the sole protein concentrate. A*ustralian Journal of Agricultural Research* 44(7) 1483 – 1499.

Jin, L. Z., Y. W. Ho, N. Abdullah, and S. Jalaludin1997. Probiotics in poultry: modes of action. World's Poultry Sci. J. 53:353-368.

Jessica D. Sherrill, Morgan Sparks, John Dennis, Mahmoud Mansour, Barbara W. Kemppainen, Frank F. Bartol, Edward E. Morrison and Benson T. Akingbemi.2010. Developmental Exposures of Male Rats to Soy Isoflavones Impact Leydig Cell Differentiation. Biology of Reproduction 83: 488-501

Kunihiko Uemura, Chieko Takahashi, Isao Kobayashi. 2010.Inactivation of Bacillus subtilis spores in soybean milk by radio-frequencyflash heating. Journal of Food Engineering 100: 622–626

Katsuki, Y.; Yasuda, K; Ueda, K; Naoi, Y. *Annu. Rep. Tokyo Metrop. Res. Lab. Public Health* 1978, 29, 26l.

Knaus WF, DH Beermann, TF Robinson, DG Fox and KD Finnerty. 1998. Effects of a dietary mixture of meat and bone meal, feather meal, blood meal, and fish meal on nitrogen utilization in finishing Holstein steers. J. Anim. Sci. 76:1481.

Kats LJ, JL Nelssen, MD Tokach, RD Goodband, JA Hansen and JL Laurin. 1994. The effect of spray-dried porcine plasma on growth performance in the early-weaned pig. J. Anim. Sci. 72:2075.

Karr-Lilienthal, L. K., N. R. Mershem, C. M. Grieshop, M. A. Flahaven, D. C. Mahan, N. D. Watts, G. C. Fahey, Jr. 2004. Ileal amino acid digestibilities by pigs fed soybean meals from five major soybean producing countries. J. Anim Sci 82:3198-3209.

Karr-Lilienthal, L. K., C. M. Grienshop, J. K. Spears, and G. C. Fahey, Jr. 2005. Amino acid, carbohydrate, and fat composition of soybean meals prepared at 55 commercial U.S. soybean processing plants. J. Agric. Food Chem. 53:2146-2150.

Karr-Lilienthal, L. K., C. N. Grieshop, N. R. Merchen, D. C. Mahan, and G. C. Fahey, Jr. 2004. Chemical composition and protein quality comparisons of soybeans and soybean meals from five leading soybean-producing countries. J. .Agric. Food Chem. 52:6193-6199.

Kunitz, M. 1946 Crystalline soybean trypsin inhibitor. J. Gen. Phygiol., S9:149-154.

Liu, N., G. H. Liu, F. D. Li,1 J. S. Sands, S. Zhang, A. J. Zheng, and Y. J. Ru.2007. Efficacy of Phytases on Egg Produ. ction and Nutrient Digestibility in Layers Fed Reduced Phosphorus Diets. Poultry Science 86:2337–2342.

Lim, H. S., H. Namkung, and I. K. Paik. 2003. Effects of phytase supplementation on the performance, egg quality, and phosphorous excretion of laying hens fed different levels of dietary calcium and nonphytate phosphorous. Poult. Sci. 82:92–99.

Lenehan, N. A., J. M. DeRouchey, R. D. Goodband, M. D. Tokach, S. S. Dritz, J. L. Nelssen, C. N. Groesbeck, and K. R. Lawrence. 2007. Evaluation of soy protein concentrates in nursery pig diets. J. Anim. Sci. 85:3013–3021.

Liener, I. E. 1953. Soyin, a toxic protein from the soybean. J. Nutrition, 49: 527.

Liener, I. E. 1994. Implications of antinutritional components in soybean foods. Crit. Rev. Food Sci. Nutr. 34:31–67.

Liu, Jiang-Gong and Lin Tser-KeShun, 2008. Survival of *Listeria monocytogenes* inoculated in retail soymilk products Food Control, 19 (2008) 862-867.

Leske, K. L., C. J. Jevne, and C. N. Coon. 1993. Effect of oligosaccharide additions on nitrogen-corrected truemetabolizable energy of soy protein concentrate. Poult. Sci. 72:664–668.

Lienei, I. E. and M. L. Kakade. 1969. Protease inhibitors. *In* I. E. Liener (Ed.) Toxic Constituents of Plant Foodstuffs. Academic Press, New York.

Liener, I. E. 1981. Factors affecting the nutritional quality of soy products. J. Am. Oil. Chem. Soc. 58:406-415.

Liener, L. E., and M. L. Kakade. 1980. Protease Inhibitors. In: I. E. Liener (Ed) Toxic Constituents of Plant Foodstuffs. pp7-71. Academic Press, New York.

Linz, A. L., R. J. Xiao, M. Ferguson, T. M. Badger, and F. A. Simmen. 2004. Developmental feeding of soy protein isolate to male rats inhibits formation of colonic aberrant

crypt foci: Major response differences to azoxymethane (AOM) in Sprague-Dawley rats from two colonies. FASEB J. 18:A127.

Madden, R.H., K.A. Murray, and A.Gilmore. 2004. Determination of principal points of product contamination during beef carcass dressing process in Northern Ireland. *J. of Food Prot.* 67: 1494-1496.Foley, S.L., D. G. White, P.F. McDermott, R.D. Walker, B.

McAndrews, G.M., M. Liebman, C.A. Cambardella, and T.L. Richard. 2006. Residual effects of composted and fresh solid swine (*Sus scrofa* L.) manure on soybean [*Glycine max* (L.) Merr.] growth and yield. Agron. J. 98: 873-882.

Montgomery, R. D. In "Toxic Constituents of Plant Foodstuffs", 2nd ed.; Liener, I. E., Ed.; Academic Press: New York, 1980; Chapter 5.

Maciorowski, K. G., Jones, F. T. Pillai, S. D., Ricke, S. C. 2004. Incidence, sources, and control of foodborne Salmonella spp. in poultry feed. World's Poultry Sct. J.60, 446-457

Montazer-Sadegh, R., Y. Ebrahim-Nezhad, and N. Maheri-Sis. 2008. Replacement of different levels of rapeseed meal with soubean meal on broiler performance. Asian Journal of Animal and Vet. Advances 3(5):278-285.

McCue, P., and K. Shetty. 2004. Health benefits of soy isoflavonoids and strategies for enhancement: A review. Crit. Rev.Food Sci. Nutr. 44:361–367.

Michael B. Zimmermann. 2009. Iodine Deficiency Endocrine Reviews, June 2009, 30(4):376–408

Messina M, Redmond G 2006 Effects of soy protein and soybean isoflavones on thyroid function in healthy adults and hypothyroid patients: a review of the relevant literature. Thyroid 16:249–258

Manavalan, L. P. Satish K. Guttikonda, Lam-Son Phan Tran, and Henry T. Nguyen. 2009. Physiological and Molecular Approaches to Improve Drought Resistance in Soybean. Plant Cell Physiol 50 (7): 1260-1276.

Medlock KL, Branham WS, Sheehan DM. Effects of coumestrol and equol on the developing reproductive tract of the rat. Proc Soc Exp Biol Med 208:67–71, 1995.

Moizzudin, S. 2003. Soybean meal quality in US and World markets. MS. Thesis, IA Iowa State University.

Marty, B. J., E. R. Chavez, and C. F. M. De Lange. 1994. Recovery of amino acids at the distal ileum for determining apparent and true ileal amino acid digestibilities in growing pigs fed various heat-processed full-fat soybean products. J. Anim. Sci. 72:2029-2037.

Nahashon, S. N., Harry S. Nakaue, and Larry W. Mirosh, 1996. Performance of Single Comb White Leghorns given a diet supplemented with a live microbial during the growing and egg laying phases. Animal Feed Science and Technology 57:25-38.

Nahashon, S. N., H. S. Nakaue, and L. W. Mirosh, 1994a. Phytase activity, phosphorus and calcium retention and performance of Single Comb White Leghorn layers fed diets containing two levels of available phosphorus and supplemented with direct-fed microbials. Poultry Science 73:1552-1562.

Nahashon, S. N., H. S. Nakaue, and L. W. Mirosh, 1994b. Production variables and nutrient retention in Single Comb White Leghorn laying pullets fed diets supplemented with direct-fed microbials. Poultry Science 73:1699-1711.

Nahashon, S. N., H. S. Nakaue, S. P. Snyder, and L. W. Mirosh, 1994c. Performance of Single Comb White Leghorn layers fed corn-soybean meal and barley-corn-soybean meal diets supplemented with a direct-fed microbial. Poultry Science 73:1712-1723.

Naylora, R. L., 1, Ronald W. Hardyb, Dominique P. Bureauc, Alice Chiua, Matthew Elliottd, Anthony P. Farrelle, Ian Forstere, Delbert M. Gatlinf,g, Rebecca J. Goldburgh, Katheline Huac, and Peter D. Nicholsi. 2009. Feeding aquaculture in an era of finite resources. PNAS 106 36: 15103-15110.

Nelson, A. I., W. B. Wijeratne, S. W. Yeh, T. M. Wei, and L. S. Wei. 1987. Dry extrusion as an aid to mechanically expelling of oil from soybean. J. Am. Oil Chem. Soc. 64:1341-1347.

NRC. 1998. Nutrient requirements of swine. 10th ed. National Academy Press, Washington, DC.

Nejaty H, Lacey M, Whitehead SA. Differing effects of endocrine disrupting chemicals on basal and FSH-stimulated progesterone production in rat granulose-luteal cells. Exp Biol Med 226:570–576, 2001.

Opapeju, F. O., A. Golian, C. M. Nyachoti, and L. D. Campbell. 2006. Amino acid digestibility in dry extruded-expelled soybean meal fed to pigs and poultry. J. Anim. Sci. 84:1130-1137.

Oakwndull, D. 1981. "Saponins in Food - A Review" Food Chem 6:19-40.

Opapeju, F. O., A. Golian, C. M. Nyachoti, and L. D. Campbell. 2006. Amino acid digestibility in dry extruded-expelled soybean meal fed to pigs and poultry. J. Anim. Sci. 84:1130-1137.

Odunsi AA. 2003. Blend of bovine blood and rumen digesta as a replacement for fishmeal and groundnut cake in layer diets. Inter. J. Poult. Sci. 2:58.

Onwudike OC. 1981. Effect of various protein sources on egg production in a tropical environment. Trop. Anim. Prod. 6:249.

Opapeju, F. O., A. Golian, C. M. Nyachotu and L.D. Campbell. 2006. Amino acid digestibility in extruded-expelled soybean meal fed to pigs and poultry. J. Anim. Sci. 84:1130-1137.

Panda, A. K., S. V. R. Rao, M. V. L. N. Raju, and S. K. Bhanja. 2005. Effect of microbial phytase on production performance of White Leghorn layers fed on a diet low in non-phytate phosphorus. Br. Poult. Sci. 46:464–469.

Payne, R. L., T. D. Bidner, L. L. Southern, and K. W. McMillin. 2001. Dietary Effects of Soy Isoflavones on Growth and Carcass Traits of Commercial Broilers. Poultry Science 80:1201–1207.

Peganova S and K Eder. 2002. Studies on requirement and excess of isoleucine in laying hens. Poult. Sci. 81:1714.

Powell , S. T. D. Bidner , and L. L. Southern. 2011 Phytase supplementation improved growth performance and bone characteristics in broilers fed varying levels of dietary calcium 1Poultry Science 90 :604–608

Palacios, M. F., R. A. Easter, K. T. Soltwedel, C. M. Parsons, M.W. Douglas, T. Hymowitz, and J. E. Pettigrew. 2004. Effect of soybean variety and processing on growth performance of young chicks and pigs. J. Anim. Sci. 82:1108–1114.

Peisker, M. 2001. Manufacturing of soy protein concentrate for animal nutrition. Pages 103–107 in Feed Manufacturing in the Mediterranean Region. Improving Safety: From Feed to Food. J. Brufau, ed. CIHEAM-IAMZ, Reus, Spain.

Pillai, P. B., T. O'Connor-Dennie, C. M. Owens, and J. L. Emmert. 2006. Efficacy of an *Escherichia coli* Phytase in Broilers Fed Adequate or Reduced Phosphorus Diets and Its Effect on Carcass Characteristics. Poultry Science 85:1737–1745

Perilla, N.S., Cruz, M.P., de Belalcazar, F. and Diaz, G.J. 1997. Effect of temperature of wet extrusion on the nutritional value of full-fat soyabeans for broiler chickens. British Poultry Science 38(4): 412-416.

Rackis, J. J. 1975. Oligosaccharides of food legumes: Alpha-galactosidase activity and the flatus problem. In: A. Jeanes and J.Hodges (ed.) Physiological Effects of Food Carbohydrates. Am. Chem. Soc., Washington, DC.

Ravindran, V., S. Cabahug, G. Ravindran, and W. L. Bryden. 1999. Effects of phytase supplementation, individually and in combination with glycanase, on the nutritive value of wheat and barley. Poult. Sci. 78:1588–1595.

Rahman, SM, T Kinoshita, T Anai and Y Takagi. 1999. Genetic relationships between loci for palmitate contents in soybean mutants. J Hered (1999) 90 (3): 423-428.

Ravindran, V., P. C. Morel, G. G. Partridge, M. Hruby, and J. S. Sands. 2006. Influence of an *Escherichia coli*-derived phytase on nutrient utilization in broiler starters fed diets containing varying concentrations of phytic acid. Poult. Sci. 85:82–89.

Rhodes, P.J. Fedorka-Cray, S., Simjee, and S. Zhao. 2006. Comparison of subtyping methods for differentiating *Salmonella enterica* serova Typhimurium isolates obtained from animal food sources. *J. Clin. Microbiol.* 44: 3569-3577.

Roman S. Temler, Charles A. Dormono, Eliane Simon, Brigette Morel and Christine Mettraux. 1984. Proteins, Their Hydrolysates and SoybeanTrypsin Inhibitor. J. Nutr. 114: 270-278, 1984.

Rangngang MB, ML Nelson and SM Parish. 1997. Ruminal undegradability of blood meal and effects of blood meal on ruminal and postruminal digestion in steers consuming vegetative orchard grass hay. J. Anim. Sci. 75:2788.

Rosselli M, Reinhard K, Imthurn B, Keller PJ, Dubey RK. Cellular and biochemical mechanisms by which environmental estrogens influence reproductive function. Hum Reprod. Update 6:332–350, 2000.

Raboy, V., D. B. Dickinson. 1984. Effect of phosphorus and zinc nutrition on soybean seed phytic acid and zinc. Plant Physiol. 75:1094-1098.

Raboy, V. , D. B Dickinson, F. E. Below. 1984. Variation in seed total phosphorus, phytic acid, zinc, calcium, magnesium, and protein among lines of Glycine Max and G. Soja. Crop Sci. 24:431-434.

Rackis, J. J., W. J. Wolf, and E. C. Baker. 1986. Protease inhibitors in plant foods: Content and inactivation. In: M. Friedman (Ed) Nutritional and Toxicological Significance of Enzyme Inhibitors in Foods. Pp 299-347. Plenum Publishing, New York.

Rackis, J. J. 1972. Biologically active components. *In* Smith and Circle (Ed.) Soybeans: Chemistry and Technology Vol. I. The Avi Publ. Co., Inc.,Westport, Conn.

Savage, J.H. et al., 2010. The natural history of soy allergy. J. Allergy Clin. Immunol. 125, 683–686

Sicherer, S.H., Sampson, H.A., 2010. Food allergy. J. Allergy Clin. Immunol. 125, S116–S125.

Smiricky, M. R., C. M. Grieshop, D. M. Albin, J. E. Wubben. 2002. The influence of soy oligosaccharides on apparent and true ileal amino acid digestibilities and fecal consistency in growing pigs. J. Anim. Sci. 2002. 80:2433–2441.

Sampson HA. Update on food allergy. J Allergy Clin Immunol; 113:805–19. 2004.

Sicherer SH. Food allergy. Lancet; 360:701–10. 2002.

Slominski, B. A. 1994. Hydrolysis of galactooligosaccharides by commercial preparations of a-galactosidase and β-fructofuranose:Potential for use as dietary additives. J. Sci. Food Agric. 65:323–330.

Smiricky-Tjardes, M. R., E. A. Flickinger, C. M. Grieshop, L. L. Bauer, M. R. Murphy, and G. C. Fahey Jr. 2003. In vitro fermentation characteristics of selected oligosaccharides by swine fecal microflora. J. Anim. Sci. 81:2505-2514.

Sahn, K. S., C. V. Maxwell, L. L. Southern, and D. S. Buchanan. 1994. Improved soybean protein sources for early weaned pigs.:Ii. Effect on ileal amino acid digestibility. J. Anim. Sci. 72:631-637.

Smiricky-Tjardes, M. R., C. M. Grieshop, E. A. Flickinger, L. L. Bauer, and G. C. Fahey, Jr. 2003. Dietary galactooligosaccharides affect ileal and total0-tract nutrient digestibility, ileal and fecal bacteria concentrations, and ileal fermentative characteristics of growing pigs. J. Anim. Sci. 81:2535-2545.

Smiricky-Tjardes, M. R., C. M. Grieshop, D. M. Albin, J. E. Wubben, V. M. Gabert, and G. C. Fahey, Jr. 2002. The influence of soy oligosaccharides on apparent and true ileal amino acid digestibilities and fecal consistency in growing pigs. J. Anim. Sci. 80:2433-2441.

Schor A and GA Gagliostro. 2001. Undegradable protein supplementation to early-lactation dairy cows in grazing conditions. J. Dairy Sci. 84:1597.

Sindt MH, RA Stock, TJ Klopfenstein and DH Shain. 1993. Effect of protein source and grain type on finishing calf performance and ruminal metabolism. J. Anim. Sci. 71:1047.

Selle, P. H., and V. Ravindran. 2007. Microbial phytase in poultry nutrition. A review. Anim. Feed Sci. Technol. 135:1–41.

Smits, C. H. M., and G. Annison. 1996. Non-starch plant polysaccharides in broiler nutrition—towards a physiologically valid Soy oligosaccharides and pigs 2441proach to their determination. World's Poult. Sci. J. 52:204–221.

Stott, J.A., J. E, Hodgson, J C Chaney. 1975. Incidence of *Salmonella* in animal feed and the effct of pelleting on content of *enterobacteriaceae*. J Appl. Bact.39:41-46.

Sands, J. S., D. Ragland, C. Baxter, B. C. Joern, T. E. Sauber, and O.Adeola. 2001. Phosphorus bioavailability, growth performance, and nutrient balance in pigs fed high available phosphorus corn and phytase. J. Anim. Sci. 79:2134–2142.

Subuh, A. M. H., M. A. Moti, C. A. Fritts, and P. W. Waldroup. 2002. Use of various ratios of extruded full fat soybean meal and dehulled solvent extracted ;soybean meal in broiler diets. Int. J. Poult. Sci. 1:9-12.

Southern, L. L., J. E. Ponte, K. L. Watkins and D. F. Coombs. 1990. amino acid-supplemented raw soybean diets for finishing swine. J. *Anim.* Sci. 1990. 68:2387-2393.

Tyus, II, James, Samuel N. Nahashon, Nathaniel Adefope, and Darren Wright. 2008. Growth Performance of Single Comb White Leghorn Chicks Fed Diets Containing Blood Meal Supplemented with Isoleucine. J. Poult. Sci. 45:31-38.

Tyus, II, James, Samuel N. Nahashon, Nathaniel Adefope, and Darren Wright. 2009. Production Performance of Single Comb White Leghorn Chickens Fed Growing Diets Containing Blood Meal and Supplemental Isoleucine. J. Poult. Sci. 46:313-321.

Tharkur, M. and C. R. Hurburgh, 2007. Quality of US soybean meal compared to the quality of soybean meal from other origins. J. Am Oil Chem Soc 84:835-843.

Traylor, S. L., G. L. Cromwell, M. D. Lindermann, and D. A. Knabe. 2001. Effects of level of supplemental phytase on ileal digestibility of amino acids, calcium, and phosphorus in dehulled soybean meal for growing pigs. J. Anim. Sci. 79:2634–2642.

U.S. Soybean Industry: Background Statistics and Information. United States Department of Agriculture, Economic Research Service. March 28, 2010.

Vucenik, I. and A. M. Shamsuddin. 2003. Cancer Inhibition by Inositol Hexaphosphate (IP6) and Inositol:From Laboratory to Clinic. J. Nutr. 133: 3778S–3784S, 2003.

van Kempen, T. A. T. G., I. B. Kim, A. J. M. Jansman, M. W. A. Verstegen, J. D. Hancock, D. J. Lee, V. M. Gabert, D. M. Albin, G. C. Fahey Jr., C. M. Grieshop, and D. Mahan. 2002. Regional and processor variation in the ileal digestible amino acid content of soybean meals measured in growing swine. J. Anim. Sci. 80:429–439.

Williams, J.E., 1981. Salmonella in poultry feeds a world-widereview. World's Poult. Sci. J. 37, 97– 105.

Wu, G., Z. Liu, M. M. Bryant, and D. A. Roland, Sr. 2006. Comparison of Natuphos and Phyzyme as phytase sources for commercial layers fed corn-soy diet. Poult. Sci. 85:64–69.

Wilhelms, K. W., C. G. Scanes, and L. L. Anderson. 2006. Lack of Estrogenic or Antiestrogenic Actions of Soy Isoflavones in an Avian Model: The Japanese Quail. Poultry Science 85:1885–1889

Wenzel, G. 2008. "The Experience of the ZKBS for Risk Assessment of Soybean." Journal fuer Verbraucherschutz und Lebensmittelsicherheit/Journal of Consumer Protection and Food Safety. 3.Suppl. 2: 55-59

Woyengo, T. A., B. A. Slominski, and R. O. Jones. 2010. Growth performance and nutrient utilization of broiler chickens fed diets supplemented with phytase alone or in combination with citric acid and multicarbohydrase. Poultry Science 89:2221–2229.

World production of osoybean…Source: FAS/USDA, United States Department of Agriculture, Foreign Agricultural Service -http://www.fas.usda.gov/psdonline/ psdReport.aspx?hidReportRetrievalName=Table+07%3a+Soybeans%3a+World+Su pply+and+Distribution&hidReportRetrievalID=706&hidReportRetrievalTemplateI D=8 November 2009.

Woodworth, J. C., M. D. Tokash, R. D. Goodband, J. L. Nelssen, P. R. O'Quinn, D. A. Knabe, and N. W. Said. 2001. Apparent ileal digestibility of amino acids and the digestible and metabolizable energy content of dry extruded-expelled soybean meal and its effect on growth performance. J. Anim. Sci. 79:1280-1287.

Xiao, C. W. 2008.Health Effects of Soy Protein and Isoflavones in Humans. Journal of Nutrition, 138:12445-12495.

Xiurong Wang, X., Xiaolong Yan and Hong Liao. 2010. Genetic improvement for phosphorus efficiency in soybean: a radical approach. Ann Bot (2010) 106 (1): 215-222.

Yi, S. Y., Jee-Hyub Kim, Young-Hee Joung, Sanghyeob Lee, Woo-Taek Kim, Seung Hun Yu and Doil Choi. 2004. The Pepper Transcription Factor *CaPF1* Confers Pathogen and Freezing Tolerance in Arabidopsis. *Plant Physiology* 136:2862-2874.

Yang, S., Barbara Vanderbeld, Jiangxin Wan and Yafan Huang. 2010. Narrowing Down the Targets: Towards Successful Genetic Engineering of Drought-Tolerant Crops. Mol. Plant (2010) 3 (3): 469-490.

Zimmermann, M. B. 2009. Iodine Deficiency. Endocrine Reviews, June 2009, 30(4):376–408.

Soya, Human Nutrition and Health

Sylvia Burssens, Ine Pertry, Delphin Diasolua Ngudi,
Yu-Haey Kuo, Marc Van Montagu and Fernand Lambein
Institute Plant Biotechnology for Developing Countries (IPBO),
Ghent University, Ghent,
Belgium

1. Introduction

Popular advice for healthy diets that may promote health and longevity include the daily consumption of at least three servings of fruits or vegetables and the variation of foods to include items derived from different plants and those plants should belong to different botanical families (Thompson H.J. et al., 1999).

Ancient civilisations in the Middle East and in America included grain legumes and cereals in well-balanced diets. In the funeral offerings found in the Egyptian pyramids various legume seeds were present, including lentils and grass pea. Apparently, legumes were a food of special consideration to be offered to kings, in contrast to the present day reputation of being the meat of the poor, with 75% of all legumes now being produced in developing countries. Excavations of ancient settlements indicate the use of both cereals and legumes (Mahler-Slasky & Kislev, 2010). A well balanced food basket promoting health and strength may have given an evolutionary advantage. The benefits of legume cultivation for soil fertility were already recognised in the 4th century BC (Flint-Hamilton, 1999).

Legumes are important factors in the natural cycle of nitrogen, being able to fix atmospheric nitrogen in symbiosis with *Rhizobium* bacteria. This enables the leguminous plants to thrive on poor soil, which makes them essential partners in the maintenance of soil fertility, and to produce protein-rich seeds. However, maintenance of optimum rates of nitrogen fixation requires continued attention by plant breeders (Provorov & Tikhonovich, 2003).

Legumes are also unusually diverse in their defence against predators by producing a large array of secondary metabolites forming their chemical armoury. Those metabolites include anti-nutrients such as inhibitors of digestion and compounds interfering with predator's metabolism reaching as far as brain function and hormonal control (Rozan et al., 2000). Interestingly, some of these metabolites are beneficial by their inhibition of human cancer cells or by antioxidant activity that can delay ageing.

Although legumes have many beneficial properties, they are not a well balanced food by themselves because of deficiencies in some essential amino acids, and should not be the sole component of the food basket. In combination with cereals that are richer in those essential amino acids which are deficient in legumes such as methionine, cysteine and tryptophan, legumes are beneficial for human health and for the world's ecology. The optimum protein quality is approximated when 60-70% cereals are mixed with 30-40% cooked legumes. This would produce a combined quality of protein comparable with meat (Bressani & Elias, 1974).

Deficiency of the sulphur amino acids methionine and cysteine in the diet during extended periods can have detrimental health effect. Cassava roots (*Manihot esculenta*) and grass pea seeds (*Lathyrus sativus*) are used as staple food, especially in drought prone areas of Asia and Africa. Both foodstuffs are notorious for their deficiency in essential sulphur amino acids and overconsumption during an extended period in unbalanced diets can result in irreversible crippling neurodegenerative diseases konzo or neurolathyrism (Bradbury & Lambein, 2011). Oxidative stress is an important factor in the aetiology of these diseases.

Both germination and fermentation have positive effects on the nutritional quality of legumes. Anti-nutritional factors such as trypsin and chymotrypsin inhibitors and the galactosidases as flatulence factors are reduced by germination or fermentation. Germination also reduces the level of fats and carbohydrates while increasing the dietary fibres and vitamins (Vidal-Valverde et al., 2002). Fermentation can increase the amino acid score as the micro-organisms involved can also produce essential amino acids. In some cases toxins present in the seeds can be reduced by fermentation (Kuo et al., 2000). Grain legumes are well known to contain deficient levels of sulphur amino acids. While this fact is widely recognised, a closer examination of several groups of antinutritional factors reveals that many of these factors target metabolic systems involving sulphur amino acids in their predators (Enneking, 2011).

Proteins that act as anti-nutritional factors such as trypsin inhibitor, chymotrypsin inhibitor and alpha-amylase inhibitor are destroyed by simple cooking (moist heat treatment) and only pose a problem when the seeds are consumed raw or are cooked insufficiently. Soybean seedlings (or more often mungbean seedlings sold as soy sprouts) are consumed raw in salads in Western cuisine, while in the East, from where the practice originated, seedlings are always heated either by deep-frying or in a wok before consumption.

The present chapter gives an overview of health and nutritional aspects of soybean for human consumption and the general description of soy foods. The potential of genetically improved varieties of soybean (GM soy) and the hurdles for its commercialization in Europe and the rest of the world are discussed.

2. Soybean, the healthiest legume

Soybean (*Glycine max*) is the legume with the highest amino acid score and closest to the standard set by the Food and Agriculture Organization (FAO) and World Health Organization (WHO). The content of the sulphur amino acids methionine and cysteine is double when compared to grass pea (*Lathyrus sativus*), the commercial legume with the lowest content of these essential amino acids (Kuo et al., 1995). Nevertheless, American farmers are spending an estimated 100 million dollars each year to supplement the soybean based feed with synthetic methionine for optimal performance by their domestic animals (Imsande, 2001). Compared to other grain legumes, soybean produces the highest yields of seeds per ha. The reason for this may in part be the allocation of better land for soybean than for other cheaper pulses.

The recent update of the 'dietary guidelines', the US-government's evidence based nutritional guidance to promote health and reduce the risk for chronic diseases and the prevalence of obesity through improved nutrition, recommends increasing soy intake as fortified beverages and other soy products (USDA, 2010). An industry-sponsored Newsletter 'Soy & Health' is devoted to the popularization of information on soy foods (Soy & Health, 2011).

The main advantage of soybean for human health, besides the nutritional value provided by its energy and protein content, is the high level of isoflavones genistein and daidzein present in the seeds. These secondary metabolites play a role in the symbiosis of the plant with *Rhizobium* but also have beneficial effects on human health. The concentration of these isoflavones in soybean is several orders of magnitude higher than in other commercial legumes. This includes the mungbean seedlings that are marketed as soybean sprouts in some European countries.

Soybean isoflavones genistein and daidzein are used in medicine at daily doses of 50 mg for the prevention of prostate cancer and breast cancer. This is the equivalent of about 50 g of soybean products or about 30 kg of other legumes (Liggins et al., 2000). Isoflavones are also antioxidants that can regenerate vitamins E and C, and are considered as a factor in the longevity of people who regularly consume soybean products in various forms. In some Asian countries with high life expectancy, the daily intake of soy-isoflavones is estimated at 50-100 mg. These soy-products include the seeds used in various recipes, soybean milk, many products made from precipitated protein such as tofu, fermented foods such as soy sauce, tempeh and also the germinated seeds. Especially the incidence of pre-menopausal breast cancer is reduced by high consumption of soybean products during adolescence and adulthood (Lee et al., 2009). It is well established that the incidence of prostate cancer is reduced in populations regularly consuming soy products. In vitro, genistein inhibits a wide range of cancer cells and inhibits several critical enzymes involved in signal transduction (Messina et al., 1994). Although more controversial, the regular consumption of soy products also seems to lower the incidence of type 2 diabetes, at least in some populations (Villegas et al., 2008).

2.1 Nutritional benefits of soya

Soybean (*Glycine max*) is one of the most important crops worldwide for producing oil and protein.

The seeds of the soya plant are an excellent source of macronutrients (protein, carbohydrate and lipid). Its composition may vary depending on the varieties and the growing conditions. The proximate composition of the whole seed of soybean, soy milk, tofu and soy sprouts is shown in Table 1.

2.1.1 Protein

Characterization. As in all legumes, globulins are the bulk of soybean proteins. Based on sedimentation, four major fractions can be distinguished:

- β-conglycinin, the principal component and sugar containing globulin;
- glycinin, the principal protein of soybeans;
- trypsin inhibitors: Soybeans contain two types of trypsin inhibitors: Kunitz trypsin inhibitor (KTI) and Bowman Birk inhibitor. KTI is reported to be one of the abundant anti-nutritional proteins in soybean seeds and it has been characterized as a food allergen in humans (Quirce et al., 2002).
- enzymes and hemaglutinins: lipoxygenase is technologically the most important enzyme found in soybean because it catalyses the oxidation of the poly-unsaturated fatty acid by molecular oxygen, leading to the development of rancidity and beany flavor. Hemaglutinins (lectins), carbohydrate–binding proteins, have been found to exert specific physiological effects as antinutritional protein factors (Natarajan, 2010) but they are easily inactivated by heat.

per 100 g	Soybean	Soy milk	Tofu	Soy sprouts
Proteins (g)	**38.0**	**3.7**	**12.0**	**5.5**
Fat (total) (g)	**18.0**	**2.2**	**7.0**	**1.0**
saturated fatty acids (g)	2.5	0.4	-	-
mono-unsaturated fatty acids (g)	4.0	0.5	-	-
poly-unsaturated fatty acids (g)	10.7	1.3	-	-
-linoleic acid (Ω-6) (g)	9.8	1.2	-	-
-alpha-linolenic acid (Ω-3) (g)	0.9	0.2	-	-
Carbohydrates (g)	6.3	2.8	1.0	4.7
Fibers (g)	22.0	0.6	-	2.38
Calcium (mg)	201	120.0	87.0	32.0
Magnesium (mg)	220	-	99.0	19.0
Potassium (mg)	-	-	94.0	235.0
Vit B2 (mg)	-	0.2	-	-

Table 1. Overview of the nutritional composition of soybean, soymilk, tofu and soy sprouts (Adapted from: http://www.sojanet.com/nutritional-benefits.html#c3501)

β-conglycinin and glycinin are storage proteins found in the protein bodies within the cells of the cotyledons. Severe allergic reactions have been reported after human consumption of those soy proteins (Bardare et al., 1988). The main allergens found in soy protein are Gly m Bd 60K (a subunit of β-conglycinin), Gly m Bd 30K (previously described as the 34-KD vacuolar protein, P34) and Gly m Bd 28K (Ballmer-Weber et al., 2007).

Besides these allergens, soybeans like other legumes contain a number of anti-nutritional factors such as trypsin and chymotrypsin inhibitors. These enzyme inhibitors are associated with the depletion of sulfur amino acids in the consumer's metabolism. A significant portion of the low cysteine content in grain legume seeds can be found in proteins designed to be active in hostile extracellular environments, where cysteine functions to stabilize these proteins by disulfide bridges. This cysteine depletion is enhanced by these digestive enzymes and manifests itself by an enlargement of the pancreas (pancreatic hypertrophy) and a depletion of sulfur amino acids, since digestive enzymes also require elevated levels of cysteine for stabilization in a hostile extra-cellular environment (Enneking, 2011). Generally, these stabilized proteins can be destroyed by moist heat during food preparation. However, depending on the effectiveness of the heat treatment [heat, moisture, time], some activity, particularly for the chymotrypsin inhibitors, may remain.

Nutritional value. The proximate protein content of soybean is very high (38%) but it is not only the total amount of protein that is important; the quality of the protein also has to be taken into account. All eight essential amino acids which are necessary for human nutrition and are not produced naturally in the body are found in soybean protein. When comparing the essential amino acids of soybean with those from the reference protein (FAO/WHO), methionine and cysteine (sulfur containing amino acids) are the limiting factor with a chemical score of 47, compared to 100 for an ideal protein for human nutrition. While sulfur amino acids are the limiting factor in soybean, lysine content is very high, and that makes soybean an excellent complement for cereals that are deficient in lysine but excellent sources of S-containing amino acids (Lambein et al., 2005).

The chemical score alone is not sufficient to evaluate the protein quality since it does not take into account the digestibility and the biological availability of the amino acid. The protein in most soybean products has a Protein Digestibility Corrected Amino Acid Score

(PDCAAS) that approaches 1.0 for soy protein and 0.92 for whole soybeans, indicating that both amino acid pattern and digestibility of soya protein are excellent for human nutrition. Consuming soya foods is an excellent way to increase the protein content of the diet. Although high protein diets may increase the risk of developing kidney disease in susceptible individuals, some studies indicate that soya protein favorably affects renal function in comparison to animal proteins (Sarwar, 1997).

	Soybean	FAO/ WHO reference protein
ILE	4.6	4
LEU	7.9	7
LYS	6.5	5.5
MET + CYS	2.6	3.5
PHE + TYR	8.2	6
THR	3.9	4
TRY	1.3	1
VAL	4.9	5

Table 2. Soybean Essential Amino acids composition as % of total amino acids versus FAO/ WHO reference protein (adapted from Lecerf & Fressin, 1995)

2.1.2 Lipids
The lipids (crude oil) content of soybeans is approximately 20% which consists of:
- Triglycerides representing about 96 % of the soybean lipids
- Phospholipids or lecithins (2%) which are used in medical and food industries as emulsifiers
- Unsaponifiable lipids (1.6 %) mainly tocopherols (Vit E) and sterols
- Free Fatty acids (0.5 %)

Soybean contains a heart-healthy balance of fatty acids, high in mono and polyunsaturated fatty acids (80 % of total fatty acids primarily in the form of linoleic acid) and low in saturated fatty acids (20 %) (Kris-Etherton et al., 1988). Linoleic acid, when substituting saturated fatty acids in the diet reduces blood cholesterol levels. Furthermore, soybean is one of the few good plant sources of the essential fatty acid, alpha-linolenic acid (ALA), an omega-3 fatty acid that may have independent coronary benefits (See the fatty acid profile of soybean in Table 3).

Fatty acid	%
Lauric acid (C12:0)	4.5
Myristic acid (C14: 0)	4.5
Palmitic acid (C16:0)	11.6
Stearic acid (C18:0)	2.5
Oleic acid (C18:1)	21.1
Linoleic acid (C18:2), Ω-6	52.4
Linolenic acid (C18:3), Ω-3	7.1

Table 3. Fatty acid profile of soybean oil (adapted from Weber et al., 2007).

The health claim that "Intake of 25 grams of soya protein a day, as part of a diet low in saturated fat, may reduce the risk of heart disease" has been approved by the American Food and Drug Administration and the British Joint Health Claim Initiative. Moreover, the important role of soy foods in displacing higher-saturated-fat foods from the diet, thereby helping to lower blood cholesterol levels was highlighted by the American Heart Association (Sacks et al., 2006). The cholesterol lowering effect of the poly-unsaturated fatty acids of soybean is mainly affecting the LDL-cholesterol, and also decreases the triglyceride level. Indeed, the excellent fatty acid composition of soya offers various advantages.

2.1.3 Carbohydrates

The total carbohydrate content of soybean is about 30%. Unlike other beans, soya is low in high molecular weight carbohydrates in soya. Soybeans contain 10-13% soluble carbohydrates of which sugars (sucrose, fructose, saccharose, raffinose and stachyose) represent 10-12% and starch 1%. Raffinose and stachyose are tri and tetrasacchide, respectively which are not broken down by human digestive enzymes but by the bacteria present in the small intestine with production of intestinal gas (flatulence). Soybean also contains 18% fibers, a mixture of cellulosic and noncellulosic structural components (cellulose, hemicellulose and pectin substances).

Considerable efforts are underway to further improve the nutritional quality of this healthy grain legume. For example of the 3 major oligosaccharides present in soybean only sucrose can be digested by monogastric animals. As stachyose and raffinose are indigestible and are considered as anti-nutritional factors which cause a reduction of energy in the diet and flatulence, research has focused on their removal from soybean meals. Recently, alleles of soybean raffinose synthase genes have been identified which are associated with a low stachyose and raffinose content in soybean plants and seeds. This identification in combination with molecular marker breeding is a promising tool to introduce the trait in soybean varieties (Dierking & Bilyeu, 2008; Schillinger et al., 2010). In cases where traditional breeding techniques have not yet succeeded, genetic transformation can be the method of choice for this endeavour.

2.1.4 Minerals

The mineral content of soybeans is about 5%. Major constituents are calcium, potassium and magnesium. Soybean milk is poor in calcium, only 12 mg/100 g. But the content of soya drinks is always enriched with calcium to be comparable to cow's milk (Zhao et al., 2005). Iron, copper and zinc are other minerals that are present in soybeans as trace elements.

2.2 Uses of soybeans as food

Raw soybeans cannot be eaten as such because of the presence of trypsin inhibitors that can disrupt digestion activities in the stomach, leading to cramps and associated discomfort. (Mccue & Shetty, 2004).

Soybean foods are typically divided into three categories: non-fermented, fermented and fortified.

Traditional non-fermented soya foods include fresh green soybeans, whole dry soybeans, soy nuts, soy sprouts, whole-fat soy flour, soymilk and soymilk products, tofu, okara and yuba.

Traditional fermented soy foods include tempeh, miso, soy sauces, natto and fermented tofu and soymilk products.

	General description	Uses
Nonfermented soya foods		
Soy milk	Heated water extract of soybeans after grinding and filtration. Resembles dairy milk	Served hot or cold, as breakfast, beverage, or with other foods
Tofu	White protein curd precipitated from soya milk with salt or acid	Cooked with or without meat, vegetable, and seasonings and served as a main dish or soup
Soy sprouts	Germinated soybeans in dark yellow cotyledons with white sprouts	Cooked as vegetable or in soup
Soy film – Yuba	Creamy, yellowish protein-lipid film formed from surface of boiling soya drink. Sheets, sticks, or flakes	As delicacy, cooked with meat or vegetables, or in soups
Edamame	Green immature soybeans	Cooked in pod or pod removed. Served as snack or vegetable
Roasted soya beans	Dry roasted soy beans, seasoned or non-seasoned.	As a snack or made into powder.
Fermented oriental soya Foods		
Miso	Heated water extract of soybeans after grinding and filtration. Resembles dairy milk	All purpose seasoning for dishes or soups
Natto	Cooked whole soya beans fermented with *Bacillus natto*. Soft beans covered by viscous, sticky polymer, distinct aroma.	Seasoned and eaten with cooked rice.
Tempeh	Cooked and dehulled soya beans fermented with *Rhizopus oligosporus*. Soft beans bound by white mycelia, cakelike, nutty flavor	Fried or cooked as part of meal, snack, or in soups.
Shoyu (Soya sauce)	Dark-brown liquid extracted from a fermented mixture of soybeans and wheat and is based on the use of the *Aspergillus oryzae* strain.	All-purpose seasoning

Table 4. Different soya foods (Source: http://www.sojanet.com/the-plant-the-food/different-soya-food.html)

Fortified soy foods are frequently used in humanitarian programs to prevent and address nutritional deficiencies, partially precooked and milled soybean is used to provide protein in **Corn Soya Blend** (CSB), the main fortified blended food distributed to affected people but sometimes soya is also mixed with wheat in Wheat Soya Blend (WSB). Those fortified blended foods are also generally used to provide extra micronutrients and to complement the general ration in Supplementary Feeding, Mother and Child Health programs and during disaster or emergency operations by the World Food Program (2006)

2.3 Soybean sprouts or mung bean sprouts?
Sprouts of mung bean (*Vigna radiata*, called green gram in India, green bean in China) are often marketed as 'soy sprouts', creating confusion among nutritionists. In the East, mung bean and soybean sprouts are sold side by side and correctly labeled. In the West, the real

soybean sprouts are rare and mung bean sprouts are labeled as soy sprouts. Mung beans are easier to sprout, cheaper and more widely available than soy sprouts. Real soybean sprouts are much richer in the isoflavones genistein and daidzein and also have more protein than any other sprouts plus an appealing flavor and taste. Health conscious consumers with the intention to lower the risks for some cancers by consumption of high isoflavone containing foodstuffs are in fact cheated by this mislabeling. For sprouting purposes, small-seeded soybeans are preferred because of the relatively thicker seed coat than the large-seeded soybeans and also because of the higher rate of germination. One gram of dry beans yields about 8-10 grams of sprouts after 4-5 days of germination. The sprout (hypocotyl) is then about 3 cm long. In mung bean the cotyledons shrink after one week germination and the first leaves become visible. The cotyledons of soybean sprouts are bigger and more yellow. Although the level of anti-nutritional factors such as trypsin inhibitors is reduced during germination, while mungbean sprouts are often used in Western cuisine uncooked together with a variety of other sprouts, in the East the sprouts are always boiled in soups or fried in a wok. Unfortunately, prolonged heat also destroys some of the vitamin C (Wai et al., 1947). According to the history book by Gavin Menzies (2002) soybean was one of the foods taken on long ocean voyages by the Chinese in 1421, and sprouted on board. This may have protected those seamen from vitamin deficiency related diseases. Indeed, germination is a simple post-harvest processing technique that increases the levels of riboflavin by up to 642%, niacin by up to 443% and ascorbic acid by up to 467% (Kaushik et al., 2010).

Fig. 1. Comparison of sprouts of mungbean (*Vigna radiate*) and soybean (*Glycine max*) (left picture), with a commercial product of mungbean sprouts sold as soysprouts, (right picture). Notice the shrunken cotyledons in the commercial product and the intact yellow cotyledons in the soybean sprouts.

An early detailed scientific report was published by Schulze in 1889 "On Some Nitrogen-Containing Constituents in Soy Sprouts (or Etiolated Soy Shoots)" (cited in Shurleff & Aoyagi, 2011). These observations were more botanical than culinary and were done with soybeans germinating in a field. Although the culinary use of soybean sprouts has an almost one thousand year tradition in China, the first known reference to soy sprouts for food use was by Li Yu-Ying in 1910 and 1912 (cited in Shurleff & Aoyagi,. 2011). By late 1910 or early 1911, he was producing soy sprouts in his soy foods plant near Paris. A detailed history of soy seedling production and consumption is described by Shurtleff and Aoyagi (2011). Bau et al. (1997) found that the trypsin inhibitor activity of soybeans was reduced by 30% during

sprouting and that the vitamin C content increased from 0 to 25 mg/100 gm. Fernandez-Orozco et al. (2008) compared the antioxidant capacity in soybean sprouts and mung bean sprouts and found a greater increase during sprouting of mung bean than in soybean, while the total tocoferol (vit E) content increased much more in soybean sprouts.

3. GM soy

3.1 History

Since the introduction of genetically modified (GM) crops in the nineties GM soy has been one of the most prominently cultivated GM crops. The first traits to be introduced in soy by means of genetic engineering were herbicide resistance to glyphosate and glufosinate. The GM soy plant acquires glyphosate resistance through the action of a genetically introduced enzyme from bacterial origin (Thompson C.J. et al., 1987) that acts as an inhibitor to the herbicide. Glyphosate (N-(phosphonomethyl)glycine), inhibits the 5-enylpyruvylshikimate-3-phosphate synthase (EPSPS) of plants and certain bacteria. This enzyme catalyses a key step in the synthesis pathway of the aromatic acids, hormones, plant metabolites, lignins and other phenolic compounds (Dill, 2005). Glufosinate (dl-homoalanin-4-yl(methyl)phosphinic acid) on the other hand inhibits glutamine synthase, required for the conversion of L-glutamic acid to L-glutamine in the presence of ammonia. Blocking this pathway in plants results in toxic ammonia levels and cell death (Duke, 1990).

Monsanto and Bayer CropScience have commercially developed and launched respectively GM glyphosate and glufosinate resistant soy lines, both of which have been approved for human and animal consumption. Since its first commercial cultivation in the US in 1996, Roundup Ready soy developed by Monsanto still is the principal biotech crop at global scale. The total herbicide resistant GM soybean area is cultivated on 73.3 million hectares, representing 50% of the total area of GM crops in the top-ten biotech crops. Countries that are growing GM soy are the USA, Brazil, Argentina, Paraguay, Uruguay, Bolivia, Mexico, Chile, and Costa Rica. In Paraguay and Bolivia GM soy accounts even for the total area of GM crops (James, 2010).

Herbicide tolerance is the only GM soybean trait that has been commercialized to date (see Table 5). Worldwide, only 3 different events of genetic modification can be found, commonly known as Roundup ready soy and Roundup ready 2 soy (Monsanto Company), both resistant to glyphosate, and Liberty Link soy (Bayer Crop Science) which confers resistance to glufosinate. Eleven additional GM soybean events have received regulatory approval in at least one country and are ready for commercialization. Amongst these are also the first GM soybeans modified to increase the value for industry by having an increased oleic acid content, and which are expected to be ready for global use by 2012 (Plenish, Dupont). Three other lines (Optimum GAT, Cultivance, Bt Roundup Ready 2 Yield) are also expected to be commercially launched within the next two to five years (Dupont, BASF Plant Science and Monsanto Company). Other GM soybean lines like 260-05 (Dupont), W62, W98, GU262 (Bayer CropScience) have already been regulatory approved in the nineties but have not yet been commercialized (James, 2010; Stein and Rodriguez-Cerezo, 2009).

3.2 Biotech soy in the pipeline

Besides the first generation of traits that have been commercialized in GM soybean, many other GM varieties with interesting traits have been developed in the lab, most of them in the private sector. Remarkably most of the notifications that have been submitted but have

not yet made it to the market. Four new lines are in advanced research phases, while 6 lines are pending regulatory approval in at least one country resulting in a total of 10 lines that could join the market within the coming years (Stein and Rodriguez-Cerezo, 2009, United States Department of Agriculture – Animal and Plant Health Inspection Service (USDA-APHIS), 2011) (see Table 5). So far only one stacked (with more than one genetic modification event) soybean variety, Dupont's DP305423 x GTS40-30-02, has been regulatory approved and is ready for commercialization (see Table 5). If in addition, all available single events would be combined into stacked varieties, this would result in a theoretical market of over 100 GM soybean lines (Stein and Rodriguez-Cerezo, 2009). Besides new herbicide tolerant soybean events, two new nutritionally enhanced soybean lines (Monsanto Company) are in the process of regularization in February 2011 (see Table 6). SDA omega-3 soybeans are developed to produce oil rich in omega-3 fatty acids, while the oil end product derived from Vistive Gold soybeans would contain levels of mono-unsaturated fatty acids similar to olive oil (Monsanto).

3.3 Nutritional fortification strategies

Biotechnology with its current high throughput bioinformatics technologies such as genomics, proteomics, phenomics, and metabolomics offers even more potential for developing useful GM crops. Recently the 1.1 gigabase soybean genome has been completely sequenced. This will allow the association of mapped phenotype effectors with the causal DNA sequence of important crop production traits and those important to seed quality for human nutrition, animal production, and biofuel production (Schmutz et al., 2010).

Apart from GM soy for industrial application, nutritionally ameliorated or enhanced GM soy will be the next generation. Despite the widespread use of soybeans and derived products in food and feed, associated nutritional problems remain an issue. Soybean contains multiple allergenic proteins: Gly m Bd 60 K, Gly m Bd 30 K, and Gly m Bd 28 K are the 3 main soy proteins triggering allergenic reactions. Since mutagenesis and breeding could not reduce or remove all three allergens, genetic engineering was used to address removal of Gly m Bd 30 K by transgene-induced silencing of the corresponding gene. This approach can allow sensitive individuals to make use of soybean products in the future (Herman et al., 2003).

In addition to allergenicity, amino acid deficiencies are known to limit soybean nutritional quality as methionine, cysteine and threonine levels are below FAO/WHO recommended values. Genetic engineering could provide a solution for these issues by designing nutritionally fortified soybeans. Despite the fact that these amino acids can be supplemented in the diet, a more attractive solution would be to produce soybeans with an improved amino acid profile. One successful approach to increase the methionine content of soybean was the introduction of the Brazil nut albumin gene. However, as the Brazil nut albumin was identified as a major allergen, this product was not further developed and new approaches were explored (Clarke & Wiseman, 2000). A successful approach is the introduction of cereal storage proteins rich in methionine and cysteine (Li et al., 2005).

Although soy is a known phosphorus source, most phosphorus is present as phytic acid, which cannot be digested by monogastric animals such as poultry and swine. This does not only result in non-optimal levels of phosphorus and minerals, but also contributes to wastewater pollution. As supplementation is costly and seems to contribute to the pollution

problem, one might prefer to make use of a soybean strain expressing a bacterial phytase, resulting in the production of utilizable inorganic phosphorus (Bilyeu et al., 2008). Soybeans are also a known source of vitamin E and folate. Although vitamin E deficiency in humans does not result in a typical disease state or disorder, it might lead to an increased risk of atherosclerosis and other degenerative diseases (Bramley et al., 2000). Folate deficiency is known to cause anemia, high levels of plasma homocysteine hereby increasing the risk for

Product name	Developer	Trait	Status of commercialization
Commercialized			
Roundup Ready	Monsanto	Herbicide tolerance (to glyphosate)	commercialized
Genuity Roundup Ready 2 Yield	Monsanto	Herbicide tolerance (to glyphosate)	commercialized
Liberty Link (event A2704-12)	Bayer CropScience	Herbicide tolerance (to glufosinate)	commercialized
Authorized in at least 1 country, ready for commercialization			
260-05	Dupont	Crop composition (altered fatty acid and oil content)	not commercialized
Liberty Link (event A5547-127)	Bayer CropScience	Herbicide tolerance (to glufosinate)	2012
Optimum GAT	Pioneer Hi Bred	Herbicide tolerance (to ALS inhibitors and glyphosate)	2011-2012
Cultivance	BASF Plant Science and Embrapa	Herbicide tolerance (to imidazolinone)	2012
Bt Roundup Ready 2 Yield	Monsanto	Insect resistance and herbicide tolerance (to glyphosate)	2013
Plenish	Dupont	Crop composition (high oleic acid content)	2012
MON87701	Monsanto	Insect resistance (to Lepidoptera)	n/a
DP305423 x GTS40-30-02	Dupont	Crop composition (altered fatty acid and oil content) and herbicide tolerance	n/a
W62	Bayer CropScience	Herbicide tolerance (to glufosinate)	not commercialized
W98	Bayer CropScience	Herbicide tolerance (to glufosinate)	not commercialized
GU262	Bayer CropScience	Herbicide tolerance (to glufosinate)	not commercialized

Table 5. Commercial status of authorized GM soybeans worldwide (n/a: not available)

Product name	Developer	Trait
Pending regulatory approval		
n/a	n/a (China)	Insect resistance
SDA Omega 3	Monsanto	Crop composition (stearidonic acid content)
Vistive Gold	Monsanto	Crop composition (improved fatty acid profile)
Dicamba tolerant	Monsanto	Herbicide tolerance (to dicamba)
DHT	Dow AgroSciences	Herbicide tolerance (to 2,4D and glufosinate)
n/a	Bayer CropScience	Herbicide tolerance (to isoxaflutole and glyphosate)
Commercial R&D pipeline		
n/a	Syngenta	Nematode resistance
n/a	Syngenta	Herbicide tolerance (to HPPD inhibitors)
n/a	Bayer CropsScience	Herbicide tolerance (to isoxaflutole and glufosinate)
n/a	Monsanto	Higher yield

Table 6. GM soybeans in the pipeline worldwide (n/a: not available)

cardiovascular disease or stroke and, in association with the maternal status, spina bifida in infants (Scott et al., 2000). Therefore it might be interesting to look for strategies to enhance vitamin E and/or folate synthesis and accumulation.

Soybean has several other interesting nutritional properties. Its high level of isoflavones as antioxidants and phytooestrogens, have received much attention because of their positive effect on osteoporosis, cardiovascular disease and cancer prevention (Cassidy et al., 1994; Messina, 1999; Setchell & Cassidy, 1999). Although the beneficial health effects of soy isoflavones are strongly being debated (Erdman et al., 2000; Sebastian, 2005), soybean consumption is considered a factor in the longevity of people in the Orient. One may think of increasing the isoflavone content in soy as an edible disease-preventing drug. Also the calcium bioavailability, naturally present in beans (Messina, 1999), could be the subject for a GM upgrade in soybeans. All together these data indicate that soybean has much potential for further development, which may result in the availability of a variety of nutritionally enhanced soybeans in the future as an important source of functional food.

3.4 Public acceptance

Many independent studies have been published demonstrating the positive agro-economic impact and socio-environmental effects of biotech crops, including GM soy, in different parts of the world (Brookes & Barfoot, 2009; Carpenter, 2010; James, 2010; Park et al., 2011; Trigo & Cap, 2006). In most countries GM soy accounts for the largest share of benefits, mainly due to the fact that soy bean is the major biotech crop with the largest percentage of

acreage. Direct and indirect benefits, with as major beneficiaries the growers and technology developers, include higher farmer incomes, job generation, lower price for the consumers and environmental benefits, e.g. less tilling, better second-cropping practices, better water, pesticide and diesel usage a well as CO_2 measures. Despite the fact that biotech crops are the most fast adopted technology in modern agriculture (James, 2010), and have clear benefits, the global public acceptance still tends to be rather low. Although approved GM varieties that went through the regulatory systems are proven to be equal in nutrient composition to, and as safe as their traditional counterparts in terms of allergenicity and toxicity and that no significant harm to human, animal health and the environment has been reported, the public at large is still very reluctant and not aware of potential benefits of GM crops. The public perception however does strongly vary between different continents and from country to country. Key findings from the last Eurobarometer on Life Sciences and Biotechnology (Gaskell et al., 2010) on the attitude of European citizens towards GM food show that the values of acceptance of GM food are much higher in Member States where the crops are grown, demonstrating a clear link between private attitudes and public policies.

When it comes to the adaption of novel, innovative and, more importantly, life-saving technologies such as GM crops, an interesting paradigm shift is occurring which most likely will be reinforced during the next decades. Out of the 29 biotech crop countries in 2010, only 10 were industrialized countries. Strikingly the lead developing countries for the cultivation of GM crops are China, India, Brazil, Argentina, and South-Africa (James, 2010), that are equally emerging and rapidly expanding economies. Developing countries are also predicted to exceed the industrial countries in percentage of biotech crops cultivated before 2015 (James, 2010). Developing countries with the largest acreages of GM crops are also amongst the biggest growers of GM soy.

3.5 EU policy in contrast to global policy development

The EU Policy, with its more than five year moratorium (1998-2004) on GM crops under the pressure of NGOs and the public opinion, has always been in strong contrast with the US Policy since the beginning of the biotech crops era. While the USDA allowed unlimited planting of GM herbicide resistant soy and recently has approved GM soy with a higher oleic acid content developed by DuPont Canada Agricultural Products, Romania even had to stop growing Roundup Ready soya after having entered the EU. Until today only two GM crops are approved for cultivation (Bt maize and Amflora potato). In addition the EU has adopted a zero-tolerance policy towards imports containing even trace amounts of non-approved GMO's, which can cause disruptions on the agricultural import and export market.

Based on the principle of 'Freedom of Choice' the EU has introduced its labeling policy for food stuff that has been produced with the aid of genetic engineering. The Regulation (EC) No 1830/2003 of the European Parliament and of the Council of 22 September 2003 concerns the traceability and labeling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms and amending Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms (http://eur-lex.europa.eu). Despite the fact that processing of raw material often renders identification of GM traces in the end product impossible, labeling depends on the origin of the ingredients of the product (GM or non-GM). As a result all soy products must be labeled if the raw material at least partially consisted of GM soybeans. Technical unavoidable or unintentional admixtures of regulatory approved GM content below the 0.9

% threshold limit value do not fall into this category. Baked goods (bread, biscuits and snacks) containing flour, for example, and/or oil from GM soybean, sweets containing lecithin from GM soy, and fat products such as margarine, vegetable oils, mayonnaise require labeling (For a complete overview see Table 7) (GMO compass, 2011).

Soy ingredient	Use	Processing and testing products for GM content	Labeling
Oils and fats	Margarine, vegetable oils, mayonnaise, etc.	Refining soy oil by heating to 120°C under a vacuum to remove solvent residues and other unwanted substances. DNA and proteins destroyed during the process to such an extent that no GM testing is possible.	Yes
Lecithin, other emulsifiers	Chocolate, desserts, baked goods, other processed foods	Lecithin is extracted from soy oil. GM trace identification maybe possible when using non-refined soy oil as a source.	Yes
Tocopherol/Vitamin E	In fatty foods to prevent oxidation, vitamin fortified products	By-product of plant oils. GM trace identification identical to lecithin.	Yes
Soy protein additives, soy isolate	Prepared foods (soups, sauces), meat substitutes, diet foods, imitation milk products	From roasted, de-oiled soy flakes. Detection possible, but more difficult along with the processing into the final product.	Yes
Soy meal, semolina flour	Bread, snacks, pasta	Similar to soy additives Baking destroys often traces of GM content	Yes
Hydrolyzed soy protein	Soy sauce, seasonings	Protein chemically changed by acids or enzymes DNA usually destroyed along the process.	Yes
Products from whole soybeans	Tofu, soy drinks, miso, soy flour	GM traces detectable in products derived from whole soybeans	Yes
Animal feed	Indirectly for animal products as meat, eggs and milk	Animals that have been fed or not with GM feed cannot be distinguished because all DNA (GM and non-GM) is degraded during digestion.	Feed: Yes, Animal products: No

Table 7. Overview soy-based food and feed (Adapted from GMO compass)

The EU imports each year 18 million tons of soybeans and 20 million tons of soy meal from Brazil, the US, and Argentina (www.gmo-compass.org), the largest GM soy producers. According to the EU Observer (www.euobserver.com) the EU imported' more than 51 million tons of animal feed of which half was GM soy from Argentina and Brazil. Despite the high dependence of the EU livestock industry on feed import, in 2009 Europe blocked 180,000 tons of GM soy from the US as a result of its zero-tolerance policy. Although the GM soy was approved for import, traces of non-approved maize were detected (Wager & McHughen, 2010). The current situation of asynchronous approvals, where the EU has only two approved GM soy varieties and one ongoing renewal of authorization (EU register of genetically modified food and feed), while 5 new soybean events are ready for commercialization (see Table 6) might even worsen the situation for EU imports. The zero-tolerance policy could lead to a net shortage between 3.3 million (minimal impact scenario) and 25.7 million tons (worst case scenario) of soybean meal resulting in increased feed costs and meat prices for consumers (Wager & McHughen, 2010). Therefore, the EU committee recently voted for an easing of the labeling policy with the allowance of 0.1% unapproved GM material but only in animal feed imports. Concerns are however being raised that it will be extremely costly to separate the material according to intended use (Dunmore, 2011).

3.6 Costs of compliance with biotechnology regulations

Despite the great potential of GM crops, only a limited number of traits have been commercialized (James, 2010, Table 6). The major reason for this is, aside from the often lacking regulatory framework in developing countries, the regulatory costs related to bringing a GM product to the market. These costs can be the direct result of the necessary compliance with biosafety regulations or can arise from delays during the regulatory approval process (Bayer et al., 2010). While the average time to gain import approval for example in North America and Brazil is one and even less than one year respectively. Europe lags behind requiring an average of 3 years to authorize GM imports (Wager & McHughen, 2010). The regulatory compliance costs can vary from tens of thousands to millions of dollars (Van Montagu, 2010) depending on the country, the GM specific event and the intended use or the developer (Bayer et al., 2010). Direct compliance for herbicide-tolerant corn in top producing and importing countries including US, Canada, Argentina and the EU, can go up to $15,510,000. Sixty percent of these costs arise from the production of tissues to perform the biosafety studies, the compositional assessment, protein production and characterization and the molecular characterization. These costs do not even include preregulatory safety assessments, which are considered as research or private compliance costs resulting from regulatory delays (Kalaitzandonakes et al., 2007). The whole process starting from discovery and research over validation to the market phase of a GM crop is estimated to cost between 52 and 100 million US$ (Monsanto). The costly regulatory framework renders the marketing of GM crops far more expensive than traditionally bred crops leaving public institutions and spin-offs, especially in developing countries, merely out of the loop when it comes to commercializing GM crops. As a consequence, the development of GM crops is mostly in hands of large private companies which turn to commercially interesting crops as corn and soybean, and successful traits with broad applications. As such, orphan crops with non attractive returns of investments but of importance to local populations in least developed countries are easily left behind (Van Montagu, 2010). Crops that are considered a life-insurance during drought for poor subsistence farmers such as grass pea (*L. sativus*), but without global economic importance, are ignored.

One example of a public research institution project that faced a more than 12 years delay as a result of regulatory burdens is the Golden Rice. Golden Rice was developed in 1999 in the lab to produce vitamin A precursor by inserting two genes, phytoene synthase and phytoene double-desaturase, would provide a way to reduce vitamin A deficiencies amongst rice-dependent poor populations prone to night-blindness. Although it was ready in 1999, it will only be commercially launched in 2012. The delay faced was due to long time required for field-testing approval (more than 2 years) and subsequent data gathering for the regulatory dossier (more than 4 years). In addition, the project had to be taken up into a public-private partnership with Syngenta to move the product development forward (Potrykus, 2010).

3.7 Soybean as a life saver on marginal lands
Due to climate change and water shortage, addressing water scarcity in arid and semi-arid rural areas will be one of the priorities in the coming decades in order to secure lives and secure their livelihoods (WHO and UNICEF, 2006; FAO/UN-Water, 2007). Moreover a significant increase for water irrigation of agricultural lands has been predicted (Bruinsma, 2009). Water shortage is also an important restrictive factor for crop yield. In the case of soya, drought stress strongly reduces vegetative branch and reproductive growth (Frederik et al., 2001), increases the rate of pod abortion during early development stage (Liu et al., 2004) and can as a result limit the total yield by up to 40% (Specht et al., 1999). Several physiological and biochemical approaches can be used for the development of drought resistant soy bean varieties such as modulation of root related traits (root morphology and plasticity, nitrogen fixation) or shoot related traits (stomatal conductance, epidermal conductance, leaf pubescence density, water use efficiency, osmotic adjustment) and soybean seed and grain-filling practices. Progress in (marker assisted selection) breeding strategies has however been rather limited as the main focus for soybean breeding programs was on biotic resistance in the past. Moreover drought resistance is a complex trait not only by the genotype, but also by the environment and their interaction. A constant selective pressure is required to be able to directly select for this trait which is not easy to achieve when it comes to drought stress (Lakshmi et al., 2009). By breeding for example, two soy lines were achieved which had an improved symbiotic nitrogen fixation and a yield advantage in environments with moderate soil water shortage. Unfortunately this trait would not allow maintaining the yield gain under very severe drought conditions (Sinclair et al., 2007). Therefore, genetically introducing drought resistance into soy may also be a promising strategy to cope with environmental changes, soil erosion and population growth.

Drought tolerance in soy could be addressed by genetic engineering of regulatory genes, analogous to the examples obtained by introducing the transcription factors ZmNF-YB2 and SNAC1 in maize and rice (Hu et al., 2006; Nelson et al., 2007). A different approach could be to genetic engineer functional genes, as for example trehalose-6-phosphate synthase/phosphatise in rice resulting in the accumulation of trehalose and increased drought stress tolerance (Garg et al., 2002). Soybean was genetically engineered to over-express L-Δ1-pyrroline-5-carboxylate reductase resulting in proline accumulation and improved drought stress tolerance (De Ronde et al., 2004). Additional research will be required to identify and use drought stress related genes for a successful engineering strategy.

An alternative interesting source for such abiotic stress resistance genes would be grass pea (*L. sativus*) that is the most environment tolerant legume that thrives on poor soil with

minimal inputs without irrigation and the most efficient nitrogen fixer among commercial legumes (Campbell, 1997). The plant is also considered for phyto-remediation (Brunet et al., 2009) and was suggested to help removing heavy radioactive metals from the human body after the Tchernobyl accident (pers. comm.). The plant is also more resistant to biotic and abiotic stress than other legumes and needs little or no inputs. It is considered a life-saver during droughts and famine in areas of Africa and Asia prone to droughts (Campbell, 1997). This controversial legume has been cultivated since the Neolithic era and is appreciated by farmers for its easy cultivation and for its production of tasty seeds when other crops fail due to drought. However, overconsumption during extended periods in socio-economic settings dominated by illiteracy and poverty can lead to irreversible crippling or neurolathyrism. Recent epidemiological research has identified protective factors as literacy, the use of at least one third of cereals in the diet and the addition of antioxidant-rich condiments such as onion, garlic and ginger to the grass pea preparations (Getahun et al., 2003, 2005). In a well balanced diet grass pea is harmless. However, because of its toxic reputation grass pea is a neglected crop that receives little attention from major research institutions. The genes for this important and sometimes live-saving environmental tolerance are not yet known. The possibility that this environmental tolerance is linked to the presence of the neuro-excitatory amino acid β-ODAP (β-N-oxalyl-α,β-diaminopropionic acid) cannot be ruled out. This unusual amino acid is a good chelator for copper and zinc and may play a role in phytoremediation (Brunet et al, 2008).

The alternative strategy could be to introduce the biosynthetic pathway for genistein into legumes more tolerant to environmental stress. Grass pea (*L. sativus*) could then be genetically improved to become a health promoting crop that at the same time can improve marginal soil with minimal ecological cost. Efforts to improve the nutritional quality of grass pea are now directed at the lowering or elimination of a neuroexcitant amino acid beta-ODAP and at the improvement of the amino acid score by increasing methionine and cysteine in the seeds (Vaz-Patto et al., 2006).

4. Conclusions

We can safely propagate that the presence of various preparations of soybean in a food basket containing also a variation of cereal products and vegetables belonging to other plant families than the legumes is beneficial for human health and for the world ecology. Soybean has particular and proven advantages on human health and longevity. The consumption of soybean products such as tofu and soymilk are becoming more popular in the West, but it is disturbing that mungbean seedlings continue to be mislabeled as soy sprouts, giving fundamentally wrong information to health conscious consumers.

Untreated grain legume seeds can contain significant levels of digestive enzyme inhibitors to cause harm in the consumer. Especially for the poor who can not afford a well-balanced diet, prolonged overconsumption of foods that are deficient in essential sulfur amino acids, such as legumes or cassava, can have detrimental medical consequences (Bradbury & Lambein, 2011).

Several high-level reports have demonstrated the need for higher food productivity in the coming years on less arable land (Foresight, 2011; The Royal Society, 2009). Especially those living in drought prone areas of Africa and Asia need the attention of the scientific community and the authorities to break the vicious cycle of malnutrition, illiteracy and underdevelopment. GM crops, including GM soy, have been recognized to have the

potential to considerably contribute to the alleviation and reduction of poverty through the provision of food, feed and fibre security (FAO International Technical Conference 2010; James, 2010). Soybean and other legumes are important contributors for maintaining soil fertility and sustain agricultural production through their ability to fix atmospheric nitrogen, which is the most expensive fertilizer consuming most energy for its production.

Due to its nutritional qualities, especially high oil and protein content, soy is part of many humanitarian programs as one of the most important grain legumes. With the use of modern genetic technologies, soy has even more potential as functional food and a complete food like the WHO/FAO reference food. The GMO-soy presently commercialized or in the pipeline is mostly directed at improving economic benefits for the producer while there is little or no attempt to improve the nutritional quality of the seed as functional food. Technology transfer of biotechnologies and public sector research initiatives as well as government incentives are needed to develop soy with traits that are of importance to human health and to poor regions and less arable soil. GM crops may also facilitate sustainable agriculture development with reduced environmental footprint and helping the mitigation of climate change and reducing greenhouse gases (FAO International Technical Conference 2010; James 2010; Organization for Economic Co-operation and Development (OECD) 2009, World Wildlife Fund (WWF) report 2009). More in particular soybean could be the subject for genetic engineering to acquire drought stress tolerance and to be able to grow on marginal lands.

5. Acknowledgement

6. References

Ballmer-Weber, B.K.; Holzhauser, T.; Scibilia, J.; Mittag, D.; Zisa, G.; Ortolani, C.; Oesterballe, M.; Poulsen, L.K.; Vieths, S. & Bindslev-Jensen, C. (2007). Clinical characteristics of soybean allergy in Europe: a double-blind, placebo-controlled food challenge study. *Journal of Allergy and Clinical Immunology,* Vol. 112, No. 6, (June 2007), pp. 1489–1496.

Bardare, M.; Magnolfi, C. & Zani, G. (1988). Soy sensitivity: Personal observation on 71 children with food intolerance. *Allergy and Immunology,* Vol. 20, No. 2, (Februari 1988), pp. 63–66.

Bayer, J.C.; Norton, G.W. & Falck-Zepeda, J.B. (2010). Costs of compliance with biotechnology regulation in the Philippines: implications for developing countries. In: *AgBioForum.* Vol. 13, No. 1, pp. 53-62. Available from: <http://www.agbioforum.org>.

Bau, H.M.; Villaume, C.; Nicolas, J.P. & Mejean, L. (1997). Effect of Germination on Chemical Composition,

Biochemical Constituents and Antinutritional Factors of Soya Bean (*Glycine max*) Seeds. *Journal of the Science of Food and Agriculture,* Vol. 73, No. 1, (January 1997), pp 1-9.

Bilyeu, K.D.; Zeng, P.; Coello, P.; Zhang, Z.J.; Krishnan, H.B.; Bailey, A.; Beuselinck, P.R. & Polacco, J.C. (2008). Quantitative conversion of phytate to inorganic phosphorus in

soybean seeds expressing a bacterial phytase. *Plant Physiology.* Vol. 146, No. 2, (February 2008), pp. 468-477.

Bradbury, J.H. & Lambein F. (2011). Konzo and neurolathyrism: Similarities and dissimilarities between these crippling diseases of the poor. *Food and Chemical Toxicology.* Vol. 49, No. 3, (March 2011), pp. 537-538.

Bramley, P.M.; Elmadfa, I.; Kafatos, A.; Kelly, F.J.; Manios, Y.; Roxborough, H.E.; Schuch, W.; Sheehy, P.J.A. & Wagner K.-H. (2000). Vitamin E. *Journal of the Science of Food and Agriculture.* Vol. 80, No. 7, (May 2000), pp. 913-938.

Bressani, R. & Elias, L.G. (1974). Legume Foods. In "*New Protein Foods. Vol. 1A. Technology*" pp. 231-297. Altschul, A.M. ed., Academic Press, New York.

Brookes, G. & Barfoot, P. (2009). Global impact of biotech crops: income and production effects 1996-2007. In: *AgBioForum* Vol. 12 No. 2 pp. 184-208. Available from: <http://www.agbiofurum.org>.

Brouwer, I.A.; Katan, M.B.& Zock, P.L.(2004). Dietary alpha-linolenic acid is associated with reduced risk of fatal coronary heart disease, but increased prostate cancer risk: a meta-analysis. *The Journal of Nutrition*, Vol. 134, No. 4, (April 2004), pp. 919-922.

Bruinsma, J. (2009). The Resource Outlook to 2050. By How much do land, water and crop yield need to increase by 2050? Paper presented at the FAO Expert Meeting, 24-26 June 2009, Rome on "How to Feed the World in 2050"

Brunet, J.; Repellin, A.; Varrault, G.; Terryn, N. & Zuily-Fodil. (2008). Lead accumulation in the roots of grass pea (*Lathyrus sativus* L.): a novel plant for phytoremediation systems? Comptes Rendus Biologies. Vol. 331, No. 11, (November 2008), pp.859-864.

Campbell, C. G. (1997). Grass pea. *Lathyrus sativus* L. Promoting the conservation and use of underutilized and neglected crops. 18. Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute, Rome, Italy. 92 pp.

Carpenter, J.E. (2010). Peer-reviewed surveys indicate positive impact of commercialized GM crops. *Nature Biotechnology.* Vol. 28, No. 4, (April 2010), pp. 319-321.

Cassidy, A.; Bingham, S. & Setchell K.D.R. (1994). Biological effects of a diet of soy bean protein rich in isoflavones on the menstrual cycle of premenopausal women. *American Journal of Clinical Nutrition.* Vol. 60, No. 3, (September 1994), pp. 333-340.

Clarke, E.J. & Wiseman J. (2000). Developments in plant breeding for improved nutritional quality of soya beans I. Protein and amino acid content. *Journal of Agricultural Science* Vol. 134, No. 2, (August 2000), pp. 111-124.

De Ronde, J.A.; Cress, W.A.; Krüger, G.H.J.; Strasser, R.J. & Van Staden J. (2004). Photosynthetic response of transgenic soybean plants, containing an *Arabidopsis P5CR* gene, during heat and drought stress. *Journal of Plant Physiology*. Vol. 161, No. 11, (November 2004), pp. 1211-1224.

Dierking, E.C. & Bilyeu, K.D. (2009). Raffinose and stachyose metabolism are not required for efficient soybean seed germination. *Journal of Plant Physiology.* Vol. 166, No. 12, (August 2009), pp. 1329-1335.

Dill, G.M. (2005). Glyphosate-resistant crops: history, status, and future. *Pest Management Science*. Vol. 61, No. 3, (March 2005), pp. 219-224.

Duke, S.O. (1990). Overview of herbicide mechanisms of action. *Environmental Health Perspectives*. Vol. 87, (July 1990) pp. 263-271.

Dunmore, C. (2011). EU experts approve trace GM in feed imports: official. Source: Reuters (February 22, 2011). Available from: <http://www.reuters.com/article/2011/02/22/us-eu-gmo-imports-idUSTRE71L3V420110222>

Enneking, D. (2011). The nutritive value of grasspea (Lathyrus sativus) and allied species, their toxicity to animals and the role of malnutrition in neurolathyrism. *Food and Chemical Toxicology.* Vol. 49, No. 3, (March 2011), pp. 694-709.

Erdman, J.W.; Stillman, R.J. & Boileau, R.A. (2000). Provocative relation between soy and bone maintenance. *American Journal of Clinical Nutrition.* Vol. 72, No. 3, (September 2000), pp. 679-80.

EU observer. 'New dawn' for GMOs after EU vote, green groups warn. 29.03.2011, Available from: http://euobserver.com/885/31856?print=1

FAO International Technical Conference (2010) Report, Agricultural biotechnologies in developing countries: Options and opportunities in crops, forestry, livestock, fisheries, and agro-industry to face the challenges of food insecurity and climate change (ABDC-10). Available from: http://www.fao.org/biotech/abdc/en/

FAO/UN 2007 FAO/UN Water: Coping with water scarcity, challenge of the twenty-first century, World Water Day 22nd March 2007,http://www.fao.org/nr/water/docs/escarcity.pdf.

Foresight. The Future of Food and Farming (2011). Final Project Report. The Government Office of Science, London.

Fernandez-Orozco, R.; Frias, J.; Zielinski, H.; Piskula M.K.; Halina Kozlowska, H. & Vidal-Valverde, C. (2008).

Kinetic study of the antioxidant compounds and antioxidant capacity during germination of *Vigna radiata* cv. *emmerald, Glycine max* cv. *jutro* and *Glycine max* cv. *Merit.* Food Chemistry, Vol. 111, No. 3, (December 2008), pp. 622-630.

Flint-Hamilton K.B. (1999). Legumes in ancient Greece and Rome: food, medicine of poison? Hesperia, Vol. 68, No. 3, (July-September 1999), pp. 371-385.

Frederick, J.R.; Camp, C.R. & Bauer, P.J. (2001). Drought-stress effects on branch and mainstem seed yield and yield components of determinate soybean. *Crop science.* Vol. 41, No. 3, (May-June 2001), pp. 759-763.

Gaskell, G.; Stares, S.; Allansdottir, A.; Allum, N.; Castro, P.; Esmer, Y.; Fischler, C.; Jackson, J.; Kronberger, N.; Hampel, J.; Mejlgaard, N.; Quintanilha, A.; Rammer, A.; Revuelta, G.; Stoneman, P.; Torgersen, H. & Wagner W. (2010). Europeans and biotechnology 2010 – Winds of Change?. *Report to the European Commission's Directorate-General for Research* EUR 24537 EN (October 2010). Available from: <http://ec.europa.eu/public_opinion/archives/ebs/ebs_341_winds_en.pdf>

Garg, A.K.; Kim, J.-K.; Owens, T.G.; Ranwala, A.P.; Do Choi, Y.; Kochian, L.V. & Wu, R.J. (2002). Threhalose accumulation in rice plants confers high tolerance levels to different abiotic stresses. *Proceedings of the National Academy of Sciences of the United States of America.* Vol. 99, No. 25, (December 2002), pp. 15898-15903.

Getahun, H.; Lambein, F.; Vanhoorne, M. & Van der Stuyft, P. (2003). Food-aid cereals to reduce neurolathyrism related to grass-pea preparations during famine. *Lancet,* Vol. 362, No. 9398, (November 29, 2003), pp. 1808-1810.

Getahun, H.; Lambein, F.; Vanhoorne, M. & Van der Stuyft, P. (2005). Neurolathyrism risk depends on type of grass pea preparation and on mixing with cereals and antioxidants. *Tropical Medicine and International Health.* Vol. 10, No. 2, (February 2005), pp. 169-178.

GMO compass. 19.03.2011, Available from: <www.gmo-compass.org>.

Herman, E.M.; Helm, R.M.; Jung, R. & Kinney, A.J. (2003). Genetic modification removes an immunodominant allergen from soybean. *Plant Physiology,* Vol. 132, No. 1, (May 2003), pp. 36-43.

Hu, H.H.; Dai, M.Q.; Yao, J.L.; Xiao, B.Z.; Li, X.H.; Zhang, Q.F. & Xiong L.Z. (2006) Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. *Proceedings of the National Academy of Science of the United States of America,* Vol. 103, No 35, (August 29, 2006), pp. 12987-12992.

Imsande, J. (2001). Selection of soybean mutants with increased concentrations of seed methionine and cysteine. *Crop Science,* Vol. 41, No. 2, (March-April 2001), pp. 510-515.

James, C. (2010). Global Status of Commercialized Biotech/GM Crops: 2010. *ISAAA Brief* No. 42. ISAAA: Ithaca, NY.

Kalaitzandonakes, N.; Alston, J.M. & Bradford, K.J. (2007). Compliance costs for regulatory approval of new biotech crops. *Nature Biotechnology,* Vol. 25, No. 5, (May 2007), pp. 509-511.

Kaushik G.; Satya S. & Naik S.N. (2010). Effect of domestic processing techniques on the nutritional quality of soybean. *Mediterranean Journal of Nutrition and Metabolism,* Vol. 3, No. 1, (March 2010), pp. 39-46.

Kris-Etherton, P.M.; Krummel, D.; Russell, M.E.; Dreon, D.; Mackey, S;. Borchers, J.& Wood, P.D..(1988). The effect of diet on plasma lipids, lipoproteins, and coronary heart disease. *Journal of the American Dietetic Association.* Vol. 88, No.11, (November 1988), pp.1373-1400.

Kuo Y.-H.; Bau H.M.; Quemener B.; Khan J.K. & Lambein F. (1995).: Solid state fermentation of *Lathyrus* seeds using *Aspergillus oryzae* and *Rhizopus oligosporus* sp T3 to eliminate the neurotoxin ß-ODAP without loss of nutritional value. *Journal of the Science of Food and Agriculture,* Vol. 69, No 1, (September 1995), pp 81-89.

Kuo, Y.-H.; Bau, H.-M.; Rozan, P.; Chowdhury, B. & Lambein, F. (2000) Reduction efficiency of the Neurotoxin β-ODAP in low toxin varieties of *Lathyrus sativus* seeds by solid-state fermentation with *Aspergillus oryzae* and *Rhizopus microsporus* var. *chinensis. Journal of the Science of Food and Agriculture,* Vol. 80, No. 15, (December 2000), pp. 2209-2215.

Lakshmi, P.M.; Guttikonda, S.K.; Phan Tran, L.-S. & Nguyen, H.T. (2009). Physiological and Molecular Approaches to improve drought resistance in soybean. *Plant & Cell Physiology,* Vol. 50, No. 7, (July 7, 2009) pp. 1260-1276.

Lambein, F.; Kuo, Y-H.; Ikegami, F.; Kusama-Eguchi, K. & Enneking, D. (2005). Grain legumes and human health. *Proceedings of the Fourth International Food Legumes Research Conference (IFLRC-IV)*, New Delhi, India, October 18–22, 2005.

Lecerf, J-M. & Fressin, C. (1995). L'intérêt nutritionnel du soja. *Nutrition Clinique et Métabolisme,* Vol. 9, No 3, (n.d.) pp. 137-144.

Lee, S.A.; Shu, X.O.; Li, H,. Yang, G.; Wen, W.; Yi, B.T.; Gao, J.; Gao, Y.T. & Zheng.W. (2009). Adolescent and adult soy food intake and breast cancer risk: results from the Shanghai women's health study. *American Journal of Clinical Nutrition.* Vol. 89, (June 1, 2009), pp. 1920-1926.

Li, Z.W.; Meyer, S.; Essig, J.S.; Liu, Y.; Schapaugh, M.A.; Mukhukrishnan, S.; Hainline, B.E. & Trick, H.N. (2005). High-level expression of maize gamma-zein protein in transgenic soybean (*Glycine max*) *Molecular Breeding,* Vol. 16, No. 1, (August 2005), pp. 11-20.

Liggins, J.; Bluck, L.J.C.; Runswiick, S.; Atkinson, C.; Coward, W.A. & Bingham, S.A. (2000). Daidzein and genistein contents of vegetables. *British Journal of Nutrition,* Vol. 84, No. 5, (November 2000), pp. 717-725.

Liu, F.; Jensen C.R. & Andersen, M.N. (2004). Drought stress effect on carbohydrate concentration in soybean leaves and pods during early reproductive development:

its implication in altering pod set. *Field Crops Research,* Vol.. 86, No. 1, (February 20, 2004), pp. 1-13.

Mahler-Slasky, Y. & Kislev, M.E. (2010). *Lathyrus* consumption in late Bronze and Iron age sites in Israel: An Aegean affinity. *Journal of Archaeological Science,* Vol. 37, No. 10 (October 2010), pp. 2477-2485.

Mccue, P. & Shetty, K. (2004). Health Benefits for Enhancement of Soy Isoflavonoids and Strategies A Review. *Critical Reviews in Food Science and Nutrition,* Vol. 44, No 5, (n.d.) pp. 361–367, ISSN: 1040-8398

Menzies G. (2002). 1421: The year China discovered the world. (p96) 650 pp. Bantam books, London, New York, ISBN 0-553-81522-9

Messina, M.J.; Persky, V.; Setchell, K.D. & Barnes, S. (1994). Soy intake and cancer risk: a review of the ni vitro and in vivo data. *Nutrition and cancer,* Vol. 21, No. 2, (n.d.), pp. 113-131.

Messina, M.J. (1999). Legumes and soybeans: overview of their nutritional profiles and health effects. *American Journal of Clinical Nutrition,* Vol. 70, No. 3, (September 1999), pp. 439S-450S.

Monsanto homepage. Available from: <http://www.monsanto.com>

Natarajan, S. S.; Xu, C., Cregan, P.; Caperna, T.J.; Garrett, W. M. & Luthria, D. (2009). Utility of proteomics techniques for assessing protein expression. Regulatory Toxicology and Pharmacology, Vol. 54, No. 3, Suppl. 1, (August 2009), pp. S32-S36.

Natarajan, S.S.   (2010). Natural variability in abundance of prevalent soybean proteins. *Regulatory Toxicology and Pharmacology,* Vol. 58, (December 2010), pp.S26–S29.

Nelson, D.E.; Repetti, P.P.; Adams, T.R.; Creelman, R.A.; Wu, J.; Warner, D.C., Anstrom, D.C.; Bensen, R.J.; Castiglioni, P.P.; Donnarummo, M.G.; Hinchey, B.S.; Kumimoto, R.W.; Maszle, D.R.; Canales, R.D.; Krolikowski, K.A.; Dotson, S.B.; Gutterson, N.; Ratcliffe, O.J. & Heard J.E. (2007). Plant nuclear factor Y (NF-Y) B subunits confer drought tolerance and lead to improved corn yields on water-limited acres. *Proceedings of the National Academy of Science of the United States of America,* Vol. 104, (August 29, 2007), pp. 16450-16455.

Organization for Economic Co-operation and Development (OECD). (2009). The bioeconomy to 2030: designing a policy agenda. ISBN 978-92-64-03853-0 © OECD 2009.

Park, J.R.; McFarlane, I.; Phipps, R.H. & Ceddia, G. (2011) The role of transgenic crops in sustainable development. *Plant Biotechnology Journal.* Vol. 9, No. 1, (January 2011), pp. 2-21.

Potrykus, I. (2010). Regulation must be revolutionized. *Nature, Vol.* 466, No. 7306, (July 28, 2010), p. 561.

Provorov, N.A. & Tikhonovich, I.A. (2003). Genetic resources for improving nitrogen fixation in legume-rhizobia symbiosis. *Genetic Resources and Crop Evolution,* Vol. 50, No. 1, (February 2003), pp. 89-99.

Quirce, S.; Fernandez-Nieto, M.; Polo, F. & Sastre, J. (2002). Soybean trypsin inhibitor is an occupational inhalant allergen. *Journal of Allergy and Clinical Immunology*, Vol. 109, No. 1, (January 2002), pp. 178-178.

Rozan, P.; Kuo, Y.H. & Lambein, F. (2000) Free amino acids present in commercially available seedlings sold for human consumption. A potential hazard for consumers. *Journal of Agricultural and Food Chemistry,* Vol. 48, No. 3, (March 2000), pp. 716-723.

Sacks, F.M.; Lichtenstein, A.; Van Horn, L.; Harris, W. Kris-Etherton, P.&, Winston M. (2006). Soy protein, isoflavones, and cardiovascular health: an American Heart

Association Science Advisory for professionals from the Nutrition Committee. *Circulation,* Vol. 113, No. 7, (February 21, 2006), pp.1034-1044.

Sarwar, G. (1997). The protein digestibility-corrected amino acid score method overestimates quality of proteins containing antinutritional factors and of poorly digestible proteins supplemented with limiting amino acids in rats". *Journal of Nutrition,* Vol.127, No. 5, (May 1997), pp.758–764.

Schillinger, J.A.; Dierking, E.C. & Bilyeu, K.D. (2011). Soybeans having high germination rates and ultra-low raffinose and stachyose content. *United States Patent Application Publication,* Pub. No. US 2011/0003045 A1, Pub. Date Jan. 6, 2011.

Schmutz, J.; Cannon, S.B.; Schlueter, J.; Ma, J.; Mitros, T.; Nelson, W.; Hyten, D.L.; Song, Q.; Thelen, J.J.; Cheng, J.; Xu, D.; Hellsten, U.; May, G.D.; Yu, Y.; Sakurai, T.; Umezawa, T.; Bhattacharyya, M.K.; Sandhu, D.; Valliyodan; B.; Lindquist, E.; Peto, M.; Grant, D.; Shu, S.; Goodstein, D.; Barry, K.; Futrell-Griggs, M.; Abernathy, B.; Du, J.; Tian, Z.; Zhu, L.; Gill, N.; Joshi, T.; Libault, M.; Sethuraman, A.; Zhang, X.-C.; Shinozaki, K.; Nguyen, H.T.; Wing, R.A.; Cregan, P.; Specht, J.; Grimwood, J.; Rokhsar, D.; Stacey, G.; Shoemaker, R.C. & Jackson, S.A. (2010). Genome sequence of the palaeopolyploid soybean. *Nature, Vol.* 463, No. 7294, (January 2010), pp. 178-183.

Scott, J.; Rébeille, F. & Fletcher, J. (2000). Folic acid and folates: the feasibility for nutritional enhancement in plant foods. *Journal of the Science of Food and Agriculture,* Vol. 80, No. 7, (May 15, 2000), pp. 795-824.

Sebastian, A. (2005). Isoflavones, protein and bone. *American Journal of Clinical Nutrition,* Vol. 81, No. 4, (April 2005), pp. 733-735.

Setchell, K.D.R. & Cassidy, A. (1999). Dietary isoflavones: biological effects and relevance to human health. *Journal of Nutrition,* Vol. 129, No. 3, (March 1999), pp. 758S-767S.

Shurtleff, W.& Aoyagi, A. (2011). History of soy sprouts. A special report on the history of traditional non-fermented soya foods. A Chapter from the Unpublished Manuscript, History of Soybeans and Soyfoods: 1100 B.C. to the 1980s. In: *Soyinfo Center*, 01.02. 2011, Available from http://www.soyinfocenter.com/HSS/ sprouts.php

Sinclair, T.R.; Purcell, L.C.; King, C.A.; Sneller, C.H.; Pengyin, C. & Vadez, V. (2007). Drought tolerance and yield increase of soybean resulting from improved symbiotic N_2 fixation. *Field Crops Research,* Vol. 101, (February 20, 2007), pp. 68-71.

Sojanet (n.d). Soya, the plant, the food. In: *Sojanet*, 02.02.2011, Available from http://www.sojanet.com/the-plant-the-food/the-plant.html

Soy & Health, (2011). Available from http://www.soyconference.com/magazines/ apr11.pdf.

Specht, J.E.; Hume, D.J. & Kumudini, S.V. (1999). Soybean yield potential – A genetic and physiological perspective. *Crop Science,* Vol. 39, No. 6, (November 1999), pp. 1560-1570.

Stein, A.J. & Rodrigues-Cerezo, E. (2009). The global pipeline of new GM crops - Implications of asynchronous approval for international trade. *JRC Scientific and Technical reports* EUR 23486 EN. Available from http://ftp.jrc.es/ EURdoc/JRC51799.pdf

The Royal Society (2009). U.K. Reaping the benefits: Science and the sustainable intensification of global agriculture. ISBN: 978-0-85403-784-1

Thompson, C.J.; Movva, R.; Tizzard, R.; Crameri, R.; Davies, J.E.; Lauwereys, M. & Botterman, J. (1987). Characterisation of a herbicide-resistance gen bar from *Streptomicus hygroscopicus*. *The Embo Journal,* Vol. 6, No. 9, (September 1987), pp. 2519-2523.

Thompson H.J.; Heimendinger, J.; Haegele, A.; Sedlacek, S.M.; Gillette, C.; O'Neill, C.;Wolfe, P. & Conry C. (1999). Effect of increased vegetable and fruit consumption on markers of oxidative cellular damage. *Carcinogenesis,* Vol. 20, No. 12, (December 1999), pp. 2261-2266.

Trigo, E. & Cap, E. (2006) Ten years of genetically modified crops in Argentine agriculture. Argentine *Council for information and Development of Biotechnology – ArgenBio*: 1-52. Available from: http://www.efb-central.org/images/uploads/Ten_Years_GM_Crops_in_Argentine_Agric.pdf

United States Department of Agriculture – Animal and Plant Health Inspection Service (USDA-APHIS) (2011) Petitions for Non-regulated Status Granted or Pending by APHIS as of February 15, 2011. Available from: <http://www.aphis.usda.gov/biotechnology/not_reg.html>

U.S. Department of Agriculture and U.S. Department of Health and Human Services. *Dietary Guidelines for Americans, 2010.* 7th Edition, Washington, DC: U.S. Government Printing Office, December 2010. Available from: <http://www.cnpp.usda.gov/Publications/DietaryGuidelines/2010/PolicyDoc/PolicyDoc.pdf>

Van Montagu, M. (2010) Challenges and responsibilities for public sector scientists. New Biotechnology Vol. 27, No. 5 (November 2010) pp. 641-644.

Vaz Patto M.C., B. Skiba, E.C.K. Pang, S.J. Ochatt, F. Lambein and D. Rubiales. 2006. *Lathyrus* improvement for resistance against biotic and abiotic stresses: from classical breeding to marker assisted selection. *Euphytica,* Vol. 147, No. 1-2, (n.d.), pp. 133-147.

Vidal-Valverde, C.; Frias, J.; Sierra, I.; Blazquez, I.; Lambein, F. & Kuo, Y.-H. (2002). New functional foods by germination: Effect on the nutritive value of beans, lentils and peas. *European Food Research and Technology,* Vol. 215, No. 6, (December 2002), pp. 472-477.

Villegas, R.; Gao,Y.T.; Yang, G.; Li, H.L.; Elasy, T.A.; Zheng, W. &. Shu, S.O. (2008). Legume and soy food intake and the incidence of type 2 diabetes in the Shanghai women's health study. *American Journal of Clinical Nutrition,* Vol. 87, No 1, (n.d.), pp. 162-167.

Wager, R. & McHughen, A. (2010). Zero sense in European approach to GM. *EMBO reports* Vol. 11 No. 4, (n.d.), pp. 258-262.

Wai, K.N.T.; Bishop J.C.; Mack P.B. and Cotton R.H. (1947). The vitamin content of soybeans and soybean sprouts as a function of germination time . Plant physiology, Vol. 22, No 2, (April 1947), pp. 117-125.

World Food Program (2006), Food and nutrition Handbook. WFP. (http://foodquality.wfp.org/FoodNutritionalQuality/WFPNutritionPolicy/tabid/362/Default.aspx?PageContentID=537, accessed on February 25, 2011)

World Wildlife Fund (WWF) Report Bang, J.K.; Follér, A. & Buttazzoni, M. (2009) Biotechnology, more than green fuel in a dirty economy. WWF Denmark (September 2009). Available from <www.wwf.dk>.

World Health Organisation and UNICEF (2006). Meeting the MDG drinking water and sanitation target; the urban and rural challenge of the decade. WHO Library Cataloguing in Publication Data.

Zhao, Y.; Martin, B.R. & Weaver, C.M. (2005). Calcium Bioavailability of Calcium Carbonate Fortified Soya milk is Equivalent to Cow's Milk in Young Women. *The Journal of Nutrition,* Vol. 135; No.10, (October 2005), pp. 2379-2382.

Feeds with Probiotics in Animals' Nutrition

Joanna Biernasiak[1], Katarzyna Ślitewska[2] and Zdzisława Libudzisz[2]
[1]Institute of Chemical Technology of Food, Technical University of Lodz
[2]Institute of Fermentation Technology and Microbiology, Technical University of Lodz
Poland

1. Introduction

The discovery of penicillin by Alexander Fleming in 1928 was a turning point which fundamentally revolutionized human and veterinary medicine. Antibiotics in veterinary medicine have been used to prevent and control bacterial infections and as growth promoters. Prevention and control of bacterial infections have been achieved by a therapeutic, metaphylactic or prophylactic application of antibiotics. Therefore, the substances, predominantly of the same class as in human medicine, have been used in veterinary medicine. Antibiotics regularly administered to animals in order to improve their growth, to better the use of feed and to reduce the number of falls have been defined as antibiotic growth promoters. The use of AGP in the European Union was approved by the Council Directive of 23 November 1970 concerning feed additives (70/524/EEC).
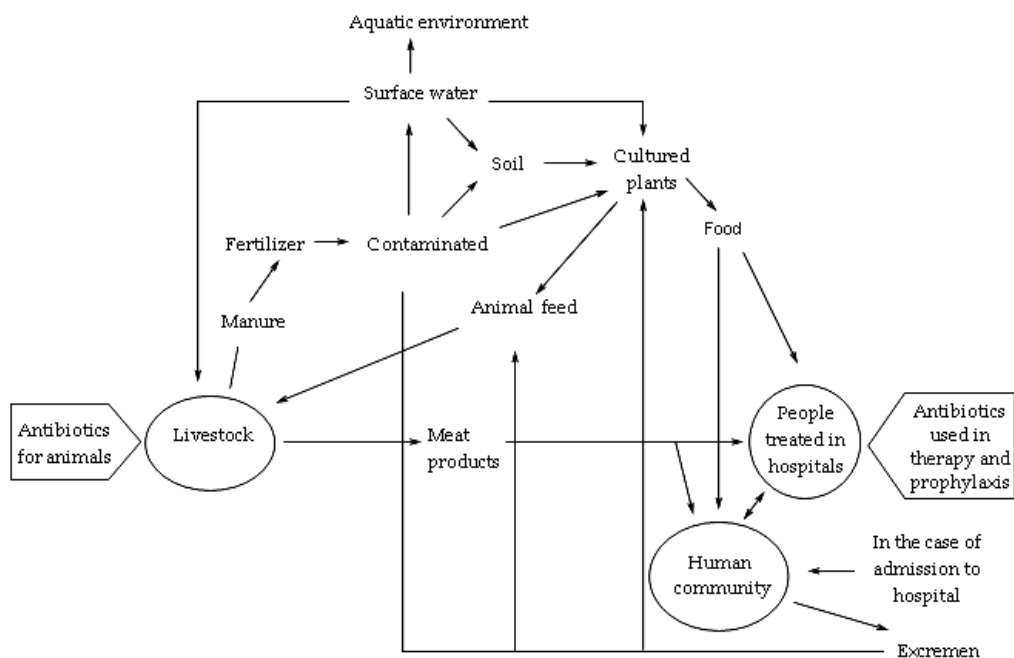
Fig. 1. Routes of spread of antibiotic-resistant strains of bacteria and drug resistance genes

The use of additives in feed has caused a number of negative changes. It influenced, among others, the environmental degradation and development of drug resistance in bacteria. Livestock are a major reservoir of bacteria resistant to antibiotics. These pathogens contained in animal meet entered human body and quickly spread in human society. Resistant bacteria also spread thanks to the use of manure as natural fertilizer. Such fertilizer, rich in drug-resistant bacteria, contaminated water and soil which had direct contact with grown plants presenting food both for humans and animals. The routs of spreading of antibiotic-resistant strains of bacteria are shown in Figure 1 (Witte, 2000).

For this reason, the number of kinds of antibiotics approved for use in animal nutrition have been consistently limited.

Beginning from 1 January 2006, the European Union introduced a total ban on the application of antibiotic growth promoters in feeds for animals bred for consumption. The ban was introduced at the same time in all countries of the Union. Since that time, antibiotics have been allowed to be used as drugs only in medicinal animal feeds or in prophylactic additives. The Regulation EC No 1831/2003 of the European Parliament and Council dated 22 August 2003, on the additives used in animal nutrition, includes, among others, probiotics as feed additives alternative to antibiotic growth promoters (Casewell et al. 2003; Berghmann et al., 2005).

2. Definition of the term "probiotic"

It is most probable that it was Ferdinand Vergin who introduced the term "probiotic" in 1954, when, in an article entitled "Anti und Probiotika" he compared the detrimental effect exerted on the flora by antibiotics and other antimicrobial substances with positive impact ("Probiotika") induced by beneficial bacteria. A few years later, in 1965, Lilly & Stillwell described probiotics as microorganisms stimulating the growth of other microorganisms. In 1974, Parker used this term for organisms and substances that contribute to balancing the intestinal microflora of the host. The definition currently in use was proposed by FAO/WHO in 2002. It defines probiotics as live microorganisms which when administered in adequate amounts provide health benefits to the host. The microorganisms used in animal nutrition in the European Union include mainly Gram-positive bacteria that belong to the genus *Bacillus* (*B. cereus, B. licheniformis, B. subtilis*), *Enterococcus* (*E. faecium*), *Lactobacillus* (*L. acidophilus*, *L. casei*, *L. farciminis*, *L. plantarum*, *L. rhamnosus*), *Pediococcus* (*P. acidilactici*), *Streptococcus* (*S. infantarius*), and yeasts of the genus *Saccharomyces* (*S. cerevisiae* and *S. boulardii*) (Anadón et al., 2006). *Saccharomyces boulardii* is a non-pathogenic yeast described in clinical literature as a biotherapeutical factor. According to taxonomic research, *S. boulardii* is considered to be a variation within the species *S. cerevisiae,* and in accordance with the agreed taxonomy it should be referred to as *S. cerevisiae var. boulardii* (Mitterdorfer et al., 2002; Van der Kühle, 2005). In contrast to bacteria of the genus *Bacillus* and yeasts of the genus *Saccharomyces*, *Lactobacillus* and *Enterococcus* bacteria belong to typical intestinal animal microflora and are present in large quantities, i.e. respectively 10^7–10^8 and 10^5–10^6 CFU/g in the intestinal content (Anadón et al., 2006).

3. Procedures of probiotics evaluation

According to the Commission Directive 94/40/EC of 22 July 1994 setting out procedures for the assessment of additives, including microorganisms used in animal nutrition, results should be presented concerning mainly the following areas:

1. Identification, characteristics, conditions of use and methods of control of the additive
   In case of microorganisms it is necessary to provide:
- name and taxonomic description in accordance with the International Code of Nomenclature
- name and place of culture collection and number of deposit
- genetic modification and all relevant characteristics for its identification
- origin
- genetic stability and purity of the cultivated strains
- properties relevant for the identification and proposed usage (e.g. vegetative form or sporulated form, CFU/g)
- resistance (loss of biological activity, e.g. viability): to weather activity (storage duration), during the preparation of premixes and feed (possible degradation products), to storage of premixes and feed in certain defined conditions (storage duration).
2. Effectiveness of the additive
   In case of microorganisms it is necessary to provide:
- information about the effects on: the nutritional value, the growth of animals, animal product features and their effectiveness, animal welfare and other parameters having a positive impact on animal production
- conditions for conducting experiments on animals. The test performed must be described, along with the statistical assessment and the methods used. The description must include the following data:
- species, breed, age and sex of animals (the method of identification)
- the test number and the number of experimental groups together with the number of animals in each of them
- the level of content of microorganism(s) in feed established by a control analysis with the use of a relevant approved method
- the place where the test was carried out, together with the description of health, physiological, nutritional and breeding conditions in accordance with the standard practice in the Community
- the date and exact duration of testing
- side effects and other negative effects which occurred during the experiment and the time when they were observed.
3. Additive safety.
   In case of microorganisms it is necessary to:
- demonstrate the lack of pathogenicity and toxicity in relation to the target species and humans under the anticipated conditions of use
- identify the antibiotic resistance
- present the results of tolerance tests in the target species. In the case of genetically modified organisms (GMO), in the understanding of Article 2 item 1 and 2 of Council Directive 90/220/EEC, the following information must be provided:
- a copy of the written consent of the competent authorities to the deliberate release of genetically modified organisms for the sake of research and development in accordance with Article 6 item 4 of Directive 90/220/EEC, and a summary of the notification, as specified in Article 9 of Directive 90/220/EEC
- complete technical documentation with information required in Annex 2 to Directive 90/220/EEC, extended, if necessary, with the data on the variety of use of the additive, including the information on the data and results obtained based on the releases of

GMOs, conducted for the sake of research and development and concerning ecosystems that might be affected by the use of the additive, and also a risk assessment in respect of human and animal health and the environment in connection with the genetically freed organism contained in the product, including the information obtained from the current stage of research and development concerning the influence of the GMO release on human health and the environment.

- conditions of introducing the additive on the market, including special conditions of use and transport, as well as a proposal for labeling and packaging, which should at least comprise the requirements set out in Annex III to Directive 90/220/EEC

4. Activity of probiotic microorganisms

The mechanism of the impact of probiotics on animals has not been fully elucidated and is still under investigation. According to the literature data, the proposed operation mechanisms of probiotic strains are as follows:

4.1 Maintenance of microbiological balance in alimentary tract

Maintenance of microbiological balance, the so-called eubiosis in the alimentary tract. The alimentary tract of animals immediately after birth is sterile and susceptible to colonization by various microorganisms, including also the pathogenic ones of the *coli* group, or of the genus *Salmonella*. Probiotic strains compete with pathogenic microorganisms for adhesion and colonization of biological membranes (Nousiainen et al., 2004). While adhering, probiotic bacteria form thin durable layers known as biofilm. Biofilm is composed of bacteria only in a small part. The remainder comprises exopolymers of these bacteria that form the so-called matrix. These include polysaccharides, proteins, nucleic acids and phospholipids. The release of these compounds is the result of adaptation to the environment. Exopolymers affect the biological, physical and chemical characteristics of the biological membrane and form its essential element. Polysaccharide exopolymers maintain the biofilm in the shape of a whole, as they fill the gaps formed among microorganisms. Usually biofilm contains 4 times more polysaccharide exopolymers than proteins. In the early stages of biofilm formation these are the polisaccharides that are released with the greatest intensity. They help the first cells to attach to the surface. Other exopolymers secreted by cells are proteins. Initially, proteins are gathered on the surface of the cells, and later, when they are released, they associate on the target surface, which helps to keep the cells on the surface. Proteins are usually a mixture of collagen and elastin. They form the extrecellural matrix to which microorganisms adhere (Czaczyk, 2003).

In the control of intestinal microflora an important role is played by metabolites of lactic acid bacteria with antagonistic activity. Among the compounds that inhibit the growth of pathogenic microorganisms, the ones considered to be the most important are organic acids, especially lactic acid and acetic acid, as well as hydrogen peroxide and bacteriocins (Salminen et al., 1998; Saarela et al., 2000; Mercenier et al., 2003). Antibacterial effect of organic acids is due to a rapid reduction in pH values beyond the optimum value range for the growth of most microorganisms, i.e. 6-7, as well as the inhibition of biochemical activity of microorganisms by undissociated acid molecules (Boris & Barbés, 2000; Messens & de Vuyst, 2002). Weak acids (lactic and acetic acid) as lipophilic compounds in the undissociated form pass into the cytoplasm, where they dissociate, which results in the reduction of pH inside the cell and the disruption of the process of moving protons through

the outer membrane and the increase in its tension. This in turn is manifested by an increase in the permeability. They can also cause denaturation of proteins (Ekuland, 1989; Caplice & Fitzgerald, 1999). The effect of lactic acid on the permeability of the outer membrane of *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* Typhimurium was examined by Alakomi et al., (2000). These researchers observed that even 5mM of lactic acid (pH 4.0) resulted in a significant increase in the permeability of the outer membrane in case of each of the strains studied by them, and the effect of lactic acid was even stronger than the effect of EDTA or HCl. The dissociation constant of lactic acid is 3.08, and in case of acetic acid it equals 4.87. Acetic acid, due to higher pKa, shows stronger antimicrobial activity than lactic acid (Cherrington et al., 1991). According to Eklund (1983), the reduction of pH of the environment to 4.0 leads to the situation where the undissociated form of acetic acid equals 85%, and in case of lactic acid it constitutes only 11%. Acetic acid is a potent inhibitor of the growth of bacteria, yeasts and molds (Blom & Mörtvedt, 1991). Ray (1992), showed that in an environment with pH of 5.0 in 1% solution of acetic acid there are enough undissociated molecules to inhibit the growth of Gram-positive and Gram-negative bacteria, while in 1% solution of lactic acid the number of undissociated molecules is enough only to inhibit Gram-negative bacteria. Thus, during the fermentation with facultative heterofermentative strains of LAB, lactic acid is mainly responsible for lowering pH of the environment, while the acetic acid acts as an antimicrobial factor (Ouwehand & Vesterlund, 2004). It should be noted, however, that lactic acid not only lowers pH, but also functions as a factor causing increased permeability of the outer membrane of Gram-negative bacteria, and thus it may increase the effectiveness of other antagonistic substances (Alakomi et al., 2000).

 Lactobacillus, and some other lactic acid-producing bacteria, in the absence of heme, do not use the cytochrome system (which reduces oxygen to water) for the final oxidation. These bacteria use flavoproteins that convert oxygen into hydrogen peroxide. The production of hydrogen peroxide is catalyzed by different enzyme systems present in the cells of lactic acid bacteria, namely the pyruvate oxidase, L-lactate oxidase, superoxide dismutase, D-lactate dehydrogenase and NADH oxidase. Hydrogen peroxide is a well-known antibacterial substance. The activity of H_2O_2 results from strong oxidizing properties. Hydrogen peroxide may inhibit the growth or kill other microbes that do not possess or have low levels of enzymes degrading H_2O_2, such as catalase and peroxidase. Studies conducted *in vitro* confirm the inhibition of different bacteria such as: *Staphylococcus aureus*, *Salmonella* Typhimurium, *Escherichia coli*, *Clostridium perfringens*, *Clostridium butyricum* and *Pseudomonas* sp. by hydrogen peroxide (Dembele et al., 1998; Tomas et al., 2004). Bacteriocins are protein substances produced by numerous strains of lactic acid bacteria and propionic acid bacteria. They make a highly diversified group of compounds in terms of physical and biochemical properties. Bakteriocins have a bactericidal or bacteriostatic effect. They attack the cell membranes of microorganisms possessing receptors capable of bonding to them. These receptors are used for the translocation of bacteriocins and other compounds across the cytoplasmatic membrane. Their construction and properties are not fully known yet. Bacteriocins may cause: poration of bacterial cytoplasmatic membrane, which leads to the dispersal of the transmembrane potential and induces leakage of K^+ ions, ATP and amino acids from the cytoplasm of affected cells; cell lysis; and they may also interfere with or inhibit the synthesis of DNA, RNA and proteins (acting as DNA-zy lub RNA-zy) (Daeschel, 1989; Klaenhammer, 1993; Grajek & Sip, 2004). Bacteriocynogenic microbes are resistant to the effect of bacteriocins produced by them. Some bacteriocins of lactic acid bacteria are active against pathogens in food, such as, for example *Listeria monocytogenes*, others inhibit the growth of Gram-positive aerobic and anaerobic bacteria of the genera

Bacillus and *Clostridium* (Schillinger & Holzapfel, 1996). Yeasts of the genus *Saccharomyces* are characterized by a high content of glucan and mannan in the cell wall and, therefore, may show an affinity to specific bacterial adhesins. Mannans have a high affinity to fimbrial structures (lectins) specific for mannose binding type 1 in pathogenic bacteria, such as *Escherichia coli* or *Salmonella* sp. They place themselves in the „hook", i.e. in the area where those undesirable microorganisms adhere to the receptors of epithelial structures of the digestive system. Then, pathogenic bacteria lose the ability to adhere to the epithelial surface and thus, given the structure of the mannan which is not digested by the endoenzymatic system of animals, they are passaged (transported) along the axis of the intestinal tract and excreted with the feces of birds. Studies have demonstrated adherence of *E. coli* to the cells of *Saccharomyces cerevisiae* var. *boulardii*, and agglutination was similar to that observed between *E. coli* and erythrocytes, phagocytes and epithelial cells (Gedek, 1999). As demonstrated by the *in vitro* and *in vivo* research, yeasts exert inhibitory effects on *Salmonella* Typhi, *Salmonella* Typhimurium, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Shigella atypical*, *Escherichia coli*, *Clostridium difficile*, *Klebsiella* sp., *Yersinia enterocolitica*, *Candida albicans*, *Candida pulcherrima*, *Candida kruzei*, *Candida pseudotropicalis*, *Torulopsis gropengiesseri* (Tasteyre et al., 2002; Van der Aa Kühle et al., 2005).

At the current stage of research, it seems that the beneficial properties of various probiotics used in animal nutrition are strain-dependent. Hence, attention is paid so that they are strictly defined. Each requires a separate set of tests in order to determine their probiotic properties (FAO/WHO, 2002).

The aim of our research was to design and evaluate the effectiveness of a probiotic preparation intended for poultry. The basis of this preparation are bacteria of the genus *Lactobacillus*: *Lactobacillus paracasei* ŁOCK 0920, *Lactobacillus brevis* ŁOCK 0944, *Lactobacillus plantarum* ŁOCK 0945 (Michałowski et al., 2004). Strains were characterized for their probiotic properties (Motyl, 2002; Motyl & Klewicka unpublished data, 2003). The study determined, among others, the resistance of the tested strains to low pH, the ability to adhere to intestinal epithelial cells and the antagonistic activity of these strains in relation to pathogenic strains that cause food poisonings and disease states in animals.

All tested cultures of *Lactobacillus* sp. showed a strong activity in respect of inhibiting the growth of both Gram-negative pathogenic strains (*Pseudomonas aeruginosa, Escherichia coli, Salmonella* Typhimurium*, Salmonella* Enteritidis*, Shigella sonnei*) and Gram-positive pathogenic strains (*Staphylococcus aureus, Enterococcus faecalis, Listeria monocytogenes, Listeria innocua*). It is worth noting that *Pseudomonas aeruginosa* bacteria were inhibited in the strongest way, and *Enterococcus faecalis* bacteria were inhibited with the weakest effectiveness (Table 1).

Due to the different sensitivity of various pathogenic bacteria to the metabolites of probiotic strains, it seems reasonable to use in animal feeding probiotic preparations made as a composition of different strains, which is consistent with literature data (Mountzouris et al., 2007).

4.2 Detoxification of mycotoxins

Mycotoxins are toxic secondary metabolites of fungi mainly belonging to the genera *Aspergillus* sp., *Penicillium* sp. and *Fusarium* sp. Chemically, they are enumerated among aromatic hydrocarbons (sometimes among aliphatic ones) with low molecular weight, which determines their resistance to environmental factors and the absence of or weak immunogenic properties (Gajęcki, 2002). The most important among the mycotoxins, from

The pathogenic strain	The antagonistic strain		
	Lactobacillus paracasei ŁOCK 0920	*Lactobacillus plantarum* ŁOCK 0945	*Lactobacillus brevis* ŁOCK 0944
	Inhibitor zone (mm)		
Pseudomonas aeruginosa ATCC 27853	18.0	9.0	19.5
Staphylococcus aureus ATCC 25923	14.0	9.5	12.5
Escherichia coli ATCC 25922	13.5	11.5	25.5
Escherichia coli ŁOCK 0836	12.0	9.0	21.5
Escherichia coli 018	13.0	11.0	21.0
Salmonella Enteritidis	14.0	9.0	8.0
Salmonella Typhimurium	9.0	10.0	7.0
Shigella sonnei	9.0	7.0	7.0
Listeria monocytogenes	12.0	14.0	12.5
Listeria innocua	11.0	12.0	10.0
Enterococcus faecalis	8.0	9.0	7.5

Table 1. Antagonistic activity of *Lactobacillus* in relation to microbial food-borne pathogens expressed as a zone of growth inhibition in mm (according to Motyl, 2002; Motyl & Klewicka unpublished data, 2003).

the toxicological point of view and also taking into account the etiology of some animal diseases are: aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2), ochratoxins (OTA and OTB), trichothecenes (DON, NIV, T-2, HT-2, DAS), zearalenone (F-2) and fumonisins (FB_1, FB_2, FB_3) . The synthesis of mycotoxins by fungi is genetically conditioned, but it is determined by environmental factors, which include: substrate composition, its texture, moisture, temperature and the presence of competitive microflora (Gourama & Bullerman, 1995; Batish et al., 1997; Fink-Gremmels, 1999). The invasion of mycotoxins into the body of animals takes place mainly via food, and the health effects, called mycotoxicoses, are poisonings with various courses - acute or chronic - resulting from the receiving small doses for a long time (Yiannikouris & Jouany, 2002). Due to the specific chemical and physical properties of mycotoxins, the spectrum of their activities in the body of animals is very different and it is characteristic for the groups of these metabolites. First of all, mycotoxins have a specific effect on the groups of body tissues (epithelia, nervous system tissue, secretory organs tissue - the pancreas, liver, kidney tissue, etc.), including the individual cells. By interfering with metabolic pathways, they may lead to abnormal replication of the cell's genetic code, which impairs the process of tissue proteins reconstruction (including the immune system – proteins of antibodies). They also damage and disrupt transport mechanisms in the cytoplasm of cells and between cells, block enzymatic reactions of cells, especially in mitochondria, as well as within cells. In addition, they may block co-factors of metabolic reactions, such as some vitamins. A significant adverse influence of numerous mycotoxins has also been confirmed (in particular of OTA, AFB_1, DON and T-2) on the antioxidant components of cells and tissues, indicating at the same time this process as on

the most dangerous for the organism's equilibrium and the occurrence of immunosuppression and oxidative stress (Sharma, 1993; Benett & Klich, 2003).

According to the current knowledge (Huwing et al., 2001; Diaz et al., 2004), removal of toxins from grains and feed can be carried out by mechanical separation of contaminated grains (sorting), addition of adsorptive materials, and using the activity of physical, chemical and biological factors.

Among the many microbes that show the potential for detoxification, special interest is aroused by lactic acid bacteria and yeasts (Shetty & Jespersen, 2006). Initial studies have shown that different strains of lactic acid bacteria can inhibit the biosynthesis of aflatoxins (Coallier-Ascah & Idziak, 1985). The concept of using yeasts to remove mycotoxins during fermentation processes appeared in the studies conducted by Benneta et al. (1981), who used corn contaminated by zearalenone as a substrate for alcohol production with the participation of yeasts of the genus *Saccharomyces*.

Literature data shows that apart from common lactic bacteria there appear varieties with different abilities to detoxify the environments from mycotoxins (Oatley, 2000). The research of EL-Nezami et al. (1996, 1998, 2000 a and b) proves that these properties are manifested by strains of *Lactobacillus rhamnosus* (LBGG and LC705). Ŝtyriak et al. (2001), examined the ability of 10 yeast strains of the genus *Saccharomyces*, *Kluyveromyces* and *Rhodotorula* to detoxify fumonisin B_1, toxin T-2 and ochratoxin A. Out of the tested organisms, yeasts of the genus *Saccharomyces* were characterized with the greatest predispositions in the area in question.

The research on the abilities of probiotic preparations for poultry manifested in the area of biological detoxification of mycotoxins, carried out under *in vitro* conditions (Biernasiak et al., 2006), showed that after six hours of incubation, the loss of aflatoxins in the control sample of feed, where there was no process of fermentation, equaled from 28 to 30%, and in case of ochratixin A it equaled from 8 to 10%. After twenty-four hours of incubation of the control sample with the addition of [5 µg/kg] ochratoxin A, a further loss was noted which equaled 10%, and 2% for the medium with the addition of [50 µg/kg]. The concentration of aflatoxins increased from 5 to 10%, probably as a result of desorption (Figure 2).
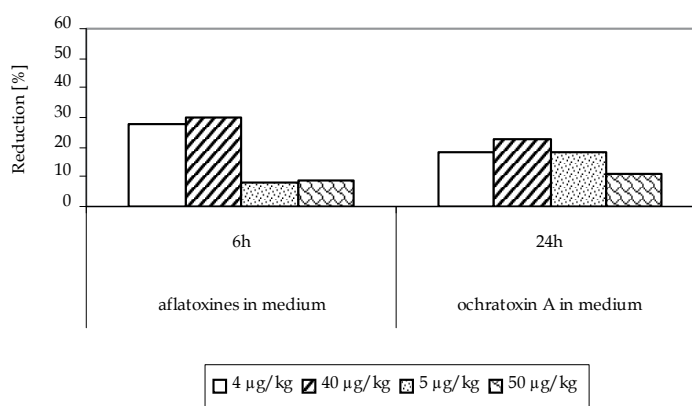
Fig. 2. Reduction of aflatoxins and ochratoxin A concentration in the sample without fermentation

After six hours of fermentation with the addition of probiotic cultures, the quantity of aflatoxins was reduced by 18 to 33% compared to that recorded in the control sample, and as for ochratoxin A the reduction equaled from 29 to 49%. After the fermentation, the loss of ochratoxin A, both at a low concentration, namely 5 µg/kg, and at a high concentration, namely 50 µg/kg, was at a similar level and equaled 50%. The same relationship was also noted for low levels of aflatoxins, i.e. for 4 µg/kg inserted into the fermentation medium. At high concentration, i.e. 40 µg/kg, the loss of aflatoxins was lower and amounted to about 30% (Figure 3).
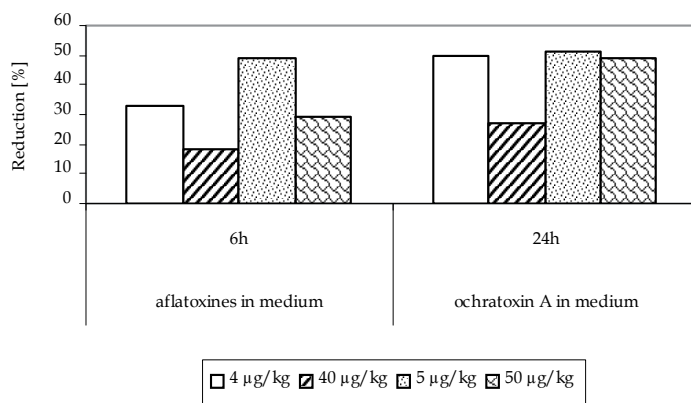
Fig. 3. Reduction of aflatoxins and ochratoxin A concentration during fermentation with the use of probiotic bacteria and yeasts

During a spontaneous fermentation, the loss of mycotoxins was much lower, and after six hours of fermentation a decrease was observed by 6–8% in the concentration of aflatoxins in relation to the initial concentration recorded in the control sample, and for ochratoxin A the decrease equaled to from 12 to 19%. After the fermentation, the loss of both aflatoxins and ochratoxin A was at a similar level from 16-24% compared with the medium in which no fermentation occurred (Figure 4). It was found that the probiotic vaccine creates a probiotic starter culture endowed with a stable feature of detoxification of mycotoxins, and particularly ochratoxin A.
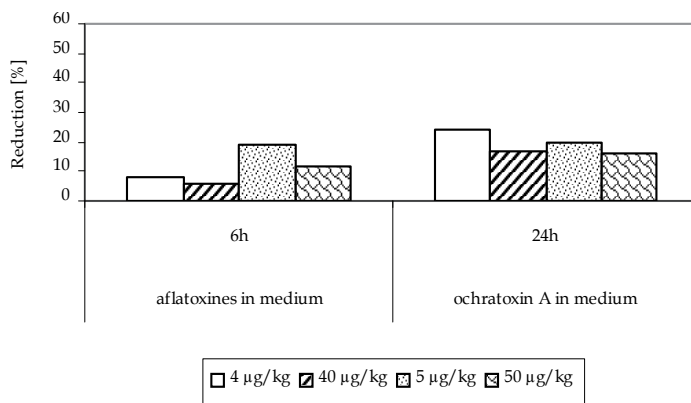
Fig. 4. Reduction of aflatoxins and ochratoxin A concentration during spontaneous fermentation

Śliżewska (unpublished data) examined the ability of a probiotic preparation to counteract the deleterious effects of aflatoxin B_1 (AFB$_1$) in broiler chickens (*in vivo* experiments). The probiotic preparation used contained (per 1 kg): 10^{10} of *Lactobacillus* cells (*L. paracasei* LOCK 0920, *L. brevis* LOCK 0944 and *L. plantarum* LOCK 0945), 10^6 of yeast *Saccharomyces cerevisiae* LOCK 0140 cells and 50g of *Yucca schidigera* extract. The Ross broiler chicks from a commercial hatchery were used in this study. The chickens were divided into four experimental groups depending on the feed administered to them: fed with the feed contaminated with 1 or 5 mg of AFB$_1$ per kg, and fed with the feed contaminated with AFB$_1$ and supplemented with the probiotic preparation. The amount of aflatoxin B_1 in the feces was determined for each week of rearing in 10 chickens in each group. After 35 days (of rearing) chickens were slaughtered and the concentration of toxins in the liver and kidneys was determined. The histopathological changes were evaluated in tissue sections obtained from the liver and kidneys of the tested animals.

The research showed that the presence of probiotics in the feed resulted in a statistically significant increase in the quantity of aflatoxin B_1 excreted in the feces of the chickens. At the end of breeding, i.e. on the 35th day, in the feces of chickens consuming feed contaminated with aflatoxin B_1 (at a concentration of 1 or 5 mg/kg) and containing the probiotic preparation, the content of toxins was higher by 67% and 31% compared to the feces of chickens consuming feed without the probiotic. The addition of the probiotic preparation prevented the accumulation of toxins in the organs (liver and kidneys) in the extent observed in the case of chickens consuming feed without the probiotic (Table 2). The toxins were not accumulated in the organs in such large amounts as in the case of chickens consuming feed without probiotics, and they were excreted to a greater extent in the feces.

Test group					
		1mg/kg AFB$_1$	1mg/kg AFB$_1$ +probiotic	5mg/kg AFB$_1$	5mg/kg AFB$_1$ +probiotic
		Concentration of aflatoxin B$_1$ [µg/kg]			
Feces	Range	0.21 - 0.47	0.52 - 1.25	2.52 - 2.88	3.68 - 7.50
	Mean*	0.29 (± 0.10)[a]	0.89 (± 0.30)[b]	2.74 (± 0.13)[c]	4.00 (± 1.61)[d]
Liver	Range	8.30 - 9.64	2.10 - 4.52	8.42 - 14.84	3.52 - 7.20
	Mean*	8.86 (± 0.49)[a]	3.69 (± 0.95)[b]	11.84 (± 2.99)[c]	5.88 (± 1.69)[d]
Kidneys	Range	4.60 - 11.62	1.24 - 3.60	10.10 - 17.16	2.94 - 4.62
	Mean*	7.93 (± 2.91)[a]	2.55 (± 0.96)[b]	13.71 (± 2.76)[c]	4.06 (± 0.68)[d]

* Results are presented as an arithmetic mean (± standard deviation)
[ab] Values in rows denoted with different letters differ considerably for $P<0.05$

Table 2. Concentration of aflatoxin B_1 in the feces, liver and kidneys

Pathological changes were observed in the livers of chickens consuming the feed contaminated with aflatoxin B_1 at both concentrations (1 and 5 mg/kg of feed). The changes were similar in type (fibrosis of the portal area and liver parenchyma, eosinophil infiltrates in the portal area, steatosis of hepatocytes), but the changes in the livers of chickens consuming the feed contaminated with aflatoxin B_1 and supplemented with probiotics were slightly less severe. The strongest toxic changes were found in the group of chickens consuming the feed contaminated with aflatoxin B_1 at 5 mg/kg of feed (Figure 5). These

changes revealed the characteristics of micronodular cirrhosis with very severe eosinophil infiltrates.

(a)   (b)
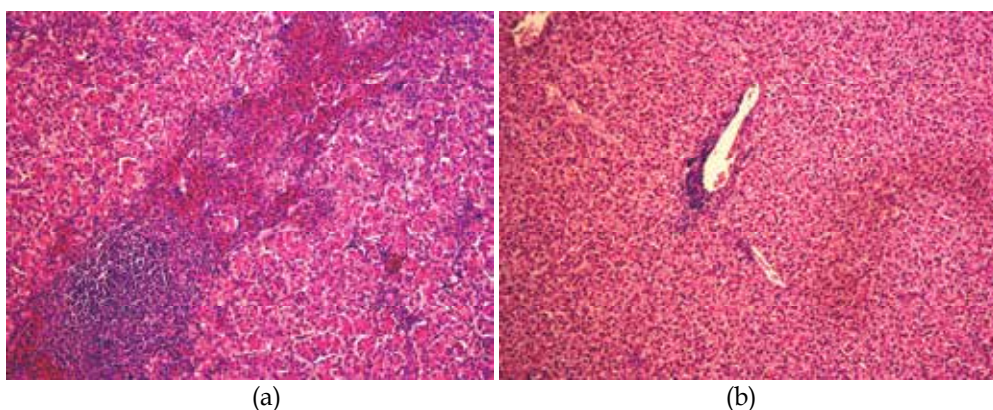
(a) Eosinophil infiltrates and enlarged lymphatic node (b) Eosinophil infiltrates

Fig. 5. Histological changes in the liver of chickens fed with the feed contaminated with AFB$_1$ (a) and supplemented with a probiotic preparation (b).

Kidney damage was found only in the chickens consuming the feed contaminated with 5 mg/kg of aflatoxin B$_1$; however, in the chickens consuming the feed with probiotics, the intensity of the changes was smaller. The changes concerned enlargement of renal glomeruli and an increase of mezangium matrix and cells (Figure 6).

While supplementation of feed with the probiotic preparation does not constitute protection against pathological changes in organs, it reduces the changes primarily in the kidneys.

(a)   (b)
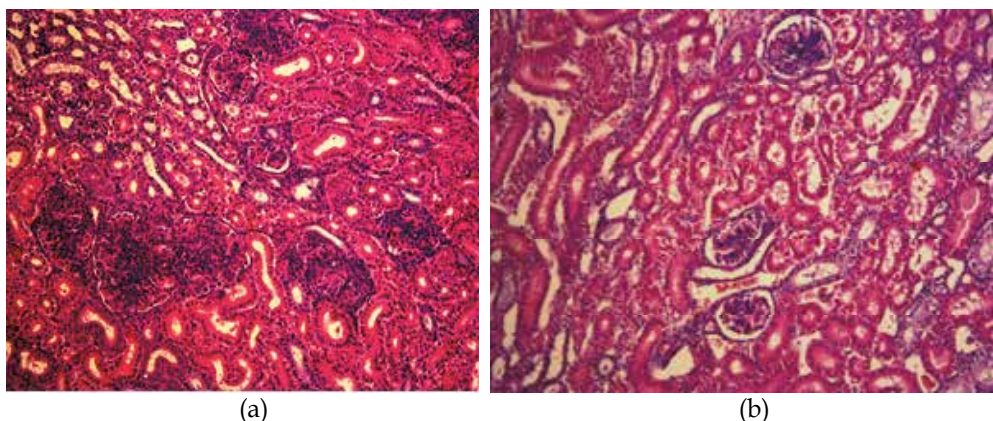
(a) Gromeruli diameter – 200-400 µm (b) Gromeruli diameter – 150-200 µm

Fig. 6. Histological changes in kidneys of the chickens fed with the feed contaminated with AFB$_1$ (a) and supplemented with probiotic preparation (b).

4.3 The immunological system stimulation

The microbes of intestinal microflora are the main factor stimulating the immune system, which is a prerequisite for the development of lymphoid structures of the system

(laboratory animals born and kept in sterile conditions do not develop them). Immunomodulating effect of intestinal microflora, including that of probiotic bacteria is based on three seemingly contradictory phenomena (Dugas et al., 1999; Isolauri et al., 2001):

- induction and maintenance of immune tolerance to environmental antigens (food and inhalants)
- induction and control of immune responses against pathogens of bacterial and viral origin
- inhibition of autoimmune and allergic reactions.

For example, in the case of chickens, GALT (gut-associated lymphoid tissue) reaches full maturity after two weeks since hatching (Bar–Shira et al., 2003). Until then, in the gastrointestinal immune system there are T and B cells, macrophages and NK cells (Lillehoj & Trout, 1996). The aim of the research conducted by Haghighi et al. (2006), was to determine the effect of probiotic bacteria on the increase of natural antibodies (IgA, IgG and IgM) in the intestinal content and blood serum of chickens. The chickens were divided into two groups. Group I was administered to the beak 0.5 ml of PBS buffer containing 10^6 of bacteria, namely *Lactobacillus acidophilus*, *Bifidobacterium bifidum* and *Enterococcus faecalis*, while Group II received 0.5 of PBS buffer without additives (control group). It was demonstrated that the intestinal content of the chickens receiving the probiotic, compared with the control group, had increased level of IgA antibodies ($P < 0.001$), reactive against the tetanus toxin (TT), alpha-toxin of *Clostridium perfringns* and bovine serum albumin (BSA). Similar relationships were recorded in relation to IgG antibodies, but they were reactive only to TT. The serum of the chickens treated with the probiotic showed an increased level of IgG antibodies ($P \leq 0.05$), but they were reactive only to TT and alfa–toxin. Similar dependencies ($P \leq 0.01$) were noted for IgM antibodies.

5. Effects of the application of probiotics in animal nutrition

The earliest from of probiotics, still widely used in animal nutrition, was based on silage, whose usefulness has been proven by many years of use. Modern probiotic preparations must be subjected to comprehensive testing in accordance with Commission Directive 94/40/EC of 22 July 1994 setting out procedures for the assessment of additives in animal nutrition.

The usefulness of probiotics in the nutrition of young pigs has been shown, although the results varied greatly from one another especially in relation to such indicators of production as growth and feed efficiency (Turner et al., 2002). The outcome of most studies indicate the beneficial effect of probiotics on the health of piglets. The most frequently observed effect is a reduction in the incidence rate of diarrhea and shortening of its duration, as well as a decrease in the mortality rate of piglets during the pre-weaning and peri-weaning period (Ross et al., 2008 & 2010). It was demonstrated that the best results are obtained when the probiotic is administered already on the first, or on the second day of life at the latest. That is why probiotics are administered to them after birth orally in the form of a special paste with the use of special dispensers (Janik et al., 2006). Literature reports on the effects of the application of probiotics in case of chickens are mixed, similarly to those referring to pigs.

The study conducted by Smulikowska et al., (2005) demonstrated that feeding broiler chickens with the feed supplemented with a probiotic preparation LABYuc-Probio (containing in 1g: 4.7×10^7 of *Lactobacillus* bacteria, 2.0×10^3 of *Saccharomyces cerevisiae* yeasts

and 50 mg of *Yucca schidigera* extract) did not result in significant changes in the body weight gains and feed utilization compared with a group of chickens receiving antibiotic feed with or without any additions.

Similar relationships were obtained in studies conducted by Watkins & Kratzer, (1983 & 1984) and Maiolino et al., (1992). However, in a group of chickens receiving a mixture supplemented with a probiotic preparation, the body mass of birds in different periods of rearing was the most uniform, as evidenced by lower standard deviation (SD). It was found that, regardless of the use of a probiotic feed additive, an antibiotic, or the total lack of supplements, the relative weights of liver, pancreas, abdominal fat and the individual sections of the gastrointestinal tract converted to % of the chickens' body weights before slaughter were similar and statistically insignificant. The research by Jin et al. (1998), showed that addition to the chickens' diet of *L. acidophilus* or a mix of bacteria of the genus *Lactobacillus*, namely *L. acidophilus* (2), *L. fermentum* (3), *L. crispatus* (1) and *L. brevis* (6), had no statistically significant effect either on the weight of the crop, liver, spleen, duodenum and small intestine expressed as % of the body weight of chickens prior to slaughter. Similar dependencies were also obtained in the studies of Fethiere & Miles, (1987) and Watkins & Kratzer, (1984).

In addition, our own study (Biernasiak et al., 2009) showed that supplementing feed with a probiotic preparation LABYuc-Probio® already during the first week of rearing resulted in a significant reduction in the number of bacteria of the genus *Clostridium* in the fecal-urate excreta of chickens. The number of these bacteria was about 10^5 CFU/g, while in the fecal-urate excreta of broiler chickens fed with the feed supplemented with an antibiotic or the feed without any additives it was higher by about two orders of magnitude. After the second week of rearing, regardless of the type of feed supplementation, there was noted a decrease by one order of magnitude in the number of bacteria of the genus *Clostridium*. After the third week of rearing, the further reduction in the number of bacteria of the genus *Clostridium* was observed, but only in the fecal-urate excreta of broiler chickens feed with the feed supplemented with the probiotic preparation. The number of bacteria then equaled around 10^3 CFU/g and it was lower by three orders of magnitude compared to that recorded in the fecal-urate excreta of the chickens included into the two remaining groups. After the fourth week of rearing, depending on the type of feed supplementation, a differentiation of the number of bacteria was observed; from 10^4 CFU/g to 10^5 CFU/g. Nevertheless, it must be emphasized that still the lowest number (10^4 CFU/g) of the studied microorganisms was recorded in the fecal-urate excreta of birds fed with the feed supplemented with the probiotic preparation. After the fifth and sixth week of rearing, the number of bacteria of the genus *Clostridium* in the fecal-urate excreta of broilers fed with the feed with the probiotic or without additional supplements was at the same level and amounted to approximately 10^5 CFU/g. The birds receiving the feed with antibiotics, compared with the other two, still manifested a higher number of these bacteria, by respectively, two orders of magnitude, and one order of magnitude (Figure 7). Analysis of variance Anova showed statistically significant differences between the kind of feed supplementation, and the number of bacteria of the genus *Clostridium* in the fecal-urate excreta of chickens in the period from the second to the fifth week of rearing ($0.01 < p < 0.05$). The obtained results are particularly important in view if the fact that after the withdrawal of antibiotic growth promoters from poultry feed mixtures an increase may be expected in the incidence of intestinal problems, especially related to *Clostridium perfringens*, i.e. necrotic

enteritis (NE). In France, the occurrence of NE increased from 4.0% in 1995 to 12.4% in 1999 and similar dependencies were also observed in other European countries (Lipiński, 2007).
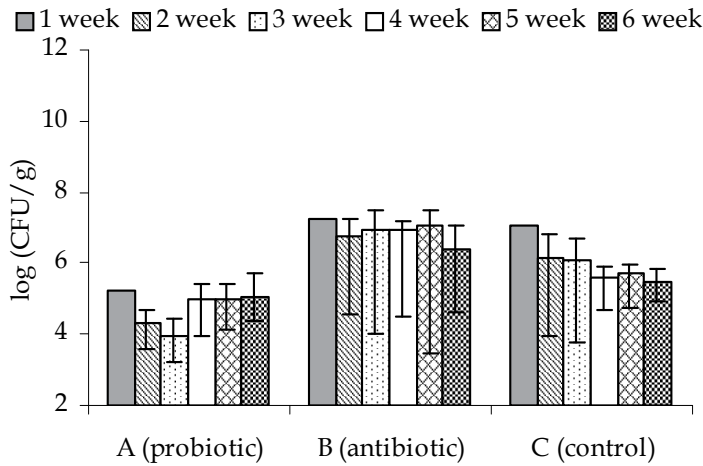
Fig. 7. Bacteria of the genus *Clostridium* in the feces of chickens

Supplementing the feed with the probiotic preparation LABYuc-Probio® also contributed to the stabilization of the number of *Enterobacteriaceae*, including bacteria from the *coli* group in the chickens' fecal-urate excreta during individual weeks of rearing (Figure 8).
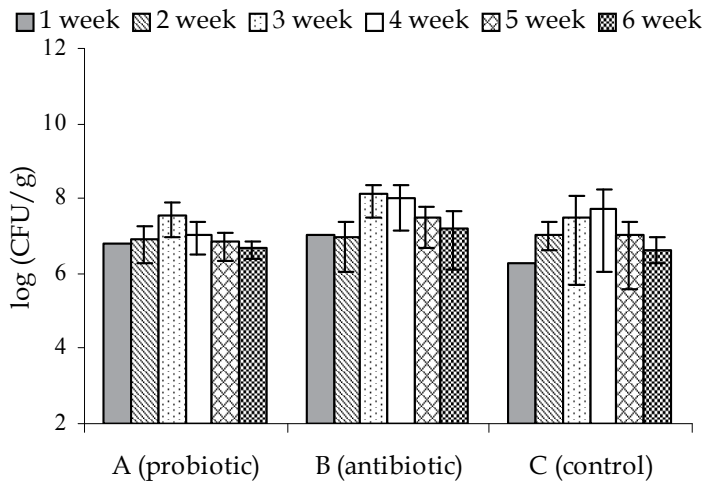
Fig. 8. Total number of bacteria of the *coli* group in the feces of chickens

Kralik et al. (2004) recorded a decrease in the number of *Enterobacteriaceae* and bacteria of the *coli* group by about 90%, compared to the control group, i.e. 1.39×10^6 and 2.72×10^5 CFU/g after 42 days of supplementing water with a probiotic containing 5×10^9 CFU/g of *Enterococcus faecium* M-74. However, he did not find statistically significant differences in the

number of bacteria of the genera *Staphylococcus* sp., *Bacillus* sp. and *Clostridium* sp. Jin et al., (1998) showed that the addition of *L. acidophilus* or a mixture of bacteria of the genus *Lactobacillus* into the chickens' diet had a statistically significant effect (P<0.05) on the decrease in the number of bacteria of the *coli* group in the blind gut, compared to the control sample, but only on the 10th and 20th day of rearing. At the same time, he registered no similar changes in the small intestine.

An average number of bacteria of the genus *Lactobacillus* in the fecal-urate excreta of all studied groups of chickens during individual weeks of rearing equaled from 10^9 to 10^{10} CFU/g (Figure 9). Data analysis showed that only after the third week of rearing there were recorded statistically significant differences (p<0.01) between the type of feed additive, and the number of bacteria of the genus *Lactobacillus* found in the birds' fecal-urate excreta (Biernasiak & Slizewska, 2009). The research of Jin et al., (1998) demonstrated that supplementation with *L. acidophilus* or a mixture of bacteria of the genus *Lactobacillus* did not have an influence on the statistically significant increase in the number of bacteria of the genus *Lactobacillus* in the blind gut during the individual weeks of rearing, and in case of the small intestine significant changes were recorded only on the 30th day of rearing. Similarly, the research of Watkins & Kratzer (1983, 1984) found no significant increase in the number of bacteria of the genus *Lactobacillus* sp. in the chickens' intestines.
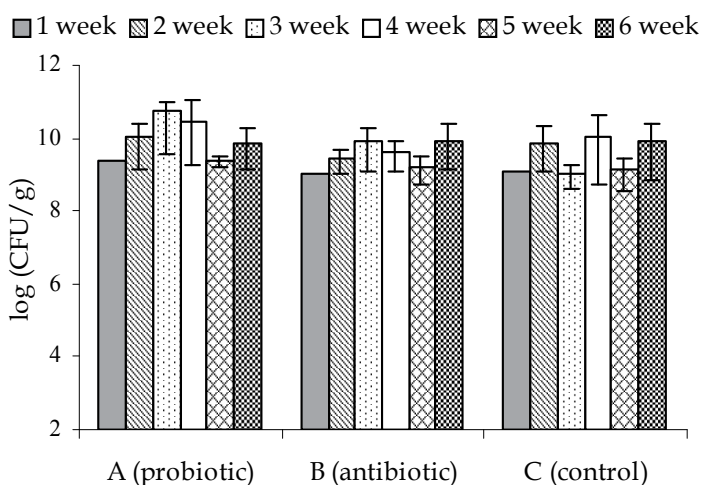
Fig. 9. Bacteria of the genus *Lactobacillus* in the feces of chickens

While using a probiotic preparation consisting of *Bacillus subtillis* CH201 and *Bacillus licheniformis* CH200 in the feed for laying hens, a statistically significant decrease was noted (P<0.05) as for the content of cholesterol and triglycerides in the serum and egg yolk. However, no statistically significant increase in the feed efficiency, egg production, or the impact on the thickness and hardness of the shell was demonstrated (Mahdari et al., 2005). On the other hand, the application of *Enterococcus faecium* M-74 in the feed for laying hens resulted in obtaining eggs with thicker and more breakage-resistant shells and in a more intense color of the yolk (Angelovicova, 1996).

It should be emphasized that the results presented above represent only a fraction of worldwide research. The divergence of the results in the presented cases indicates the need

for further research in order to clarify the questionable effects of probiotics in animal nutrition.

The future of probiotics involves aspiring to reach full explanation of mechanisms concerning their activity in relation to mutual microorganism-animal interaction and looking for new bacterial strains, as well. Delimitation of appropriate directions of research can have a great meaning for newly described principles of prophylaxis in animal nutrition without using antibiotic growth stimulators.

6. References

Alakomi, H.L.; Skytta, E.; Saarela, M.; Mattila-Sandholm, T.;, Latva-Kala, K. & Helander, I.M. (2000). Lactic acid permeabilizes gram-negative bacteria by disrupting the outer membrane. *Applied and Environmental Microbiology*, Vol. 66, No. 5, pp. 2001-2005, ISSN 1098-5336

Anadón, A.; Martinez-Larrañaga, M.R. & Martinez, M.A. (2006). Probiotics for animal nutrition in the European Union. Regulation and safety assessment. *Regulatory. Toxicology and Pharmacology,* Vol. 45, pp. 91-95, ISSN 0273-2300

Angelowicova, M. (1996). The effect of *Streptococcus faecium* M-74 based probiotic on the performance of laying hens. *Czech Journal of Animal Science*, Vol. 41, No. 9, pp. 391-395, ISSN 1212-1819

Bar-Shira, E.; Sklan, D. & Friedman, A. (2003). Establishment of immune competence in the avian GALT during the immediate post-hatch period. *Developmental & Comparative Immunology*, Vol. 27, pp. 147-157, ISSN 0145-305X

Batish, V.K.; Uptal, R.; Ram, L. & Grover, S. (1997). Antifungal attributes of lactic acid bacteria - a review. *Critical Reviews in Biotechnology*, Vol. 17, No. 3, pp. 209-225, ISSN 0738-8551

Bennett, G.A.; Lagoda, A.A.; Shotwell, O.L. & Hesseltine, C.M. (1981). Utilization of zearalenone-contaminated corn for ethanol production. *Journal of the American Oil Chemists' Society*, Vol. 58, pp. 974–976, ISSN 0003-021X

Bennett, J.W. & Klich, M. (2003). Mycotoxins. *Clinical Microbiology Reviews*, Vol. 16, No. 3, pp. 497-516, ISSN 0893-8512

Berghmann, L.R.; Abi-Ghanem, D.; Wagnela, S.D. & Ricke, S.C. (2005). Antibodies: an alternative for antibiotics? *Poultry Science*, Vol. 84, No. 4, pp. 660-666, ISSN 0032-5791

Biernasiak, J. & Śliżewska K. (2009). The effect of a new probiotic preparation on the performance and faecal microflora of broiler chickens. *Veterinarni Medicina*, Vol. 54, No. 12, pp. 525-531, ISSN 0375-8427

Biernasiak, J.; Piotrowska, M. & Libudzisz, Z. (2006). Mycotoxins by probiotic preparation for broiler chickens. *Mycotoxin Research*, Vol. 22, No. 4, pp. 230-235, ISSN 0178-7888

Blom, H. & Mörtvedt, C. (1991). Anti-microbial substances produced by food associated microorganisms. *Biochemical Society Transactions,* Vol. 19, pp. 694-698, ISSN 0300-5127

Boris, S. & Barbés, C. (2000). Role played by lactobacilli in controlling the population of vaginal pathogens. *Microbes and Infection,* Vol. 2, No. 5, pp. 543-546, ISSN 1286-4579

Caplice, E. & Fitzgerald, G.F. (1999). Food fermentations: role of microorganisms in food production and preservation. *International Journal of Food Microbiology*, Vol. 50, No. 1-2, pp. 131-149, ISSN 0168-1605

Casewell, M.; Friis, C. & Marco, E. (2003). The European ban on growth promoting antibiotics and emerging consequences for human and animal health. *Journal of Antimicrobial Chemotherapy*, Vol. 52, No. 2, pp. 159-161, ISSN 0305-7453

Cherrington, C.A.; Hinton, M.; Pearson, G.R. & Chopra, I. (1991). Short-chain organic acids at pH 5,0 kill *Escherichia coli* and *Salmonella* spp. without causing membrane perturbation. *Journal of Applied Bacteriology*, Vol. 70, No. 2, pp. 161-165, ISSN 0021-8847

Coallier-Ascah, J. & Idziak, E.S. (1985). Interaction between *Streptococcus lactis* and *Aspergillus flavus* on production of aflatoxin. *Applied and Environmental Microbiology*, Vol. 49, No. 1, pp. 163-167, ISSN 1098-5336

Commission Directive 94/40/EC of 22 July 1994 amending Council Directive 87/153/EEC fixing guidelines for the assessment of additives in animal nutrition.

Council Directive 70/524/EEC of 23 November 1970 concerning additives in feedingstuffs.

Council Directive 90/220/EEC of 23 April 1990 on the deliberate release into the environment of genetically modified organisms

Czaczyk, K. (2003). Tworzenie biofilmów bakteryjnych-istota zjawiska i mechanizmy oddziaływań. Biotechnologia, [The creating biofilms of bacteria - the creature the phenomenon and mechanisms of influences. Biotechnology] Vol. 3, pp. 180-192, ISSN 2080-8682 [in Polish]

Daeschel, M. (1989). Antimicrobial substances from lactic acid bacteria for use as food preservatives. *Food Technology*, Vol. 1, pp. 164-167, ISSN 0015-6639

Dembele, T.; Obdrzalek, V. & Votava, M. (1998). Inhibition of bacterial pathogens by lactobacilli. *Zentralblatt für Bakteriologie*, Vol. 288, No. 3, pp. 395-401, ISSN 0934-8840

Diaz, D.E.; Hagler, W.M.; Blackwelder, J.T.; Eve, J.A.; Hopkins, B.A.; Andersen, K.L.; Jones, F.T. & Whitlow, L.W. (2004). Aflatoxin binders II: Reduction of aflatoxin M1 in milk by sequestering agents of cows consuming aflatoxin in feed. *Mycopathologia*, Vol. 157, pp. 233–241, ISSN 0301-486X

Dugas, B.; Mecenier, A.; Lenoir-Wijnkoop, J.; Arnaud, C.; Dugas, N. & Postaire, E. (1999). Immunity and probiotics. *Immunology Today*, Vol. 20, No. 9, pp. 387-389, ISSN 0167-5699

Eklund, T. (1983). The antimicrobial effect of dissociated and undissociated sorbic acid at different pH levels. *Journal of Applied Bacteriology*, Vol. 54, pp. 383-389, ISSN 0021-8847

Ekuland, T. (1989). Organic acids and esters. In: Mechanisms of Action of Food Preservation Procedures, G. W. Gould (Ed.), 161-200, London, ISBN 1851662936, Elsevier Applied Science

El-Nezami, H.; Kankaanp, P.; Salminen, S. & Ahokas, J. (1998). Ability of dairy strains of lactic acid bacteria to bind a common food carcinogen, aflatoxin B_1. *Food and Chemical Toxicology*, Vol. 36, pp. 321-332, ISSN 0278-6915

El-Nezami, H.S.; Chrevatidis, A.; Auriola, S.; Salminen, S. & Mykkänen, H. (2002a). Removal of common *Fusarium* toxins in vitro by strains of *Lactobacillus* and *Propionibacterium*. *Food Additives and Contaminants*, Vol. 19, pp. 680-686, ISSN 0265-203X

El-Nezami, H.S.; Polychronaki, N.; Salminen, S. & Mykkänen, H. (2002b). Binding rather than metabolism may explain the interaction of two food grade *Lactobacillus* strains with zearalenone and its derivative α-zearalenol. *Applied and Environmental Microbiology*, Vol. 68, No. 7, pp. 3545-3549, ISSN 1098-5336

El-Nezami, H.S.; Salminen, S. & Ahokas, J.T. (1996). Biologic control of food carcinogen using *Lactobacillus* GG. *Nutrition Today*, Vol. 31, pp. 41-43, ISSN 0029-666X

FAO (2002) Guidelines for the Evaluation of Probiotics in Food. *Report of a Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food*, Londyn Ontario, Kanada, April 30 and May 1, 2002 r.

Fethiere, R. & Miles, R.D. (1987). Intestinal tract weight of chicks fed an antibiotic and probiotic. *Nutrition Reproduction Incorporation*, Vol. 36, pp. 1305-1309, ISSN 1608-3369

Fink-Gremmels, J. (1999). Mycotoxins: their impact on human and animal health. *Veterinary Quarterly*, Vol. 21, pp. 115-120, ISSN 0042-4900

Gajęcki, M. (2002). Mikotoksyny jako czynnik antyżywieniowy w produkcji zwierzęcej. Hodowca Drobiu [Mycotoxin as unnutritional factor in animal production. Poultry farmer's], Vol. 9, pp. 5-17, ISSN 1425-963X [in Polish]

Gedek, B.R. (1999). Adherence of *Escherichia coli* serogroup O 157 and the *Salmonella* Typhimurium mutant DT 104 to the surface of *Saccharomyces boulardii*. *Mycoses*, Vol. 42, No. 4, pp. 261-264, ISSN 0933-7407

Gourama, H. & Bullerman, L.B. (1995). Antimycotic and antiaflatoxigenic effect of lactic acid bacteria: a review. *Journal of Food Protection*, Vol. 57, No. 11, pp. 1275-1280, ISSN 0362-028X

Grajek, W. & Sip, A. (2004). Biologiczne utrwalanie żywności z wykorzystaniem metabolitów bakterii mlekowych. W: Bakterie fermentacji mlekowej Klasyfikacja, metabolizm, genetyka, wykorzystanie. [Biological fixation food with utilization of lactic acid bacteria metabolites In: Lactic acid bacteria. Classification, metabolism, genetiics, application]. Eds. Z. Libudzisz, P. Walczak, J. Bardowski. Politechnika Łódzka [Technical University of Lodz.], pp. 103–197 [in Polish].

Haghighi, H.R.; Gong, J.; Gyles, C.L.; Hayes, M.A.; Zhou, H.; Sanei, B.; Chambers, J.R. & Sharif, S. (2006). Probiotics stimulate production of natural antibodies in chickens. *Clinical and Vaccine Immunology*, Vol. 13, No. 9, pp. 975-980, ISSN 1556-6811

Huwing, A.; Freimund, S.; Kappeli, O. & Dutler, H. (2001). Mycotoxin detoxication of animal feed by different adsorbents. *Toxicology Letters*, Vol. 122, pp. 79–188, ISSN 0378-4274

Isolauri, E.; Sütas, Y.; Kankaanpää, P.; Arvilommi, H. & Salminen, S. (2001). Probiotics: effects on immunity. *The American Journal of Clinical Nutrition*, Vol. 73, pp. 444S-450S, ISSN 0002-9165

Janik, A.; Kaska, M.; Paluch, U.; Pieszka, M. & Borowicz, T. (2006). Probiotyki w żywieniu prosiąt. *Wiadomości Zootechniczne*, [Probiotics in pigs nutrition. *Zootechnical Message*] Vol. R.XLIV, pp. 1–39, ISSN 0209-2492 [in Polish]

Jin, L.Z.; Ho, Y.W.; Abdullah, N.; Ali, M.A. & Jalaludin, S. (1998). Effects of adherent *Lactobacillus* cultures on growth, weight of organs and intestinal microflora and volatile fatty acids in broilers. *Animal Feed Science and Technology*, Vol. 70, pp. 197-209, ISSN 0377-8401

Klaenhammer, T.R. (1993). Genetics of bacteriocins produced by lactic acid bacteria. *FEMS Microbiology Review*, Vol. 12, pp. 39-86, ISSN 0168-6445

Kralik, G.; Milaković, Z. & Ivanković, S. (2004). Effect of probiotic supplementation on the performance and the composition of the intestinal microflora in broilers. *Acta Agraria Kaposváriensis*, Vol. 8, No. 2, pp. 23-31, ISSN 1418-1789

Lillehoj, H.S. & Trout, J.M. (1996). Avian gut-associated lymphoid tissues and intestinal immune responses to *Eimerica* parasites. *Clinical Microbiology Reviews*, Vol. 9, pp. 349-360, ISSN 0893-8512

Lilly, D.M. & Stillwell, R.H. (1965). Probiotics: Growth promoting factors produced by microorganisms. *Science*, Vol. 147, pp. 747-748, ISSN 0036-8075

Lipiński, K. (2007). Żywieniowe strategie zapobiegania nekrotycznemu zapaleniu jelit. *Hodowca drobiu*, [The nutritional strategies of prevention the enteritis. *Poultry farmer's*] Vol. 2, pp. 5-17, ISSN 1425-963X [in Polish]

Mahdari, A.H.; Rahmani, H.R. & Pourreza, I. (2005). Effect of probiotic supplements on egg quality and laying hen's performance. *International Journal of Poultry Science*, Vol. 4, No. 7, pp. 488-492, ISSN 16828356

Maiolino, R.; Fioretii, A.; Menna, L.F. & Meo, C. (1992). Research on the efficiency of probiotics in diets for broiler chickens. *Nutrition Abstracts and Reviews Series B*, Vol. 62, pp. 482, ISSN 0029-6619

Mercenier, A.; Pavan, S. & Pot, B. (2003). Probiotics as biotherapeutic agents: Present knowledge and future prospects. *Current Pharmaceutical Design*, Vol. 9, No. 2, pp. 175-191, ISSN 1381-6128

Messens, W. & de Vuyst, L. (2002). Inhibitory substances produced by *Lactobacilli* isolated from sourdough - a review. *International Journal of Food Microbiology*, Vol. 72, No. 1, pp. 31-43, ISSN 0168-1605

Michałowski, P., Libudzisz, Z., Śliżewska, K., Klewicka, E., Motyl, I. & Zabierski, R. (2004). Probiotic growth promoter for poultry. Licence Number P-371363

Mitterdorfer, G.; Mayer, H.K.; Kneifel, W. & Viernstein, H. (2002). Clustering of *Saccharomyces boulardii* strains within the species *S. cerevisiae* using molecular typing techniques. *Journal of Applied Microbiology*, Vol. 18, pp. 521-530, ISSN 1364-5072

Mountzouris, K.C.; Tsirtsikos P.; Kalamara E.; Nitsch S.; Schatzmayr, G. & Fegeros, K. (2007). Evaluation of the Efficacy of a Probiotic Containing *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, and *Pediococcus* Strains in Promoting Broiler Performance and Modulating Cecal Microflora Composition and Metabolic Activities. *Poultry Science*, Vol. 86, pp. 309-317, ISSN 0032-5791

Nousiainen, J.; Jaranainen, P.; Setala, J. & von Wright, A. (2004). Lactic acid bacteria as animal probiotics. *Food Science and Technology*, Vol. 139, pp. 547-580, ISSN 0023-6438

Oatley, J.T.; Rarick, M.D.; Ji, G.E. & Linz, J.E. (2000). Binding of aflatoxin B1 to Bifidobacteria *in vitro*. *Journal of Food Protection*, Vol. 63, No. 8, pp. 1133-1136, ISSN 0362-028X

Ouwehand, A.C. & Vesterlund, S. (2004). Antimicrobial components from lactic acid bacteria. *Food Science and Technology*, Vol. 139, pp. 375-396, ISSN 0023-6438

Parker, R.B. (1974). Probiotics, the other half of the antibiotic story. *Animal Nutrition and Health*, Vol. 29, pp. 4-8, ISSN 0003-3553

Ray, B. (1992). Food and microorganisms of concern. In: Food biopreservatives of Microbial Origin, B. Ray & M. Daeschel (Ed.), pp. 103-197, Floryda, ISBN 0849349435, CRC Press

Ross, G.R.; Gusils, C. & Gonzalez, S.N. (2008). Microencapsulation of probiotic strains for swine feeding. *Biological and Pharmaceutical Bulletin*, Vol. 31, No. 11, pp. 2121-2125, ISSN 0918-6158

Ross, G.R.; Gusils, C.; Oliszewski, R.; de Holgado, S.C. & González, S.N. (2010). Effects of probiotic administration in swine. *Journal of Bioscience and Bioengineering*, Vol. 109, No. 6, pp. 545-549, ISSN 1389-1723

Saarela, M.; Mogensen, G.; Fonden, R.; Matto, J. & Mattila-Sandholm, T. (2000). Probiotic bacteria: safety, functional and technological properties. *Journal of Biotechnology*, Vol. 84, No. 3, pp. 197-215, ISSN 0168-1656

Salminen, S.; von Wright, A.; Morelli, L.; Marteau, P.; Brassart, D.; de Vos, W.M.; Fondén, R.; Saxelin, M.; Collins, K.; Mogensen, G.; Birkeland, S.E. & Mattila-Sandholm, T. (1998). Demonstration of safety of probiotics - a review. *International Journal of Food Microbiology*, Vol. 44, No 1-2, pp. 93-106, ISSN 0168-1605

Schillinger, R.G. & Holzapfel, W.H. (1996). Potential of antagonistic microorganisms and bacteriocins for the biological preservation of food. *Trends in Food Science & Technology*, Vol. 7, pp. 158-164, ISSN 0924-2244

Sharma, R.P. (1993). Immunotoxicity of mycotoxins. *Journal of Dairy Science*, Vol. 76, pp. 892-897, ISSN 2044-2432

Shetty, P.H. & Jespersen, L. (2006). *Saccharomyces cerevisiae* and lactic acid bacteria as potential mycotoxin decontaminating agents. *Trends in Food Science and Technology*, Vol. 17, pp. 48-55, ISSN 0924-2244

Smulikowska, S.; Śliżewska, K.; Biernasiak, J.; Mieczkowska, A. & Michałowski, P. (2005). The effect of a probiotic composed of Lactobacillus and yeasts, and of flavomycin on the performance and faecal microflora of broiler chickens. *Journal of Animal and Feed Sciences*, Vol. 14, pp. 483-485, ISSN 1230-1388

Ŝtyriak, I.; Čonková, E.; Kmet, V.; Böhm, J. & Razzazi, E. (2001). The use of yeast for microbial degradation of some selected mycotoxin. *Mycotoxin Research*, Vol. 174, No. 2, pp. 24-27, ISSN 0178-7888

Tasteyre, A.; Barc, M.C.; Karjalainen, T.; Bourlioux, P. & Collignon, A. (2002). Inhibition of in vitro cell adherence of *Clostridium difficile* by *Saccharomyces boulardii*. *Microbial Pathogenesis*, Vol. 32, pp. 219-225, ISSN 0882-4010

Tomas, M.S.; Otero, C.M.; Ocana, V. & Nader-Macias, E.M. (2004). Production of antimicrobial substances by lactic acid bacteria I: determination of hydrogen peroxide. *Methods in Molecular Biology*, Vol. 268, pp. 337-346, ISSN 1064-3745

Turner, J.L.; Pas, S.; Dritz, S. & Minton, J.E. (2002). Alternatives to conventional antimicrobials in swine diets. *Professional Animal Scientists*, Vol. 17, pp. 217-226, ISSN 1080-7446

Van der Aa Kühle, A.; Skovgaard, K. & Jespersen, L. (2005). In vitro screening of probiotic properties of *Saccharomyces cerevisiae* var. *boulardii* and food-borne *Saccharomyces cerevisiae* strains. *International Journal of Food Microbiology*, Vol. 101, No. 1, pp. 29-39, ISSN 0168-1605

Vergin, F. (1954). Anti- und Probiotika. *Hipokrates*, Vol. 25, pp. 16-119, ISSN 0018-2001

Watkins, B.A. & Kratzer, F.H. (1983). Effect of oral dosing of *Lactobacillus* strains on gut colonization and liver biotin in broiler chicks. *Poultry Science*, Vol. 62, pp. 2088-2094, ISSN 0032-5791

Watkins, B.A. & Kratzer, F.H. (1984). Drinking water treatment with commercial preparation of a concentrated *Lactobacillus* culture for broiler chickens. Poultry Science, Vol. 63, pp. 1671-1673, ISSN 0032-5791

Witte, W. (2000). Ecological impact of antibiotic use in animals on different complex microflora: environment. *International Journal of Antimicrobial Agents*, Vol. 14, No. 4, pp. 321-325, ISSN 0924-8579

Yiannikouris, A. & Jouany, J.P. (2002). Mycotoxins in feeds and their fate in animals: a review. *Animal Research*, Vol. 51, pp. 81-99, ISSN 0367-6722

Using Exogenous Enzymes to Increase the Nutritional Value of Soybean Meal in Poultry Diet

Tuoying Ao
Alltech Inc.
U.S.A.

1. Introduction

Soybean meal is by far the most widely used protein source in animal feed all over the world. It is estimated that about 63% of all protein sources used in animal feed is from soybean meal while 98 % of the plant protein used in poultry feeds is from soybean meal in the U.S. (Soybean meal INFO center, 2010). The major reasons for the popularity of soybean meal in poultry and swine feed include: 1) compared with other plant protein sources, soybean meal is not only rich in protein (44 - 48%), but also can provide a complete protein with almost all essential amino acids for animals (Table 1); 2) the development of heating process to denature the anti-nutritional factors, especially trypsin inhibitors in soybean; 3) the discovery and production of vitamins by using chemical synthesis and fermentation. Six decades ago, animal protein was an essential ingredient in animal feed for providing not only protein, but also minerals and vitamins, especially vitamin B12; 4) further reduction of animal protein in the feed due to the concern of the health and safety of animal byproducts and the concern of phosphorus load in animal waste; 5) modern poultry and swine producing system restricted the use of other plant protein source, such as milling byproducts in animal feed; 6) computer based least-cost feed formulation program proved that the corn and soybean meal based simple diet fortified with vitamins, minerals and methionine is the least cost in producing broiler chicks and turkeys in the U.S. The simplicity of the diet can also reduce the cost and time associated with purchasing, storing and handling more ingredients.

2. Anti-nutritional factors in soybean and its negative effect on the nutritional value of soybean meal

Soybeans contain variety of anti-nutritional factors that either adversely affect their nutritional value or are detrimental to the animals unless they are properly controlled. The deleterious effects of feeding raw soybean meal on animals have been well documented (Osborne and Mendel, 1917; Hayward et al., 1937; Almquist and Meritt, 1952). The major anti-nutritional factors in soybeans include trypsin inhibitors, phytic acid, oligosaccharides, antigenic factors and lectins.

Trypsin inhibitors in raw soybeans have been long known to reduce the digestibility of dietary proteins through inhibiting the activities of trypsin and chymotrypsin produced by

pancreas (Pusztai, 1967; Gallaher and Schneeman, 1984). It has been proved that trypsin inhibitors can overstimulate the secretion of digestive enzymes from the exocrine pancreas to cause pancreatic hypertrophy of experimental animals (Lyman and Lepkovsky, 1957; Liener 1981; Rackis, J. J. and Gumbman, 1981).

Approximately two thirds of the total phosphorus in plant feed stuff is present in the form of phytic acid or phytate and in soybean meal 75% of the phosphorus is present as phytate (CVB, 1998). Monogastric animals such as poultry and swine have very limited ability to utilize the phytate phosphorus due to the lack of significant amount of endogenous phytase that will hydrolyze phytic acid (Cooper and Gowing, 1983). Phytate is considered toxic, or

Nutrients, % as-is basis	Soybean meal	Sunflower meal	Canola meal	Peanut meal	Cottonseed meal	Sesame meal
Dry matter	88.4	89.8	88.0	91.9	90.4	90.0
Crude protein	47.5	36.8	34.8	49.0	41.4	41.0
Lysine	2.96	1.24	1.94	1.54	1.76	0.91
Methionine	0.67	0.80	0.71	0.54	0.51	1.22
Cystine	0.72	0.64	0.87	0.64	0.62	0.72
Histidine	1.28	0.87	0.93	1.07	1.10	0.99
Isoleucine	2.12	1.43	1.37	1.55	1.33	1.51
Leucine	3.74	2.22	2.47	2.97	2.41	2.68
Threonine	1.87	1.29	1.53	1.24	1.34	1.40
Tryptophan	0.74	0.41	0.44	0.48	0.52	0.62
Serine	2.48	1.49	1.53	2.25	1.78	1.72
Phenylalanine	2.34	1.66	1.44	2.41	2.23	1.93
Tyrosine	1.95	0.91	1.09	1.8	1.14	1.48
Glycine	2.05	2.03	1.82	2.67	1.69	2.04
Arginine	3.48	2.85	2.08	5.33	4.66	4.68
Valine	2.22	1.74	1.76	1.87	1.82	1.91

Table 1. Protein and amino acids content of commonly used plant protein sources for poultry, NRC 1994

antinutritive because it is capable of binding di- and trivalent cations such as Ca, Co, Cu, Fe, Mg, Mn, Ni and Zn in very stable complexes (Cosgrove, 1980; Wise, 1983) and reducing the availability of these minerals to the animal (Pallauf and Rimbach, 1997). In addition, phytate may form complexes with proteins and starches and may also reduce the availability of these nutrients from the diet (Thompson, 1986).

Soybeans contain three main types of oligosaccharides including verbascose, stachyose and raffinose that make up approximately 6% of soybean meal dry matter. These oligosaccharides cannot be digested in the small intestine of monogastric animals due to the absence of endogenous enzyme with α-galactosidase activity (Gitzelmann and Auricchio,

1965). The accumulation of these oligosaccharides in the alimentary tract results in fluid retention and an increased flow rate of digesta, which negatively affects the digestion and absorption of nutrients (Wiggins, 1984).

Feeding soy-based rations to early weaned pigs has sometimes led to reduced feed intake and slower growth rates. Studies found that soybeans contain certain types of antigenic proteins that can cause an inflammatory response in the intestine of early-weaned pigs. The allergic response is greater if pigs are exposed to soybean meal before they are weaned (Cromwell, 1999).

Lectins are glycoproteins that have the ability to bind to cellular surfaces via specific oligosaccharides or glycopeptides (Oliveira *et al*., 1989). Studies indicated that lectins can bind to small intestinal epithelium (Pusztai, 1991) and cause impairment of brush border continuity and ulceration of villi (Oliveira *et al*., 1989), which are believed major causes for increased endogenous nitrogen losses (Oliveira and Sgarbierri, 1986; Schulze *et al*., 1995) and depressed growth rate in young animals (Pusztai *et al*., 1990). Douglas et al. (1999) reported that approximately 15% of the total growth depression from raw soybeans in chicks was associated with lectins.

3. Approaches used in inactivating the anti-nutritional factors in soybean

It has long been recognized that major anti-nutritional factors in soybeans such as trypsin inhibitors and lectins are heat labile and can be destroyed with heat (Hayward et al., 1936; Borechers et al., 1948). Caskey and Knapp (1944) first reported that the heat treatment required to inactivate the urease in soybean parallels the treatment required to destroy the trypsin inhibitor. Since then, urease test, which measures the rise in pH when soybean meal is placed in a solution containing urea, has become widely used method to monitor adequacy of soybean meal heating processing. In general, good quality soybean meal with optimal nutritional value requires proper toasting condition including moisture content, temperature, shear force and duration of heating. Under-processing may result in low quality meal as substantial amount of anti-nutritional factors in soybean meal remain active. However, over-processing can also reduce the nutritive value of soybean meal by rendering unavailable several essential amino acids, particularly lysine and arginine (Hayward et al., 1936; Renner et al., 1953). Protein solubility in a KOH solution has been proved a good indicator for monitoring over-processing of soybean meal (Araba and Dale, 1990). Other methods such as chemical treatments (Sessa et al., 1990; Wu and Sessa, 1994), chemical modification of disulfide bonds (Wang et al, 2009), fermentation (Feng et al., 2007) etc. were studied to explore the possibility to inactivate the activity of trypsin inhibitors, however, these methods still stay in research level so far. Also, plant breeders have been trying to improve the nutritive value and reduce the anti-nutritional factors such as trypsin inhibitors, phytate and oligosaccharides in soybean through plant breeding.

4. The use of exogenous enzymes to increase the nutritional value of soybean meal

Although heat treatment is generally considered a very effective approach to inactivate antinutritive factors such as trypsin inhibitors and lectins in soybean meal (Campbell and van der poel, 1998), some of antinutritive factors such as phytate, oligosaccharides and antigenic proteins cannot be reduced or alleviated by heat. Also, trypsin inhibitors can be

reduced by 80 - 95% of the activity originally present by heat processing (Gumbmann et al. 1985). The residual activity of trypsin inhibitors in the toasted soybean flour was still sufficient to cause physiological effects on the performance of rats (Rackis et al., 1986). Further processing to destroy the remaining residual activity by using heat would reduce nitrogen solubility and the nutritional value of the protein. With the recent developments in feed enzyme technologies, many microbial enzymes such as phytase, amylase, protease and α-galactosidase, have been used into corn-soybean meal-based diet either to improve digestibility of nutrients or to reduce the antinutritive factors.

Enzymes play a key role in the digestive process. Although enzymes are produced by the animal itself or by the microbes naturally present in the digestive tract, specific activities necessary to break down some compounds in feed are not found or are at low levels in the digestive tract. Therefore, exogenous enzymes are added to the diet to break down these compounds. Many years ago, nutritionists had generally regarded enzyme addition to diets as a futile effort on the basis that proteolysis in the stomach and anterior small intestine would result in inactivation before they could be of significant digestive benefit. However, in 1946, Hastings first reported that addition of a diastatic enzyme material to a high fiber chick diet improved growth and feed efficiency. Later, Jensen et al. (1957) found that supplementation of barley-based poultry diets with a crude mixture containing β-glucanase activity gave a significant improvement in the performance of the birds as well as an improvement in litter quality. Since then, a lot of research work has been done about the use of exogenous enzymes in animal feed.

Phytate phosphorus is poorly available (30%) to monogastric animals, including poultry, due to the absence of adequate levels of endogenous enzyme with phytase activity. Nelson et al. (1967) first demonstrated the effectiveness of a microbially produced phytase for increasing the utilization of phosphorus from plant sources by poultry. Now, dietary supplementation with microbial phytase is well established as an effective and practical method of improving phytate digestibility in production animals (Kornegay, 2001). In poultry, microbial phytase supplementation generally results in a 20 - 45% improvement in phytate-P utilization (Ravindran *et al.*, 1995). The negative effect of phytate on mineral digestibility is ameliorated by dietary supplementation with microbial phytase. Microbial phytase supplementation in corn-soybean meal-based diets improved Ca availability and Zn utilization in poultry (Sebastian *et al.,* 1996a,b; Ao et al. 2007) and increased the apparent absorption of Mg, Zn, Cu and Fe in pigs (Adeola, 1995). Phytase supplementation also increased the ileal digestibility of crude protein, and most amino acids in both poultry and swine (Sebastian et al., 1997; Yi et al., 1996; Mroz et al., 1994).

The energy utilization of soybean meal by poultry is very poor. The digestibility of the dry matter and gross energy in soybean meal is approximately 50% when fed to poultry (Dudley-Cash, 2001). The metabolizable energy (ME) value of dehulled soybean meal suggested by the National Research Council (NRC) bulletin for swine (1998), is 3,380 kcal/kg. The NRC bulletin for poultry (1994) suggests a ME value of only 2,240 kcal/kg for dehulled soybean meal when fed to poultry. Pierson *et al.* (1980) pointed out that the low ME of soybean meal for poultry is due mainly to the very poor digestibility of the carbohydrate fraction. Soybean meal contains up to 22.7% non starch polysaccharides (NSPs) on a dry matter basis (Chesson, 1987). This includes about 6% oligosaccharides, including 1.0% raffinose and 4.6% stachyose (Trugo *et al.*, 1995). These oligosaccharides cannot be digested in the small intestine of poultry because of the absence of endogenous α-

(1,6)-galactosidase enzyme (Gitzelman and Auricchio, 1965). In addition to their indigestibility, these oligosaccharides have been shown to produce gastrointestinal gas in rats, dogs, and man (Steggerda, 1968) and produce diarrhea that may increase digesta passage rate and decrease digestion and absorption of nutrients (Kuriyama and Mendel, 1917; Wiggins, 1984). Coon *et al.* (1990) studied the effect of oligosaccharide-free soybean meal on the ME content of soybean meal and fiber digestion in adult roosters. The results showed that the removal of the oligosaccharides in soybean meal by ethanol extraction increased the nitrogen corrected true metabolizable energy (TME_n) by 21% due to increased fiber digestion and the digesta passage rate was reduced by approximately 50%. Further studies by Coon and coworkers (Leske *et al.*, 1991, 1993) also demonstrated the improved TME_n of soybean meal through alcohol extraction with both roosters and broilers. The recombination of the alcohol extract or addition of pure raffinose and stachyose to soy protein concentrate yielded TME_n values that were similar to those of soybean meal. Parsons *et al.* (2000) compared the AME_n of soybean meals varying in oligosaccharide content using roosters. The results indicated that the TME_n of low oligosaccharide soybean meal was significantly higher than that of conventional soybean meals. Ao et al. (2009) reported a significant increase of AME_n of corn-soybean meal diet by dietary supplementing α-galactosidase. However, Irish *et al.* (1995) removed up to 90% oligosaccharides from soybean meal using either ethanol extraction or exogenous α-galactosidase. No beneficial effect on the nutritional value of soybean meal was observed when the low oligosaccharide (extracted or enzyme-incubated) soybean meal was fed to broiler chicks.

Many studies have been conducted to investigate the effect of carbohydrase and protease supplementation to corn-soybean meal diets on the nutritive value of diets and performance of chicks. Swift *et al.* (1996) examined the effects of a commercial enzyme product called Allzyme Vegpro, a mixture of protease, cellulase, pentosanase, α-galactosidase and amylase, on digestibility and growth performance of broiler chicks. Enzyme treatment significantly improved nitrogen and energy digestibility and feed conversion over a 35-day feeding period. Schang *et al.* (1997) compared Vegpro in corn-soybean meal and corn full-fat soybean diets for broilers, using high and low nutrient density formulations. Addition of the enzyme product to the low density diet significantly improved body weight gain and feed conversion. Results from Ao et al. (2010) showed that the supplementation of Allzyme SSF, a naturally fermented product with activities of carbohydrase and phytase in corn-soy diet increased AME_n value of the diet by 84 Kcal/kg. Marsman *et al.* (1997) examined the effect of enzyme treatments (protease and carbohydrase) of soybean meal on growth performance and ileal nutrient digestibilities in broiler chicks. Enzyme treatment improved apparent ileal digestibility of crude protein and NSPs; however, enzyme treatment did not improve growth performance of the chicks. Zanella *et al.* (1999) investigated the effect of a commercial enzyme cocktail containing xylanase, protease and amylase on performance of broilers fed a corn-soybean meal based diet. Enzyme supplementation improved body weight gain, feed conversion ratio and ileal digestibility of crude protein. Graham *et al.* (2002) pretreated soybean meal using 4% α-galactosidase enzyme solution. Enzyme treatment degraded raffinose and stachyose in soybean meal by 69 and 54%, respectively, compared to untreated soybean meal. Enzyme treatment increased TME from 2974 to 3328 kcal/kg. However, chick growth performance was not significantly improved by enzyme treatment. Kocher *et al.* (2002) investigated the effect of two enzyme products on the nutritive value of soybean meal with emphasis on changes in composition of NSPs along the

digestive tract. They concluded that glycanases with galactanase and pectinase activities supplemented at appropriate dosages can improve the digestibility of the NSPs in soybean meal and increase the metabolizable energy content of the diet containing high levels of soybean meal. In another study, Kocher *et al.* (2003) reported that although enzyme addition to the corn-soybean meal based diet can significantly improve AME_n, the improvement depended greatly on the raw ingredients available at the time. Studies by Ghazi *et al.* (2003) demonstrated the improvement of the nutritive value of soybean meal by protease and α-galactosidase treatment in broiler chicks. They first used tube-fed chicks to measure the effect of different enzyme treatments on true metabolizable energy (TME) and true nitrogen digestibility (TND) of commercial solvent-extracted, heat-treated soybean meal. Protease and α-galactosidase improved TME and TND of the soybean meal. In other studies, they added enzymes in broiler diets and fed broilers for 21 d. Increases in chick growth rate and digestibility that were similar to those recorded in previous study were obtained when protease and α-galactosidase were included in the diets. Ao et al. (2009) did two trials to investigate the effects of α-galactosidase supplementation and acidification of diets on nutrient digestibility and growth performance of broiler chicks fed corn-soybean meal diet. The data showed that dietary supplementation of α-galactosidase significantly increased feed intake and weight gain of broiler chicks, which was further approved by increased AME_n of the diets and digestibility of CP and NDF.

Parkany-Gyarfas (1975) found a 3.6% improvement in body weight and 4.0% improvement in feed utilization in male turkeys when a corn-soybean meal diet was supplemented with α-amylase. Similar results were observed by Ritz *et al* (1995). The later study also demonstrated that the mean villus length within the jejunum and ileum was significantly increased at 2 and 3 wk of age by dietary supplementation of amylase when compared with control diet. These findings suggest that the increased growth associated with the amylase diet be explained in part by the increase in absorptive surface area, allowing for increased digestion of available nutrients coupled with increased enzyme activity. However, no physiological mechanism to explain increased villus length as a response to enzyme supplementation is known. In chicks, Noy and Sklan (1995) reported that daily net secretion of amylase was low at d 4 and steadily increased up to d 21. Uni *et al.* (1995) also reported that the secretion of amylase per gram of feed was low at d 4, increased up to d 7, and then stabilized. Burnett (1966) first reported the beneficial effects of amylase and protease preparations on growth and feed efficiency of chicks when added to diets. Gracia *et al.* (2003) studied the influence of exogenous α-amylase on digestion and performance of broilers fed a corn-soybean meal diet. At 7 d age, α-amylase supplementation improved daily gain by 9.4% and feed conversion by 4.2%. Also, α-amylase supplementation significantly improved apparent fecal digestibility of organic matter and starch and AMEn of the diet. The weight of pancreas as a percentage of body weight decreased with α-amylase supplementation, which indicates that the secretion of pancreatic enzymes might be affected by the concentration of enzymes and substrates or products of their hydrolysis in the lumen of the small intestine.

Scheideler *et al.* (1999, 2003a, b) conducted several studies to investigate the effect of enzyme addition to corn-soybean rations on pullet and laying hen performance. They used a microbial multi-enzyme package with amylase, protease and xylanase activity. The results showed that the enzyme supplementation increased pullet growth rate and improved egg production, egg mass and feed conversion ratio. Improvements were also seen in nitrogen

retention and availability of energy in pullet and layer diets supplemented with enzyme. In another study, Scheideler and Weber (2003a) investigated the role of α-galactosidase in corn-soy based layer rations. They found the addition of α-galactosidase improved egg production of hens and the ME of the diet. Hens performed very well on diet formulations reducing the energy available from fat sources and relying on more energy from soybean meal when α-galactosidase was added to the rations. Gomez *et al.* (1999) added a multi-enzyme complex containing amylase, protease and xylanase to corn-soybean meal diets with three energy densities. Enzyme supplementation improved egg mass and feed conversion ratio at all three energy levels tested.

Pretreatment of raw soybean or soybean meal with proteases was studied in many experiments. The purpose of adding proteases in soybean or soybean meal containing diet is to destroy or inactivate the anti-nutritional factors, such as residual trypsin inhibitors, lectins and antigenic protein. Huo *et al.* (1993) found that fungal and bacterial protease enzymes could inactivate trypsin inhibitors and lectins in raw soybean and low-temperature extruded soybean *in vitro*. Based on their results, the protease from bacterial source was more effective at breaking down trypsin inhibitors than the protease from fungal source. Rooke *et al.* (1998) incubated the soybean meal using 0.1% acid protease for 3 h at 50°C, pH 4.5. The soybean meal treated with protease contained fewer antigenic proteins than the other soy-containing diets. In another study, they added alkaline protease into soybean meal and incubated for 2 h at 50°C, pH 8.5. They found the composition of soybean meal was changed due to pretreatment, and soluble α-amino nitrogen concentrations were increased by treatment with protease. The antigenic protein concentration was reduced. Beal *et al.* (1998a, b) reported that the pre-incubation of raw soybean or soybean meal with protease significantly increased the *in vitro* nitrogen digestibility. Ghazi *et al.* (2002) pretreated soybean meal with two different proteases: one was alkaline protease (isolated from *bacillus* species) and the other one was acid protease (isolated from *Aspergillus*). Then they incorporated the soybean meal into the diets for broiler chicks. Acid protease treatment improved chick performance from 7 to 28 d of age and increased apparent ileal nitrogen digestibility and apparent nitrogen retention across the whole digestive tract. Also, enzyme pretreatment significantly reduced chick serum antisoya antibodies. They also conducted two tube-feeding experiments using pretreated soybean meal. The results showed that the acid protease treatment improved nitrogen digestibility and true metabolizable energy. Ao et al. (2010) did a few studies to observe the effects of commercial preparations of α-galactosidase and protease on in vitro nutrient release from soybean meal and trypsin inhibitor content in defatted whole soybeans. An in vitro model was used to simulate the chicken's digestive process in the crop, the stomach (proventriculus and gizzard) and the small intestine. Soybean meal and ground whole soybeans were used as substrates. Graded levels of either α-galactosidase (0 to 13,792 units/kg) or protease (0 to 888 units/kg) were added to the substrates. Reducing sugars and α-amino N were measured at the end of the crop phase, the stomach phase, and the whole phase (crop through small intestine). Trypsin inhibitor content was measured at the end of the stomach phase. Increasing α-galactosidase levels linearly increased the release of reducing sugars in both the crop and the whole phases (Figure 1). Linear increases in α-amino N occurred with increasing doses of protease at the crop, the stomach and the whole phases (Fig. 2). However, no effect of protease on trypsin inhibitor activity in raw soybeans was detected.
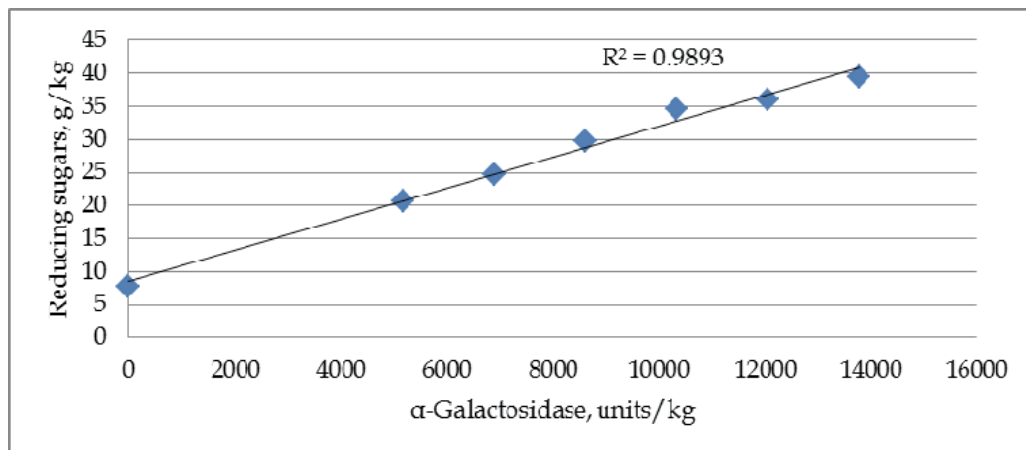
Fig. 1. Effects of α-galactosidase on the release of reducing sugars in soybean meal after in vitro digestion in GI tract phase, Ao et al. 2010.
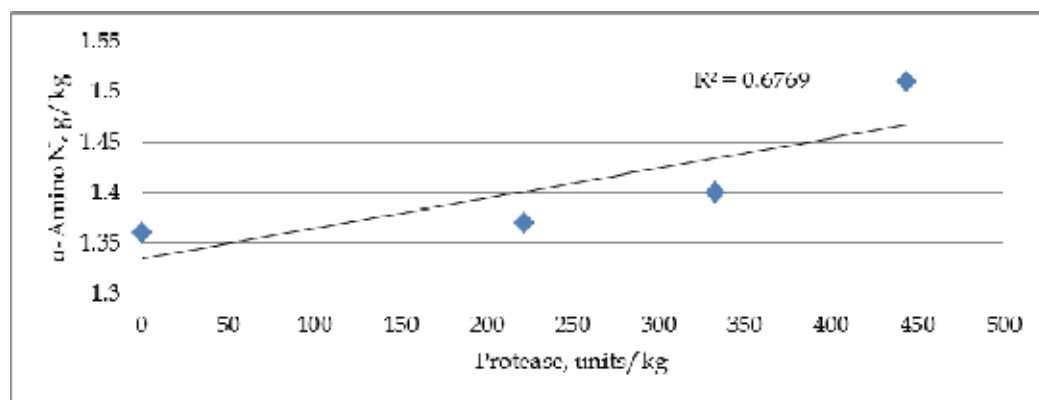
Fig. 2. Effects of protease on the release of α-amino N in raw soybean after in vitro digestion, Ao et al. 2010.

5. Conclusion

Soybean meal is the most important protein source of poultry feed all over the world. Although heat treatment has been successfully used to inactivate some antinutritional factors in soybean, the nutritional value of soybean meal to chicken is still far from reaching the maximal level due to residual antinutritional factors. Supplementing exogenous enzymes in poultry feed has been proved one of useful approaches to further increase nutritional value of soybean meal.

6. References

Adeola, O. (1995). Digestive utilization of minerals by weanling pigs fed copper- and phytase- supplemented diets. Can. J. Anim. Sci. 75:603-610.

Almquist, H. J. & Merrit, J. B. (1952). Effect of raw soybean meal on growth of the chick. Proc. Soc. Exp. Biol. Med. 79:277-279.

Ao, T., Cantor, A. H., Pescatore, A. J., Pierce, J. L. & Dawson, K.A. (2010). Effects of citric acid, alpha-galactosidase and protease on *in vitro* nutrient release from soybean meal and trypsin inhibitor content in raw whole soybeans. Anim. Feed Sci. Tech. doi: 10.1016/j.anifeedsci.2010.08014.

Ao, T., Pierce, J. L., Hoskins, B., Paul, M., Pescatore, A. J., Cantor, A. H., Ford, M.J. & King W. D. (2010). Allzyme SSF® increased AME_n of the corn-soy diet and improved performance of boilers. Poult. Sci. 89 (Suppl. 1):863.

Ao, T., Cantor, A. H., Pescatore, A. J., Ford, M.J., Pierce, J. L. & Dawson, K.A. (2009). Effect of Enzyme Supplementation and Acidification of Diet on Nutrient Digestibility and Growth Performance of Broiler Chicks. Poult. Sci. 88:111-117.

Ao, T., Pierce, J. L., Pescatore, A. J., Cantor, A. H., Dawson, K.A., Ford, M.J. & Shafer, B. L. (2007). Effects of organic Zn and phytase supplementation in a maize-soybean meal diet on the performance and tissue Zn content of broiler chicks. Brit. Poult. Sci. 48 (6) 690-695.

Araba, M. & Dale, N. M. (1990). Evaluation of protein solubility as an indicator of overprocessing soybean meal. Poult. Sci. 69:76-83.

Beal, J. D., Brook, P. H. & Schulze, H. (1998a). The effect of pretreatment with different proteases on the *in vitro* digestibility of nitrogen in raw soy bean and four different full fat soy bean meals. Page 264 in Book of Abstracts of the 49th Annual Meeting of the European Association for Animal Production, Warsaw, Poland. van Arendonk, J. A. M., ed. Wageninggen Pers, Wageningen, The Netherlands.

Beal, J. D., Brook, P. H. & Schulze, H. (1998b). The hydrolysation of protein in raw and autoclaved soybean meals by a microbial protease. Page 167 in British Society of Animal Science Winter Meeting, Scarborough, UK.

Borechers, R., Ackerson, C. W. & Mussehl, F. E. (1948). Trypsin inhibitor, VI. Effect of various heating periods on the growth promoting value of soybean oil meal for chicks. Poult. Sci. 27:601-604.

Burnett, G. S. (1966). The effect of damaged starch, amylolytic enzymes, and proteolytic enzymes on the utilization of cereals by chickens. Br. Poult. Sci. 3:89-103.

Campbell, G. L. & van der Poel, A. F. B. (1998). Use of enzymes and process technology to inactivate antinutritional factors in legume seeds and rapeseed. Pages 377-386 in Proc. Recent Advances of Research in Antinutritional Factors in Legume Seeds and Rapeseed. Wageningen Press, Wageningen, The Netherlands.

Caskey, C. D., Jr. & Knapp, F. C. (1944). Method for detecting inadequately heated soybean-oil meal. Ind. Eng. Chem. Anal. Ed. 16:640-641.

Chesson, A. (1987). Supplementary enzymes to improve the utilization of pig and poultry diets. Pages 71-89 in Recent Advances in Animal Nutrition. Haresign, W. and Cole, D. J. A., ed. Butterworths, UK.

Coon, C. N., Leske, K. L., Akavanichan, O. & Cheng, T. K. (1990). Effect of oligosaccharide-free soybean meal on true metabolizable energy and fiber digestion in adult roosters. Poult. Sci. 69:787-793.

Cooper, R. J. & Gowing, H. S. (1983). Mammalian small intestine phytase (EC 3.1.3.8). Br. J. Nutr. 50:429–435.

Cosgrove, D. J. (1980). Inositol Phosphates: Their chemistry, biochemistry and physiology. Elsevier Scientific Publishing Co., New York, NY. Denbow, D. M., V. Ravindran, E. T. Kornegay,

Cromwell, G. L. (1999). Soybean meal – the "golden standard". The Farmer's Pride, KPPA News, Vol. 11, No. 20, November 10.

CVB (Centraal Veevoederbureau), 1998. Table of feedstuffs. Information about composition, digestibility and feeding value. Centraal Veevoederbureau, Lelystad, The Netherlands.

Douglas, M. W., Parsons, C. M. & Hymowitz, T. (1999). Nutritional evaluation of lectin-free soybeans for poultry. Poult. Sci. 78:91–95.

Dudley-Cash, W. A. (2001). Soybean meal source, added enzyme affect nutritional value for chicks. Feedstuffs 73:41.

Feng, J. X., Liu, Z. R. Xu, Y., Wang, Z. & Liu, J. X. (2007). Effects of fermented soybean meal on digestive enzyme activities and intestinal morphology in broilers. Poult. Sci. 86:1149-1154.

Gallaher, D. & Schneeman, B. O. (1984). Nutritional and metabolic response to plant inhibitors of digestive enzymes. Advances in Experimental Medicine and Biology 177, 299-320.

Ghazi, S., Rooke, J. A., Galbraith, H. & Bedford, M. R. (2002). The potential for the improvement of the nutritive value of soybean meal by different proteases in broiler chicks and broiler cockerels. Br. Poult. Sci. 43(1):70-7.

Ghazi, S., Rooke, J. A., Galbraith, H. (2003). Improvement of the nutritive value of soybean meal by protease and α-galactosidase treatment in broiler cockerels and broiler chicks. Br. Poult. Sci. 44(3):410-8.

Gitzelmann, R. & Auricchio, S. (1965). The handling of soy α-galactosidase by a normal and galactosemic child. Pediatrics 36:231-232.

Gomez, S., Scheideler, S. E. & Wyatt, C. (1999). Effects of enzyme supplementation to corn-soybean rations with different energy densities on laying hen performance. Poult. Sci. (supplement) 78:103.

Graham, K. K., Kerley, M. S., Firman, J. D. & Allee, G. L. (2002). The effect of enzyme treatment of soybean meal on oligosaccharide disappearance and chick growth performance. Poult. Sci. 81:1014-1019.

Gracia, M. I., Aranibar, M. J., Lazaro, R., Medel, P. & Mateos, G. G. (2003). α-amylase supplementation of broiler diets based on corn. Poult. Sci. 82:436-442.

Gumbmann, M. R., Spa Gler, W. L., Gan, G. M. D, Rackis, J. J. A. & Lie ER L, E. D. (1985). The USDA trypsin inhibitor study. IV. The chronic effects of soy flour and soy protein isolate on the pancreas in rats after two years. Qualitas Plantarum Plant Foods for Human Nutrition, 35, 275-314.

Hastings, W. H. (1946). Enzyme supplements to poultry feeds. Poult. Sci. 25:548-586.

Hayward, J.W., Steenbock, H. & Bohstedt, G. (1936). The effect of heat as used in the extraction of soybean oil upon the nutritive value of the protein. J. Nutr. 11:219-234.

Hayward, J.W., Halpin, J.G., Holmes, C.E., Bohstedt, G. & Hart, E.B. (1937). Soybean oil meal prepared at different temperatures as a feed for poultry. Poult. Sci. 16:3-14.

Huo, G.C., Fowler, V.R. & Bedford, M. (1993). The use of enzymes to denature antinutritive factors in soybean. Pages 517- 521 in Recent Advances of Research in Antinutritional Factors in Legume Seeds. Proceedings of the Second International Workshop on 'Antinutritional factors (ANFs) in Legume seeds'. van der Poel, A.F.B., Huisman, J. and Saini, H.S., ed. Wageningen Pers, Wageningen, The Netherlands.

Irish, G. G., Balnave, D., Classen, H. L., Tyler, R. T. & Bedfrod, M. R. (1995). Removal of the α-galactosides of sucrose from soybean meal using either ethanol extraction or exogenous α-galactosidase and broiler performance. Poult. Sci. 74:1484-1494.

Jensen, L. S., Fry, R. E., Allred, J. B. & McGinnis, J. (1957). Improvement in the nutritional value of barley for chicks by enzyme supplementation. Poult. Sci. 36:919-921.

Kocher, A., Choct, M., Porter, M. D. & Broz, J. (2002). Effect of feed enzymes on nutritive value of soybean meal fed to broilers. Br. Poult. Sci. 43(1):54-63.

Kocher, A., M. Choct, G. Ross, J. Broz, and T. K. Chung, 2003. Effects of enzyme combinations on apparent metabolizable energy of corn-soybean meal-based diets in broilers. J. Appl. Poult. Res. 12:275-283.

Kornegay, E. T. (2001). Digestion of phosphorus and other nutrients: the role of phytases and factors influencing their activity. In: M. R. Bedford and G. G. Partridge. Enzymes in Farm Animal Nutrition. CABI Publishing, pp. 237-271.

Kuriyama, S., & Mendel, L. B. (1917). The physiological behavior of raffinose. J. Biol. Chem. 31:125-147.

Leske, K. L., Akavanichan, O., Cheng, T. K. & Coon, C. N. (1991). Effect of ethanol extract on nitrogen-corrected true metabolizable energy for soybean meal with broilers and roosters. Poult. Sci. 70:892-895.

Leske, K. L., Jeven, C. J. & Coon, C. N. (1993). Effect of oligosaccharide additions on nitrogen corrected metabolizable energy of soy protein concentrate. Poult. Sci. 72:664-668.

Liener, L. E. (1981). Factors affecting the nutritional quality of soya products. J. AM. Oil Chem. Soc. 58, 406-415.

Lyman, R.L. & Lepkovsky, S. (1957). The effect of raw soybean meal and trypsin inhibitor diets on pancreatic enzyme secretion in the rat. J. Nutr. 62, 269-84.

Marsman, G. J. P., Gruppen, H., Van Der Poel, A. F. B., Kwakkel, R. P., Verstegen, M. W. A. & Voragen, A. G. J. (1997). The effect of thermal processing and enzyme treatments of soybean meal on growth performance, ileal nutrient digestibility, and chime characteristics in broiler chicks. Poult. Sci. 76:864-872.

Mroz, Z., Jongbloed, A. W. & Kemme, P. A. (1994). Apparent digestibility and retention of nutrients bound to phytate complexes as influenced by microbial phytase and feeding regime in pigs. J. of Anim. Sci. 72:126-132.

Nelson, T. S. (1967). The utilization of phytate phosphorus by poultry – A review. Poult. Sci. 46:862-869.

National Research Council (1994). Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington, DC.

National Research Council (1998). Nutrient Requirements of Swine. 10th rev. ed. National Academy Press, Washington, DC.

Noy, Y. & Sklan, D. (1995). Digestion and absorption in the young chick. Poult. Sci. 74:366-373.

Oliveira, A. C. & Sgarbierri, V. C. (1986). Effect of diets containing dry beans (*Phaseolus vulgaris* L.) on the rat excretion of endogenous nitrogen. J. Nutr. 116:2387–2392.

Oliveira, A. C., Vidal, B. C. & Sgarbieri, V. C. (1989). Lesions of intestinal epithelium by ingestion of bean lectins in rats. J. Nutr. Sci. Vitaminol. 35:315–322.

Osborne, T.B. & Mendel, L. B. (1917). The use of soybean as food. J. Bio. Chem. 32:369-387.

Pallauf J. & Rimbach G. (1997). Nutritional significance of phytic acid and phytase. Archive of Animal Nutrition 50: 301-319.

Parkany-Gyarfas, A. (1975). Utilization of alpha-amylase-supplemented diet in turkey feeding tests. Acta Aliment 4:23-35.

Parsons, C. M., Zhang, Y. & Araba, M. (2000). Nutritional evaluation of soybean meals varying in oligosaccharide content. Poult. Sci. 79:1127-1131.

Pierson, E. E. M., Potter, L. M. & Brown, R. D. (1980). Amino acid digestibility of dehulled soybean meal by adult turkeys. Poult. Sci. 59:845-848.

Pusztai, A. (1967). Trypsin inhibitors of plant origin, their chemistry and potential role in animal nutrition. Nutrition Abstract and Reviews 37: 1-9.

Pusztai, A. (1991). Plant Lectins. Cambridge University Press, Cambridge, UK.

Pustzai, A., Ewen, S.W.B. Grant, G., Peumans, W. J., van Damme, E.J.M., Rubio, L. & Bardocz, S. (1990). Relationship between survival and binding of plant lectins during small intestinal passage and their effectiveness as growth factors. Digestion 46(Suppl. 2):308–316.

Rackis, J. J. & Gumbman, M. R. (1981). Protease inhibitors: physio-logical properties and nutritional significance. In: ORY, R. L. (Ed.), *Antinutrients and Natural Toxicants in Foods.* Westport, CT: Food and Nutrition Press, pp. 203-237.

Rackis, J. J., Wolf, W. J. A., Baker, E. C. D. (1986). Protease inhibitors in plant foods: content and inactivation. In: FRIEDMAN, M. (Ed.), Nutritional and Toxicological Significance of Enzyme Inhibitors in Foods. ew York and London: Plenum Publishing, pp. 299-347.

Ravindran, V., Bryden, W. L. & Kornegay, E. T. (1995). Phytates: occurrence, bioavailability and implications in Poultry nutrition. Poult. Avian Biol. Rev. 6:125-143.

Renner, R., Clandinin, D. R. & Robblee, A. R. (1953). Action of moisture on damage done during over-heating of soybean oil meal. Poult. Sci. 32:582-585.

Ritz, C. W., Hulet, R. M., Self, B. B. & Denbow, D. M. (1995). Growth and intestinal morphology of male turkeys as influenced by dietary supplementation of amylase and xylanase. Poult. Sci. 74:1329-1334.

Rooke, J. A., Slessor, M., Fraser, H. & Thomson, J. R. (1998). Growth performance and gut function of piglets weaned at four weeks of age and fed protease-treated soybean meal. Anim. Feed Sci. and Tech. 70:175-190.

Schang, M. J., Azcona, J. O. & Arias, J. E. (1997). Effects of a soya enzyme supplement on performance of broilers fed corn/soy or corn/soy/full-fat soy diets. Poult. Sci. 76 (Suppl. 1), 132 (abstract).

Scheideler, S. E., Abudabos, A. & Wyatt, C. (1999). Effects of enzyme supplementation of corn-soy based layer diet. Poult. Sci. (supplement) 77:55.

Scheideler, S. E. & Weber, T. (2003a). Role of alpha-galactosidase in corn-soy based layer rations. Pages 20-22 in The Nebraska Poultry Report.

Scheideler, S. E. & Weber, T. (2003b). Supplementation of pullet and layer diets with a multi-enzyme product. Pages 27-27 in The Nebraska Poultry Report.

Schulze, H., Saini, H. S., Huisman, J., Hessing, M., van den Berg, W. & Verstegen, M.W.A. (1995). Increased nitrogen secretion by inclusion of soya lectin in the diets of pigs. J. Sci. Food Agric. 69:501–510.

Sebastian, S., Touchburn, S. P., Chavez, E. R. & Lague, P. C. (1996a). The effects of supplemental microbial phytase on the performance and utilization of dietary calcium, phosphorus, copper and zinc in broiler chickens fed corn-soybean diets. Poult. Sci. 75:729-736.

Sebastian, S., Touchburn, S. P., Chavez, E. R. & Lague, P. C. (1996b). Efficacy of supplemental microbial phytase at different calcium levels on growth performance and mineral utilization of broiler chickens. Poult. Sci. 75:1516-1523.

Sebastian, S., Touchburn, S. P., Chavez, E. R. & Lague, P. C. (1997). Apparent digestibility of protein and amino acids in broiler chickens fed a corn-soybean diet supplemented with microbial phytase. Poult. Sci. 76:1760-1769.

Sessa, D. J., Haney, J. K. & Nelsen, T. C. (1990). Inactivation of soybean trypsin inhibitors with ascorbic acid plus copper. J. Agric. Food Chem. 38: 1469-1474.

Steggerda, F. R. (1968). Gastrointestinal gas following food consumption. Ann. N.Y. Acad. Sci. 150:57-66.

Swift, M. L., van Keyserlingk, M. A. G., Leslie, A. & Teltge, D. (1996). The effect of Allzyme Vegpro supplementation and expander processing on the nutrient digestibility and growth of broilers. 12th Annual Symposium on Biotechnology in the Feed Industry. Lexington, Kentucky. Supplement 1, enclosure code UL 2.1.

Thompson, L.U. (1986) Phytic acid: a factor influencing starch digestibility and blood glucose response. In: Graf, E. (ed.) Phytic Acid: Chemistry and Applications. Pilatus Press, Minneapolis, p. 173.

Trugo, L. C., Farah, A. & Cabral, L. (1995). Oligosaccharide distribution in Brazilian soya been cultivars. Food Chem. 52:385-387.

Uni, Z., Noy, Y. & Sklan, D. (1995). Posthatch changes in morphology and function of the small intestines in heavy and light strain chicks. Poult. Sci. 74:1622-1629.

Wang, H., Faris, R. J., Wang, T., Spurlock, M. E., Gabler, N. (2009). Increased in vitro and in vivo digestibility of soy proteins by chemical modification of disulfide bouds. J. Am. Oil Chem. Soc. DOI 10.1007/s11746-009-1449-5.

Wiggins, H. S. (1984). Nutritional value of sugars and related compounds undigested in the small gut. Proc. Nutr. Soc. 43:69-85.

Wu, Y. V. & Sessa. D. J. (1994). Confirmation of Bowman – Birk inhibitor. J. Agric. Food Chem. 42:2136-2138.

Yi, Z., Kornegay, E. T. & Denbow, D. M. (1996). Effect of microbial phytase on nitrogen and amino acid digestibility and nitrogen retention of turkey poults fed corn-soybean meal diets. Poult. Sci. 75:979-990.

Zanella, I., Sakomura, N. K., Silversides, F. G., Fqueirdo, A. & Pack, M. (1999). Effect of enzyme supplementation of broiler diets based on corn and soybeans. Poult. Sci. 78:561-568.

Soybean Peptide: Novel Plant Growth Promoting Peptide from Soybean

Yoshiki Matsumiya and Motoki Kubo
Department of Biotechnology, College of Life Sciences
Ritsumeikan University, Nojihigashi , Kusatsu, Shiga
Japan

1. Introduction

Soybean is one of the most important agricultural products and its global production was more than 200 million tons per year in 2005 (Table 1) (Ministry of Agriculture, Forestry and Fisheries (Japan), 2007; Uchida, 2007). Soybean is used mainly as a vegetable oil (31.6 million tons a year in 2005) and the production ratio is the highest (30%) among vegetable oils.
Soybean waste, which remains after extraction of vegetable oil, contains about 50% proteins, which consist of a well-balanced mix of amino acids. Therefore, soybean waste is a valuable biomass for animal feedstuffs. Soybean is used directly as food in Japan and several Asian countries but soybean proteins are used less widely elsewhere in the world.

Agricultural products	Production (Million tons)
Corn	710
Wheat	624
Rice	401
Soybean	214
Barley	153

Table 1. Amount of main agricultural production in 2005. (Ministry of Agriculture, Forestry and Fisheries (Japan), 2007)

Recently, investigations into utilization of proteins from soybean waste have been carried out for the development of high quality foods. Protein fractions, such as soy protein isolates (SPI) and whey protein are industrially produced, and these fractions are used as additives for the improvement of food nutrition (Malhotra & Coupland, 2004). Several soybean proteins have been purified and utilized as medicines for hypotension, rheumatism, and cholesterol control. Peptide inhibiting angiotensin I converting enzyme has also been developed by a protease treatment (Farzamirad & Aluko, 2008; Yonekura & Tanaka, 2003; Yonekura et al., 2004).
On the other hand, soybean waste was the most utilized N source for organic fertilizers prior to the 1940s (Okuda, 1961). Recently, utilization of organic fertilizers for the production of organic agricultural products is rising because healthcare and environmental concerns are increasing. Studies into utilization of soybean waste as a fertilizer and as

bioactive materials have been attempted (Kubo et al., 1997; Matsumiya et al., 2007; Shinano et al., 1991; Yamazaki & Roppongi, 1998).

This chapter describes utilization of soybean meal and the development of a bioactive peptide for plant growth. Moreover, mechanisms of novel bioactive peptides for root hair promotion are described in this chapter.

2. Isolation of soybean meal degrading bacteria and analysis of effectiveness of the degraded products as fertilizers

Because of increasing environmental concerns, the excessive utilization of chemical fertilizers has recently received increased attention. Therefore, the development of new fertilizers using natural materials, such as amino acids and natural nitrogen compounds, has become the focus of much research (Acea et al., 1988; Klopper et al. 1989; O'Sullivan et al., 1991).

Soybean meal, which is produced in large amounts as biomass, is rich in nitrogen compounds and has been utilized as fertilizer. Degradation and mineralization of soybean meal in the soil environment requires several reactions: proteins → peptides & amino acids → ammonia → nitrite → nitrate (Smith et al., 1977). The proteins → peptides & amino acids reaction is important for nitrogen mineralization in the soil environment (Kamimura & Hayano, 2000; Watanabe & Hayano, 1995). However, the degradation of soybean meal in the soil is too slow for mineralization.

Isolation of *Bacillus circulans* HA12, which degrades soybean meal efficiently and rapidly, is described here (Hasegawa et al., 2002; Kubo et al., 1994), and the plant growth promoting effects of the degraded soybean meal products (DSP) are also described in this section.

2.1 Isolation of soybean meal degrading bacteria

Soybean meal degrading bacteria were isolated using a 1% (w/v) soybean meal medium (Kubo et al., 1994), and about 50,000 bacteria were isolated. Protease production of all isolates were tested by LC agar medium (Matsumiya et al., 2004), resulting in 21 strains being isolated. Each isolate was further sub-cultured in soybean meal medium at 50°C for 48 h. As a result, the protease-producing bacterium HA12 was isolated. The procedure for the screening is shown in Fig. 1.

Strain HA12 was characterized and identified based on Bergey's Manual of Determinative Bacteriology (Buchanan & Gibbons, 1974). Because the strain was strictly aerobic, gram positive, catalase producing, and endospore forming, the strain belongs to genus *Bacillus*. The maximum temperature for the growth of strain HA12 was 55°C. The characteristics of the strain are listed in Table 2. Strain HA12 is identified as *B. circulans* and designated as *B. circulans* HA12.

2.2 Analysis of soybean meal degradation by *B. circulans* HA12

B. circulans HA12 formed a clear halo on LC agar medium and the strain degraded soybean meal efficiently. The protease activity was 550 U/ml after 16 h of cultivation (Kubo et al., 1997). The protease(s) from *B. circulans* HA12 was secreted into the medium and the optimum temperature of the protease was about 70°C.

Strain HA12 consumed dissolved soybean proteins for primary metabolism during the first stage. During the next stage, soybean meal was degraded and protein accumulated gradually in the medium. Subsequently, proteins were further digested to smaller molecules, including peptides and amino acids. The maximum concentrations of peptides

produced by degradation of soybean meal with *B. circulans* HA12 for 48 h were 6.5 mg/ml (1% w/v soybean meal medium) and 30 mg/ml (10% w/v soybean meal medium). The amino acid composition of DSP is shown in Table 3.

1st screening

Bacteria which grow soybean meal as a sole carbon source at 50℃

⬇ 50 thousand strains

2nd screening

Select highly protease producing strains

⬇ 21 strains

3rd screening

Strain which degraded soybean meal completely in 48 h
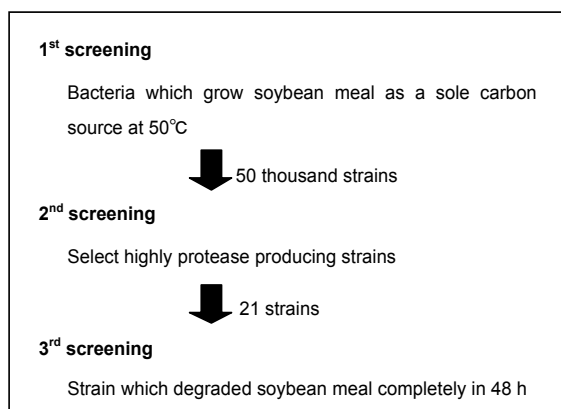
Fig. 1. Screening procedure for soybean waste-degrading bacteria.

Property	Growth	Property	Growth
Cell morphology	Rod	Growth in urease	+
Gram staining	+	Growth in NaCl 0%	+
Spore formation	+	3%	+
Motility	-	7%	-
Growth at: 25°C	+	Hemolysis	-
37°C	+	Decarboxylation from ornithine	-
Strict aerobic reaction	+	Decarboxylation from lysine	-
Oxidase reaction	+	Decarboxylation from arginine	+
Catalase reaction	+	Gas from glucose	+
Nitrate reduction	-	Gas from mannitol	+
H_2S production	-	Gas from lactose	+
Indole production	+	Gas from sucrose	+
Methyl red reaction	+	Gas from maltose	+

Table 2. Properties of strain HA12.

Amino acid	Mol. (%)	Amino acid	Mol. (%)	Amino acid	Mol (%)
Ala	5.84	His	1.37	Pro	6.74
Arg	3.35	Ile	4.75	Ser	4.27
Asp	13.33	Leu	6.29	Thr	3.32
Cys	0.00	Lys	7.66	Trp	0.00
Glu	18.40	Met	1.86	Tyr	4.64
Gly	6.61	Phe	6.25	Val	5.31

Table 3. Amino acid composition of degraded soybean meal products.

2.3 Effect of degraded soybean meal products on plant growth

Because DSP includes small molecules such as peptides, the plant growth promoting effects of DSP were investigated. The fresh weight of *Brassica rapa* was increased by 25% through addition of DSP (12 mg-peptides/kg-soil) (Table 4 & Fig. 2). The growth of *Solanum tuberosum* L., *Solanum lycopersicum*, and *Brassica juncea* were also promoted by addition of DSP. Moreover, DSP produced thicker roots than a chemical fertilizer (Fig. 3).

The total nitrogen, total phosphate (TP), and total potassium (TK) in DSP were 0.70, 0.11, and 0.28%, respectively. These TP and TK contents are not enough to act as a fertilizer in DSP, and moreover, DSP did not contain nitrate. Therefore, the plant growth promotion of DSP appears to be caused by bioactive peptides.

	Fresh weight (g)	Relative yield (%)
Water	44.2 ± 5.2	100
Chemical fertilizer	53.3 ± 6.6	121
DSP	55.1 ± 6.9	125

Table 4. Effect of degraded soybean meal products on growth of *Brassica rapa.*
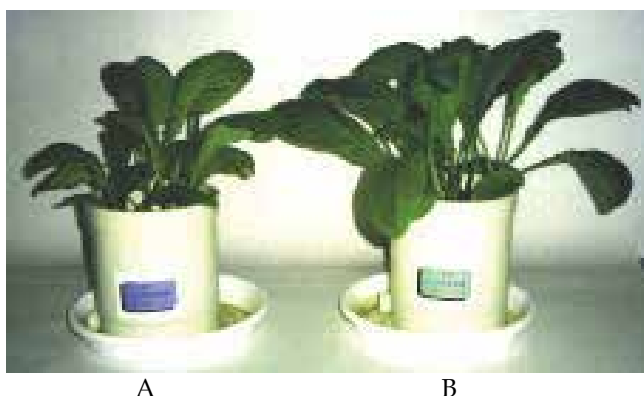
Fig. 2. Plant growth-promoting effect of degraded soybean meal products (DSP). A: *Brassica rapa* grown with chemical fertilizer, B: *B. rapa* grown with DSP.
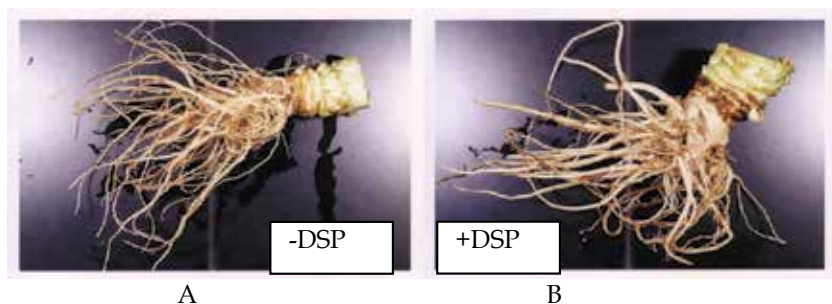
Fig. 3. Effect of degraded soybean meal products (DSP) on the root system of *Brassica juncea*. A: root of *B. juncea* grown in soil without DSP, B: *B. juncea* grown in soil with DSP.

2.4 Effect of DSP on root hair promotion

Recently, several bioactive peptides from plants have also been found to have phytohormone-like activities (Ito et al., 2006; Kondo et al., 2006; Matsubayashi and Sakagami, 1996; Matsubayashi et al., 1999; McGurl et al., 1992; Pearce et al., 1991; Schopfer et al., 1999; Suzuki et al., 1999). Phytosulfokine, systemin, SCR/SP11, and CLE are endogenous peptides produced in a variety of plants. The respective bioactivities of these peptides cause cell differentiation, protease inhibitor induction, cell division, and the pollen self-incompatibility response.

In order to analyze the plant growth promoting effect, the effect of DSP on root of *B. rapa* was analyzed. The number of root hairs was increased and elongated when DSP (30 μg/ml) was added (Fig. 4). DSP also promoted the root hair formation of *B. oleracea* L., *Lactuca sativa*, *Trifolium incarnatum* L., and *Gypsophila elegans*.

Root hair is an important plant organ for the absorption and transport of nutrients (Gilroy & Jones, 2000; Lauter et al., 1996). The enhancement of plant growth by DSP is caused by the increase of root hair numbers and length. Root hair promotion is observed with even 0.3 μg/ml of DSP, and the root hair promoting activity increases with DSP concentration.

A B
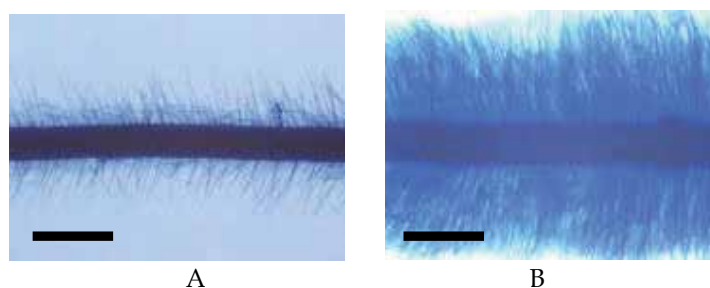
Fig. 4. Root hair promoting effect of degraded soybean meal products (DSP). A: root of *Brassica rapa* grown in plant growth medium (Matsumiya et al., 2007), B: root of *B. rapa* grown with DSP in plant growth medium. Bar denotes 1 mm.

2.5 Comparison between bioactive effects of DSP and phytohormones

Ethylene, which is a phytohormone, also promotes root hair numbers and length. The bioactive effects of DSP and ethylene were compared (Fig. 5). The root hair promotion by DSP was similar to ethylene, but spiraling of the main root was observed in the case of ethylene.

A B C
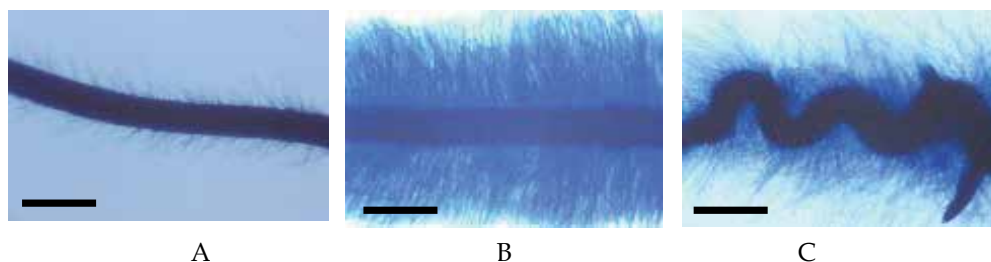
Fig. 5. Effects for main root and root hairs of degraded soybean meal products (DSP) and ethylene against *Brassica rapa*. A: plant growth medium, B: plant growth medium + DSP, C: plant growth medium + ethylene. The bar shows 1 mm.

On the other hand, adventitious root formation by DSP and ethylene were analyzed (Fig. 6). Obvious adventitious root formation was observed in the case of DSP addition. DSP and ethylene showed different effects on main root and adventitious root formation, suggesting that DSP did not induce ethylene for root hair promotion.

A   B   C
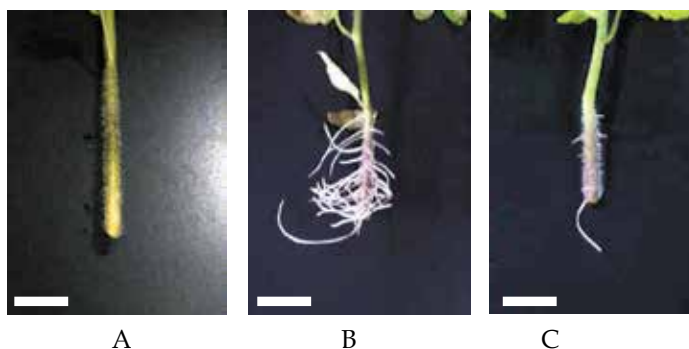
Fig. 6. Adventitious root formation with degraded soybean meal products (DSP) or phytohormones in *Lycopersicon esculentum.* The adventitious root formation assays were carried out using shoots of *L. esculentum* soaked in DSP and ethylene for 1 week at 25°C. A: water, B: water + DSP, C: water + ethylene. The bar denotes 2 cm.

	Root hair	Adventitious root	Shoot growth	Epinasty	Diapause	Callus induction	Leaf enlargement
Ethylene	+	+	-	+	-	-	-
Auxin	-	+	-	-	-	+	-
Cytokinine	-	-	-	-	-	-	+
Gibberelin	-	-	+	-	-	-	-
Abscisic acid	-	-	-	-	+	-	-
Brassinosteroid	-	-	+	-	-	-	-
Jasmoic acid	-	-	-	-	-	-	-
DSP	+	++	-	-	-	-	-

Table 5. Bioactive effects of degraded soybean meal products or phytohormones on plants.

The bioactive effects of DSP and phytohormones on plants were analyzed (Table 5) (Gaither, 1975; Masucci & Schiefelbein, 1994; Pitts et al., 1998; Tanimoto et al., 1995;). The effects of DSP on plants did not agree with those of phytohormones, and therefore DSP has different mechanisms of action on plant growth.

3. Exogenous bioactive peptides in DSP and the structural determination of the root hair promoting peptide

Systemin (McGurl et al., 1992; Pearce et al., 1991), phytosulfokine (Matsubayashi and Sakagami, 1996; Matsubayashi et al., 1999), SCR/SP11 (Schopfer et al., 1999; Suzuki et al., 1999), and CLV3 (Ito et al., 2006; Kondo et al., 2006) have been identified as endogenous peptide signals, which act as phytohormones. On the other hand, 2,3-butandiol, which is produced by several *Bacillus* strains, is known as an exogenous signal for plants (Ryu et al.,

2003). DSP seems to comprise an exogenous peptide signal and shows bioactivity for root hair promotion similar to that of phytohormones. In this section, the effect of DSP on roots and the structure of the root hair promoting peptide in DSP are described.

3.1 Effect of DSP on roots

To analyze the mechanism by which root hair numbers and length are increased by DSP, the number of trichoblasts (hair cells) and atrichoblasts (hairless cells) were counted. The trichoblast number in the presence of DSP (30 µg/ml) increased about 4.4 times over that without DSP, and the atrichoblast number also increased in response to DSP treatment by about 1.9 times (Table 6). The effect of DSP on the root hair seems to be similar to that of ethylene (phytohormone) (Dolan, 1996; Masucci, & Schiefelbein, 1994; Tanimoto et al., 1995;).

Ethylene led to an increase in root hair numbers by converting atrichoblasts to trichoblasts, while the localization pattern of the trichoblasts and atrichoblasts was not altered by addition of DSP (Fig. 7). DSP did not affect the balance of the endogenous phytohormones. DSP contains exogenous peptide signal(s) for root hair promotion and causes root hair promotion through a different mechanism than that of ethylene.

Treatment	Trichoblast number (cells/mm^2) (%)	Atrichoblast number (cells/mm^2) (%)	Length of root hair (mm) (%)	Thickness of root hair (µm) (%)	Surface area of root hair (mm^2/mm^2) (%)
- DSP	51.5 (100)	66.7 (100)	0.34 (100)	9.39 (100)	0.26 (100)
+ DSP	224.2 (435)	124.2 (186)	0.99 (290)	12.4 (132)	4.32 (1,660)

Table 6. Effects of degraded soybean meal products on root hair size and density in *Brassica rapa*.
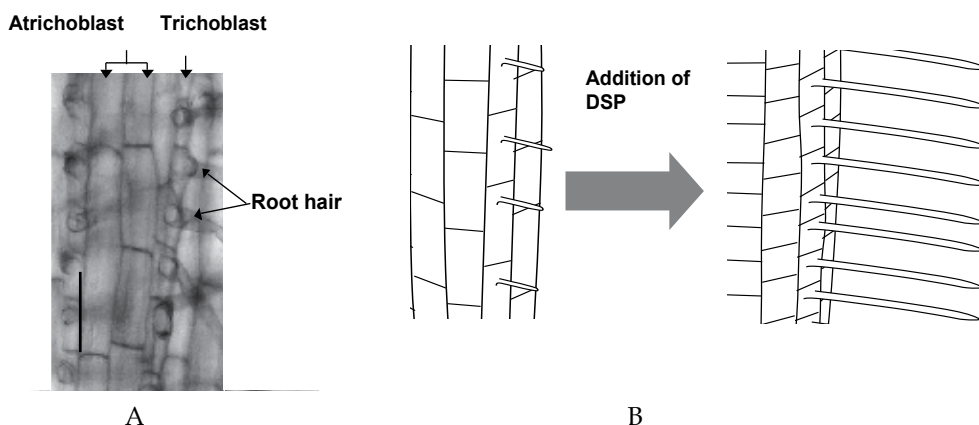
Fig. 7. Microscopic examination of root hairs grown in the presence of 30 µg/ml of degraded soybean meal products (DSP) (A) and a schematic model of the effect of DSP on trichoblast and atrichoblast (B).

3.2 Structure of exogenous peptide signal from DSP: analysis of protease from *B. circulans* HA12 for production of root hair promoting peptide(s)

The protease from *B. circulans* HA12 was purified and characterized. The N-terminal amino acid sequence (20 amino acids) of the protease produced by *B. circulans* HA12 was identical to subtilisin Carlsberg, derived from *B. licheniformis* (Jacobs et al., 1985; Jacobs, 1995). The molecular weight of the protease was about 30 kDa. The protease was inhibited by phenylmethylsulfonyl fluoride and its optimum pH was around 10. The protease from *B. circulans* HA12 was a subtilisin-like alkaline protease.
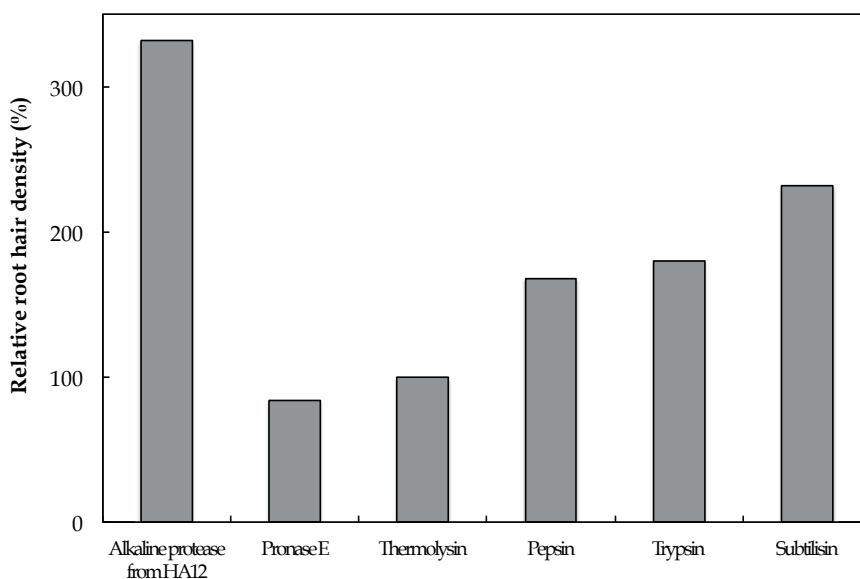
Fig. 8. Root hair promoting activity of various peptides.

Soybean meal was degraded by several proteases (pronase E, thermolysin, pepsin, trypsin, and subtilisin) and the root hair promoting activities of degraded products were analyzed. DSP by pronase E and thermolysin did not possess root hair promoting activity. Treatment by pepsin, trypsin, and subtilisin each showed root hair promoting activities, but these were lower than that of DSP (Fig. 8). The specific peptide(s) is produced by the degradation of soybean protein with an alkaline protease from *B. circulans* HA12.

3.3 Structure of exogenous peptide signal from DSP: identification of the root hair promoting peptide

Soybean contains various kinds of proteins, such as 7S globulin, 11S globulin, lectin and trypsin inhibitor (Brooks & Morr, 1985; Hamblin & Kent, 1973; Iibuchi & Imahori, 1978a; Iibuchi & Imahori, 1978b). The proteins were separated and purified by several steps, shown in Fig. 9. The separated soybean proteins were degraded by the alkaline protease from *B. circulans* HA12 and the root hair promoting activity of the degraded products from each fraction was analyzed (Table 7). Degraded products of Kunitz trypsin inhibitor (KTI), purified from whey protein, showed high root hair promoting activity, thus KTI was the origin protein for the root hair promoting peptide (Rackis et al., 1962).

The root hair promoting peptide from degraded products of KTI was purified by several chromatographic steps. The molecular mass was analyzed by matrix-assisted laser
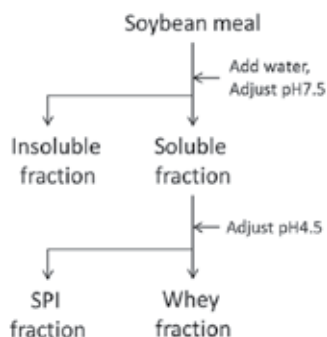
Fig. 9. Purification procedure of soybean meal proteins.

Treatment (peptides concentration)	Root hair promoting activity (%)
Without addition of peptides and amino acids	100.0
DSP (30 µg/ml)	331.1
Degraded soluble protein fraction (30 µg/ml)	337.1
Whey protein fraction (30 µg/ml)	335.5
KTI (10 µg/ml)	340.0

Table 7. Root hair promoting activity of degraded products from each protein fraction.

A

B

^{1}DFVLDNEGNPLENGGTYYILSDITAF**GGIRAAPTGNER**CP^{40}
^{41}LTVVQSRNELDKGIGTIISPSYRIRFIAEGHPLSLKFDSF^{80}
^{81}AVIMLCVGIPTEWSVVEDLPEGPAVKIGENKDAMDGWFRL^{120}
^{121}ERVSDDEFNNYKLVFCPQQREDDKCGDIGISIDDDGHTRR^{160}
^{161}LVVSKNKPLVVQFQKLDKESL^{181}
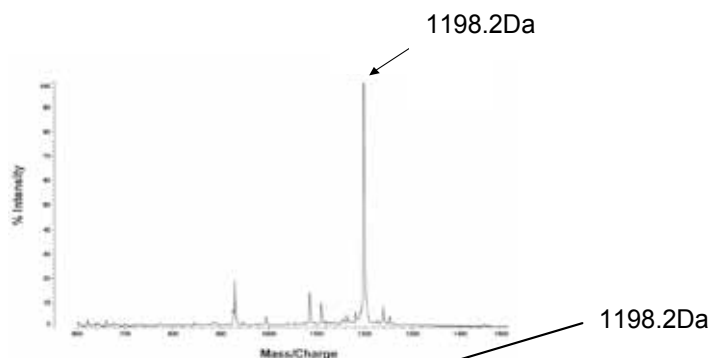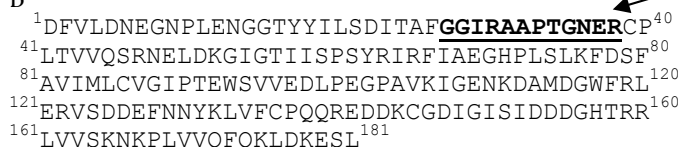
Fig. 10. Matrix-assisted laser desorption/ionization time–of-flight mass spectrometry spectra of root hair promoting peptide (A) and amino acid sequence of Kunitz trypsin inhibitor (B). The peptide sequence that is identical to 1198.2 Da is underlined.

desorption/ionization time–of-flight mass spectrometry (MALDI-TOF MS). The molecular weight of the bioactive peptide was 1,198.2 Da (Fig. 10A), and the molecular weight of the amino acid sequences in KTI was searched. Positions 27 - 38 in KTI (Gly-Gly-Ile-Arg-Ala-Ala-Pro-Thr-Gly-Asn-Glu-Arg) were identical to the molecular weight, and the peptide was thus designated root hair promoting peptide (RHPP) (Fig. 10B). RHPP was chemically synthesized and also shown to have root hair promoting activity (Fig. 11).
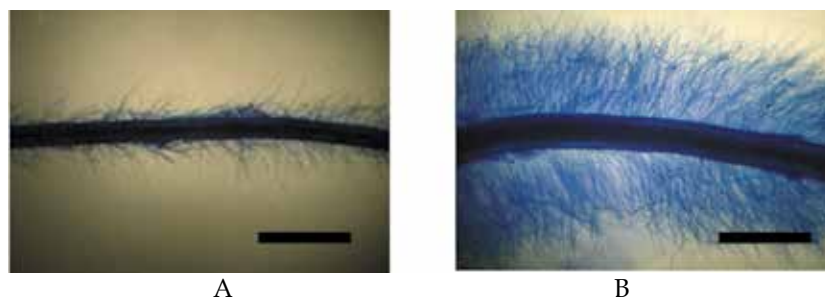
Fig. 11. Root hair promotion of chemically synthesized root hair promoting peptide (RHPP). A: plant growth medium, B: plant growth medium + chemically synthesized RHPP.

3.4 Comparison of RHPP and other endogenous peptide signals

RHPP consists of 12 amino acids and is rich in Ala, Arg, and Gly residues. The amino acid sequences of the endogenous peptide signals are shown in Table 8, and the length of each ranges from 5 to 96. The amino acid sequences of exogenous peptide, RHPP, and endogenous peptides seem to have no relationship within their structure. The mechanisms of RHPP bioactivity (root hair promotion and adventitious root formation) seem to be different from those of endogenous peptide signals.

RHPP contains four residues of α-helix breaking amino acids and the root hair promoting activity was retained after heat treatment (121°C, 15 min). Thus, secondary and tertiary structures of the peptide are not required for root hair promoting activity. On the other hand, the root hair promoting activity of RHPP decreased when one residue of the C terminus was deleted, indicating that the 12 residues of RHPP might be the minimum unit for expressing root hair promoting activity.

Peptide	Role	Amino acid sequence	Reference
CLV3	Proliferation of cells in the apical meristem	MDSKSFVLLLLLFCFLFLHDASDLTQ AHAHVQGLSNRKMMMMKMESEW VGANGEAEKAKTKGLGLHEELRTVP SGPDPLHHHVNPPRQPRNNFQLP	(Kondo, et al. 2006)
Phytosulfokine	Stimulate the proliferation of plant cells	Y(SO₃H)IY(SO₃H)TQ	(Matsubayashi et al., 1999)
SCR/SP11	Self incompatibility	NLMKRCTRGFRKLGKCTTLEEEKCK TLYPRGQCTCSDSKMNTHSCDCKSC	(Suzuki et al., 1999)
Systemin	Activates the synthesis of proteinase inhibitors	AVQSKPPSKRDPPKMQTD	(McGurl, et al., 1992)
RHPP	Root hair promotion Adventitious root formation	GGIRAAPTGNER	(Matsumiya et al., 2007)

Table 8. Characteristics of peptide signals for plants.

4. Analysis of peptide uptake in DSP and accumulation of RHPP in plant roots

Inorganic nitrogen is one of the most important elements for plant growth. Plants usually absorb and utilize ammonia and nitrate as inorganic nitrogen for biosynthesis of proteins and nucleic acids. Lately, direct utilization of organic nitrogen, such as amino acids, peptides, and proteins, for plant growth has been found (Chapin et al., 1993; Kielland et al., 2006; Paungfoo-Lonhienne et al., 2008.). Growth of a rice plant in the presence of Gln was faster than that with nitrate.

On the other hand, L-methionine is known as a precursor of phytohormone. L-Met is absorbed into plant cells from root and stoma and converted to ethylene. Moreover, bioactivity of D-Met on roots has been also found (Hasegawa et al. 2002). DSP contains various kinds of peptides, and these seem to be utilized as nitrogen sources and/or bioactive compound(s). This section describes uptake of peptides in DSP and the accumulation of RHPP in plant roots.

4.1 Uptake of peptides in DSP by *B. rapa*

The peptide uptake was analyzed using *B. rapa* in the presence of DSP solution (Fig. 12). A decrease in peptide concentration was observed. *B. rapa* absorbed about 45% of the initial water volume, and the peptide concentration decreased by about 75%, indicating that the plant seemed to positively absorb peptides in DSP.
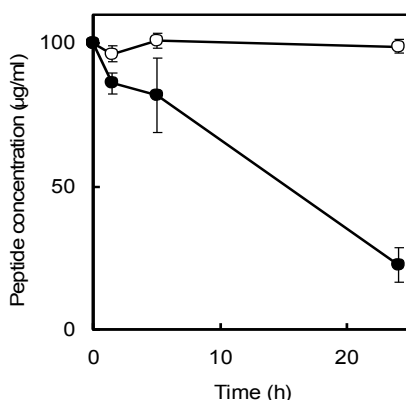
Fig. 12. Time course of peptides uptake from DSP solution by the root system of *B. rapa*. ○: without plant (control), ●: the root system of *B. rapa* was soaked in 100 μg/ml DSP solution.

Uptake of each peptide in DSP was analyzed by reversed phase HPLC (Fig. 13). All peaks decreased in intensity, suggesting that the plant absorbed many types of peptides (average uptake of peptides was 16.6%). Three specific peaks were markedly absorbed in the plant (peaks a, b, and c decreased by 54.5, 30.9, and 33.2%, respectively). Uptake of peptides by the plants seems to be influenced by the peptide lengths and amino acid sequences.

4.2 Accumulation of fluorescence labeled RHPP in roots

Carboxyfluorescein (FAM) labeled RHPP (FAM-RHPP) was synthesized for analysis of accumulation of RHPP. FAM-RHPP has root hair promoting activity at the same level as RHPP, so FAM-RHPP was used for further RHPP accumulation experiments.

Accumulation of the peptide was analyzed using a confocal laser scanning microscope. Fluorescence was observed over the whole epidermal cell (trichoblast and atrichoblast) after soaking of the root system in a FAM-RHPP solution for 24 hour (Fig. 14). The peptide was accumulated in both trichoblasts and atrichoblasts, and subsequently, FAM-RHPP seemed to increase trichoblast and atrichoblast numbers.
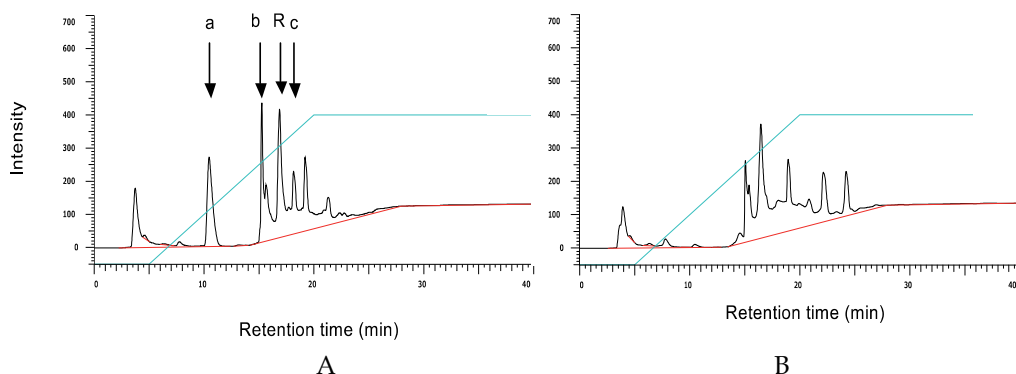
Fig. 13. Peptide uptake from degraded soybean meal products solution by the root system of *Brassica rapa*.
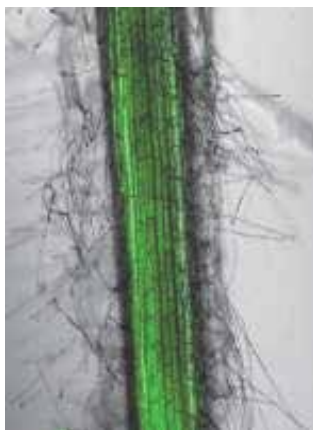
Fig. 14. Analysis of carboxyfluorescein-RHPP uptake in the root system of *Brassica rapa* by confocal laser scanning microscope.

4.3 Hypothetical root hair promotion by RHPP

Many kinds of peptides were generated by the degradation of soybean meal by the alkaline protease from *B. circulans* HA12. Several peptides in DSP are specifically absorbed into plants from the root system. RHPP seems to be absorbed into the root of *B. rapa* and accumulated in trichoblasts and atrichoblasts. RHPP in the cytoplasm of the root may affect the expression of specific gene(s) for the promotion of root hair numbers and root hair length. The surface area of the root system is increased by RHPP and consequently plant growth is stimulated by enhancement of nutrient uptake from the root system.
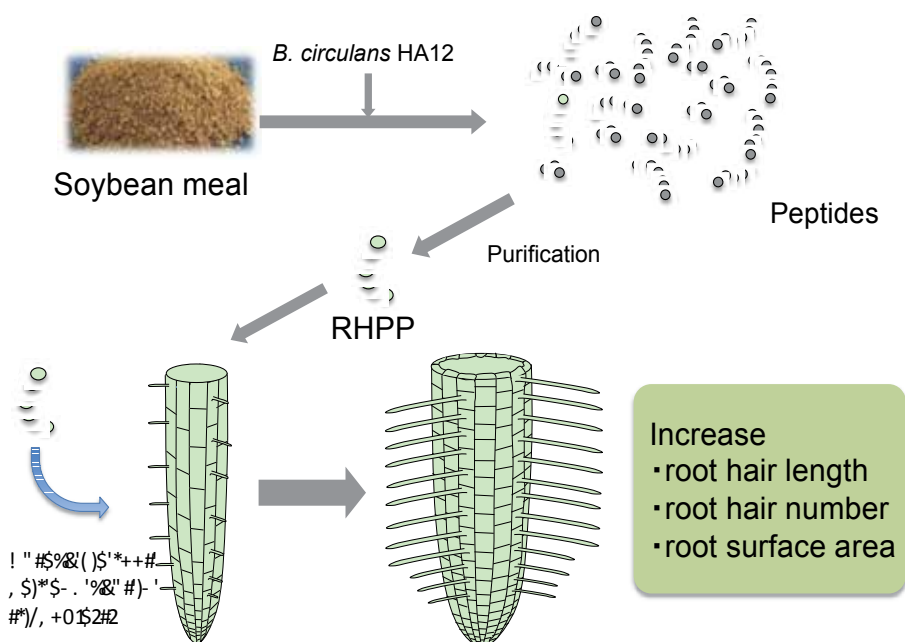
Fig. 15. Schematic model of internalization of the root hair promoting peptide.

5. Conclusion

This chapter describes utilization of soybean meal and the development of bioactive peptides for plant growth using soybean meal and the alkaline protease.

Section 1

A soybean meal degrading bacterium was isolated, identified, and designated as *B. circulans* HA12. The strain produced an alkaline protease. Soybean meal was degraded with *B. circulans* HA12, and DSP promoted various kinds of plant growth at low concentration. DSP increased root hair numbers for *B. rapa* and adventitious root was also formed from the stem of *L. esculentum* soaked in DSP solution. The bioactivity of DSP differs from that of phytohormones.

Section 2

DSP increased the number of epidermal cells without altering the localization patterns of trichoblasts and atrichoblasts. The root hair surface area was increased by about 16.6 times. The origin protein for RHPP was KTI, and RHPP was purified using KTI and the alkaline protease from *B. circulans* HA12. The structure of RHPP was analyzed by MALDI-TOF MS, and the amino acid sequence was identified (GGIRAAPTGNER; M.W. 1198.2 Da).

Section 3

Peptides in DSP were absorbed from the root system of B. rapa, but the uptake ratio was different for each peptide. RHPP was also absorbed into the plant and accumulated in both trichoblasts and atrichoblasts of the plant root. RHPP seemed to stimulate specific gene(s) that increase of number and length of root hair.

6. References

Acea, M. J., Moore, C. R., & Alexander, M. (1988). Survival and growth of bacteria introduced into soil. *Soil Biology and Biochemistry*, Vol. 20, No. 4, (December 2002), pp. 509-515, ISSN 0038-0717

Brooks, J. B. & Morr, C. V. (1985). Current aspects of soy protein fractionation and nomenclature. *Journal of the American Oil Chemists' Society*, Vol. 62, No. 9, (September 1985), pp. 1347-1364, ISSN 0003-021X

Buchanan, R. E. & Gibbons. N. E. (1974). *Bergey's manual of determinative bacteriology, 8th ed.* The Williams & Wilkins Co., ISBN0683-41051-1, Baltimore, USA

Chapin, F. S., Moilanen, L., & Kielland, K. (1993). Preferential use of organic nitrogen for growth by a non-mycorrhizal arctic sedge. *Nature*, Vol. 361, No. 6408, (January 1993), pp. 150-153, ISSN 0028-0836

Dolan, L. (1996). Pattern in the root epidermis: An interplay of diffusible signals and cellular geometry. *Annals of Botany*, Vol. 77, No. 6, (June 1996), pp. 547-553, ISSN 0305-7364

Farzamirad, V. & Aluko, R. E. (2008) Angiotensin-converting enzyme inhibition and free-radical scavenging properties of cationic peptides derived from soybean protein hydrolysates. *International Journal of Food Sciences and Nutrition*. (August 2008), Vol. 59, No. 5, pp. 428-437, ISSN 0963-7486

Gaither, D. H. (1975). Auxin and the response of pea roots to auxin transport inhibitors: morphactin. *Plant Physiology*, Vol. 55, No. 6, (June 1975), pp. 1082-1086, ISSN 0032-0889

Gilroy, S. & Jones, D. L. (2000). Though form to function: root hair development and nutrient uptake. *Trends in Plant Science*, Vol. **5**, No. 2, (February 2000), pp. 56–60, ISSN 1360-1385

Hamblin, J. & Kent, S. P. (1973). Possible role of phytohaemagglutinin in *Phaseolus vulgaris* L.. *Nature New Biology*, Vol. 245, No. 140, (September 1973), pp. 28-30, ISSN 0028-0836

Hasegawa, N., Fukumoto, Y., Minoda, M., Plikomol, A., & Kubo, M. (2002). Promotion of plant and root growth by soybean meal degradation products. *Biotechnology Letters*, Vol. 24, No. 18, (September 2002), pp. 1483-1486, ISSN 1360-1385

Iibuchi, C. & Imahori, K. (1978a). Heterogeneity and its relation to the subunit structure of the soybean 7S globulin. *Agricultural and Biological Chemistry*, Vol. 42, No. 1, (November 2008), pp. 31-36 ISSN 0002-1369

Iibuchi, C. & Imahori, K. (1978b). Interconversion between monomer and dimmer of the 7S globulin of soybean seed. *Agricultural and Biological Chemistry*, Vol. 42, No. 1, (November 2008), pp. 25-30, ISSN 0002-1369

Ito, Y., Nakanomyo, I., Motose, H., Iwamoto, K., Sawa, S., Dohmae, N., & Fukuda, H. (2006). Dodeca-CLE peptides as suppressors of plant stem cell differentiation. *Science*, Vol. 313, No. 5788, (August 2006), pp. 842-845, ISSN 0036-8075

Jacobs, M. F. (1995). Expression of the subtilisin Carlsberg-encoding gene in *Bacillus licheniformis* and *Bacillus subtilis*. *Gene*, Vol. 152, No. 1, (January 1995), pp. 69-74, ISSN 0378-1119

Jacobs, M., Eliasson, M., Uhlén, M., & Flock, J. I. (1985). Cloning, sequencing and expression of subtilisin Carlsberg from *Bacillus licheniformis*. *Nucleic Acids Research*. Vol. 13, No. 24, (December 1985), pp. 8913-8926, ISSN 0305-1048

Kamimura, Y. & Hayano, K. (2000). Properties of protease extracted from tea-field soil. *Biology and Fertility of Soils*, Vol. 30, No. 4, (January 2000), pp. 351-355, ISSN 0178-2762

Kielland, K., McFarland, J., & Olson, K. (2006). Amino acid uptake in deciduous and coniferous taiga ecosystems. *Plant and Soil*, Vol. 288, No. 1-2, (October 2006), pp. 297-307, ISSN 0032-079X

Klopper, J. W., Lifshitz, R., & Zablotowicz, R. M. (1989). Free-living bacteria inocula for enhancing crop productivity. *Trends in Biotechnology*, Vol. 7, No. 2, (February 1989), pp. 39-44., ISSN 0167-7799

Kondo, T., Sawa, S., Kinoshita, A., Mizuno, S., Kakimoto, T., Fukuda, H., & Sakagami, Y. (2006). A plant peptide encoded by CLV3 identified by in situ MALDI-TOF MS analysis. *Science*, Vol. 313, No. 5788, (August 2006), pp. 845-848, ISSN 0036-8075

Kubo, M., Okajima, J. & Hasumi, F. (1994). Isolation and characterization of soybean waste-degrading microorganisms and analysis of fertilizes effect of degraded products. *Applied and Environmental Microbiology,* Vol. 60, No. 1, (January 1994), pp. 243-247, ISSN 0099-2240

Lauter, F. R., Ninnemann, O., Bucher, M., Riesmeier, J. W., & Frommer, W. B. (1996). Preferential expression of an ammonium transporter and of two putative nitrate transporters in root hairs of tomato. *Proceedings of the National Academy of Science of the United States of America*, Vol. 93, No. 15, (July 1996), pp. 8139–8144, ISSN 1091-6490

Masucci, J. D. & Schiefelbein, J. W. (1994). The *rhd6* mutation of Arabidopsis thaliana alters root-hair initiation through an auxin- and ethylene-associated process. *Plant Physiology,* Vol. 106, No. 4, (December 1994), pp. 1335-1346, ISSN 0032-0889

Matsubayashi, Y. & Sakagami, Y. (1996). Phytosulfokine, sulfated peptides that induce the proliferation of single mesophyll cells of *Asparagus officinalis* L. *Proceedings of the National Academy of Science of the United States of America*, Vol. 93, No. 15, (July 1996), pp. 7623-7627, ISSN 1091-6490

Matsubayashi, Y., Morita, A., Matsunaga, E., Furuya, A., Hanai, N., & Sakagami, Y. (1999). Physiological relationships between auxin, cytokinin, and a peptide growth factor, phytosulfokine-α, in stimulation of asparagus cell proliferation. *Planta*, Vol. 207, No. 4, (February 1999), pp. 559–565, ISSN 0032-0935

Matsumiya, Y., Nishikawa, K., Aoshima, H., Inouye, K., & Kubo, M. (2004). Analysis of autodegradation sites of thermolysin and enhancement of its thermostability by modifying Leu155 at an autodegradation site. *Journal of Biochemistry*, Vol. 135, No. 4, (April 2004), pp. 547-553, ISSN 0021-924X

Matsumiya, Y., Sumiyoshi, S., Matsukura, T., & Kubo, M. (2007). Effect on epidermal cell of soybean protein-degraded products and structural determination of the root hair promoting peptide. *Applied Microbiology and Biotechnology*, Vol. 77, No. 1, (November 2007), pp. 37-43, ISSN 0175-7598

McGurl, B., Pearce, G., Orozco-Cardenas, M., & Ryan, C. A. (1992) Structure, expression, and antisense inhibition of the systemin precursor gene. *Science*, Vol. 255, No. 5051, (March 1992), pp. 1570-1573, ISSN 0036-8075

O'Sullivan, M. P., Stephens, M., & O'Gara, F. (1991). Extracellular protease production by fluorescent *Pseudomonas* spp and the colonization of sugarbeet roots and soil. *Soil Biology and Biochemistry*, Vol. 23, No. 7, (1991), pp. 623-627, ISSN 0038-0717

Okuda, A. (1961). *Dojou hiryou sousetu*, Yokendou, Co. LTD., Tokyo, Japan

Paungfoo-Lonhienne, C., Lonhienne, T. G., Rentsch, D., Robinson, N., Christie, M., Webb, R. I., Gamage, H. K., Carroll, B. J., Schenk, P. M., & Schmidt, S. (2008). Plants can use protein as a nitrogen source without assistance from other organisms. *Proceedings of the National Academy of Science of the United States of America*, Vol. 105, No. 11, (March 2008), pp. 4524-4529, ISSN 1091-6490

Pearce, G., Strydom, D., Jonson, S., & Ryan, C. A. (1991). A Polypeptide from tomato leaves induces wound-inducible proteinase inhibitor proteins. *Science*, Vol. 253, No. 5022, (August 1991), pp. 895-898, ISSN 0036-8075

Pitts, R. J., Cernac, A., & Estelle, M. (1998). Auxin and ethylene promote root hair elongation in *Arabidopsis. The Plant Journal*, Vol. 16, No. 5, (December 1998), pp. 553-560, ISSN 0960-7412

Rackis, J. J., Sasame, H. A., Mann, R. K., Anderson, R. L., & Smith, A. K. (1962). Soybean trypsin inhibitors: isolation, purification and physical properties. *Archives of Biochemistry and Biophysics*, Vol. 98, No. 3, (September 1962), pp. 471-478, ISSN 0003-9861

Ryu, C. M., Farag, M. A., Hu, C. H., Reddy, M. S., Wei, H. X., Pare, P. W., & Kloepper, J. W. (2003). Bacterial volatiles promote growth in *Arabidopsis. Proceedings of the National Academy of Science of the United States of America*, Vol. 100, No. 8, (April 2003), pp. 4927-4932, ISSN 1091-6490

Schopfer, C. R., Nasrallah, M. E., & Nasrallah, J. B. (1999). The male determinant of self-incompatibility in *Brassica. Science*, Vol. 286, No. 5445, (November 1999), pp. 1697-1700, ISSN 0036-8075

Shinano, T., Osaki, M., & Tadano, T. (1991). Effects of nitrogen application on reconstruction of nitrogen compounds during the maturation stage in several field crops. *Soil Science and Plant Nutrition*, Vol. 37, No. 2, (June 1991), pp. 259-270, ISSN 0038-0768

Smith, S. J., Young, L. B., & Miller, G. E. (1977). Evaluation of soil nitrogen mineralization potentials under modified field conditions. *Soil Science Society of America Journal*, Vol. 41, (January-February 1977), pp. 74-76, ISSN 0361-5995

Suzuki, G., Kai, N., Hirose, T., Fukui, K., Nishio, T., Takayama, S., Isogai, A., Watanabe, M., & Hinata, K. (1999). Genomic organization of the S locus: Identification and characterization of genes in SLG/SRK region of S^9 haplotype of *Brassica campestris* (syn. rapa). *Genetics*, Vol. 153, No. 1, (September 1999), pp. 391-400, ISSN 0016-6731

Tanimoto, M., Roberts, K., & Dolan, L. (1995). Ethylene is a positive regulator of root hair development in *Arabidopsis thaliana. The Plant Journal*, Vol. 8, No. 6, (December 1995), pp. 943-948, ISSN 0960-7412

Uchida, M. (2007). Prospects for World Trade in the Soybean Sector. *Journal of Nagoya Bunri University*, Vol. 7, (March 2007), pp. 97-102, ISSN 1346-1982

Watanabe, K. & Hayano, K. (1995). Seasonal variation of soil protease activities and their relation to proteolytic bacteria and *Bacillus* spp in paddy field soil. *Soil Biology and Biochemistry*, Vol. 27, No. 2, (February 1995), pp. 197-203, ISSN 0038-0717

Yamazaki, H. & Roppingi, K. (1998). The effect of organic matters application for leaf vegetable yield and quality. *Saitamaen Shikenhou*, Vol. 21, (March 1998), pp. 7-20, ISSN 0385-3675

Yonekura, M. & Tanaka, A. (2003). Isolation and application of physiologically active peptides from soybean whey and okara proteins. *Soy Protein Research, Japan,* Vol. 6, No. 24, (2003), pp. 88-93, ISSN 1344-4050

Yonekura, M. & Yamamoto, A. (2004). Isolation and application of physiologically active peptides from soybean whey and okara proteins. *Soy Protein Research, Japan,* Vol. 7, No. 25, (2004), pp. 79-84, ISSN 1344-4050

Dietary Effect of Soybean (*Glycine max*) Products on Gut Histology and Microbiota of Fish

Daniel L. Merrifield[1], Rolf Erik Olsen[2],
Reidar Myklebust[3] and Einar Ringø[4*]
[1]Aquaculture and Fish Nutrition Research Group, The University of Plymouth,
[2]Institute of Marine Research, Matre Aquaculture Station, Matredal,
[3]Molecular Imagining Centre (MIC), Institute for Biomedicine,
University of Bergen, Bergen,
[4]Norwegian College of Fishery Science, Faculty of Biosciences,
Fisheries and Economics, University of Tromsø,
Breivika,
[1]UK
[2,3,4]Norway

1. Introduction

On a global scale, fisheries landings have remained constant at about 90 million tons of fish per annum for the last decade whereas aquaculture output has been increasing at a rate of around 8% per annum and now supplies about 65 million tons (FAO 2010). Indeed, more than half of the fish products produced for human consumption come from commercial aquaculture. To sustain high rates of increased production, a matching increase in the volume of fish feeds is required. In 2009, compound aquafeed useage was ca. 30 million tons but this is expected to more than double by 2020 (FAO 2010; Tacon 2010). Although this is only a small portion of global animal feed production (ca. 620 million tons) the very specific nature of aquafeeds, e.g. higher protein and lower carbohydrate levels compared to other animal feeds, results in special challenges as traditional sources of fish - protein (fishmeal; FM) and oil (FO) are not sustainable. Worldwide annual production of FM (about 6 million tons) and FO (less than 1 million tons) has remained fairly stable for the last 20 years. FM and FO are produced from designated pelagic fisheries, mainly from the Atlantic, Chile and Peru. Efforts are constantly underway to ensure that these marine fisheries on which FM and FO depend remain sustainable and are not over-exploited. FM and FO are also produced from trimmings, offal and/or by-catch, although to a limited extent. Considering the known benefits of seafood, as a rich source of omega-3 fatty acids, in regards to human health (e.g. cardiovascular health, brain development, lowering cholesterol and blood pressure etc) the continued provision of this protein source is essential.

As there will be a limitation in global supplies of FM and FO in the near future sustainable alternatives must be explored. Soybean meal (SBM) and soybean oil (SBO) are considered to

be suitable alternatives for the partial replacement of FM and FO and indeed are utilised in commercial aquafeeds. However, even when heat-treated, standard (solvent-extracted) and full-fat SBM-containing feeds supplemented with limiting amino acids, can lead to decreased growth in salmonids (Davies & Morris, 1997). More specifically, dietary SBM inclusion causes lower feed intake, weight gain, fecal dry matter, and energy and fat digestibilities in all salmonid species studied (Krogdahl et al., 2003). Dehulled SBM as the sole protein source may lead to growth arrest and increased mortality in rainbow trout *Oncorhynchus mykiss* (Walbaum) (Rumsey et al., 1994). Full-fat SBM, however, appears to support better growth than solvent-extracted SBM in rainbow trout (Olli & Krogdahl, 1994) and Atlantic salmon *Salmo salar* (Olli et al., 1994 b). Dehulled, solvent-extracted SBM causes similar negative effects on growth and nutrient digestibility in Atlantic salmon as SBM produced from hulled soybeans (Olli et al., 1994 b). White flakes, which are dehulled, moderately toasted solvent-extracted SBM, has been reported to cause similar reductions in growth, feed efficiency ratio, and nutrient digestibilities as standard SBM in Atlantic salmon (Refstie et al., 2005).

Thus, the change from FM and FO to soybean products presents several metabolic and health challenges for the farmed fish. When using high dietary levels of plant derived materials, particularly those derived from soybean, it is important to consider the impacts on gut microbiota and gut histology as the gastrointestinal (GI) tract can be one of the important infection routes for some pathogens in fish.

2. Effect of soybean products on gut histology

The primary purpose of the digestive tract is to digest foodstuffs into molecules suitable for absorption via the various transport mechanisms of the epithelial border cells of the GI sections. In essence, the fish GI tract is a tube-like structure that varies in complexity depending on the various feeding habits of the species. It can be subdivided into the mouth, oesophagus, stomach, pyloric ceaca, mid intestine, distal intestine and the rectum; however, not all of these distinctive regions are present in all fish. According to the early work of Suyehiro (1942), stomachs (if present) are classified into five categories according to their appearance: I – shape, U – shape, V – shape, Y – shape and Γ - shape. Distal to the stomach, in the mid gut region, some fish have pyloric ceaca. These finger-like pouches can be completely absent or present in numbers ranging from few (e.g. flounders) to more than 1000 (e.g. gadoids) (Suyehiro, 1942; Olsen & Ringø, 1997). Distal to the pyloric ceaca is the intestine which also varies in physiology across fish species. Typically, the GI tract of carnivorous fish species is short, often less than one body length long, whereas the GI tract of herbivorous and omnivorous fish species is much longer and can be found to be greater than 20 times the body length (Suyehiro, 1942; Olsen & Ringø, 1997). Distinctive physiological differences are also observed between fresh water and marine species. The surface cells exposed to the luminal contents are the enterocytes, which with their microvilli structures, comprise the epithelial brushborder. Between the epithelial cells goblet cells excrete a continuous layer of mucus which forms an effective protective barrier. Beneath the inner epithelium we find the cellular connective tissues which comprise the lamina propria and the submucosa. Beyond the submucosa lie a circular muscular layer and a longitudinal muscular layer. Finally, the serosa forms the outer layer of the GI tract.

From an electron microscopy (EM) point of view, the GI tract is a fascinating organ as illustrated in Figure 1.
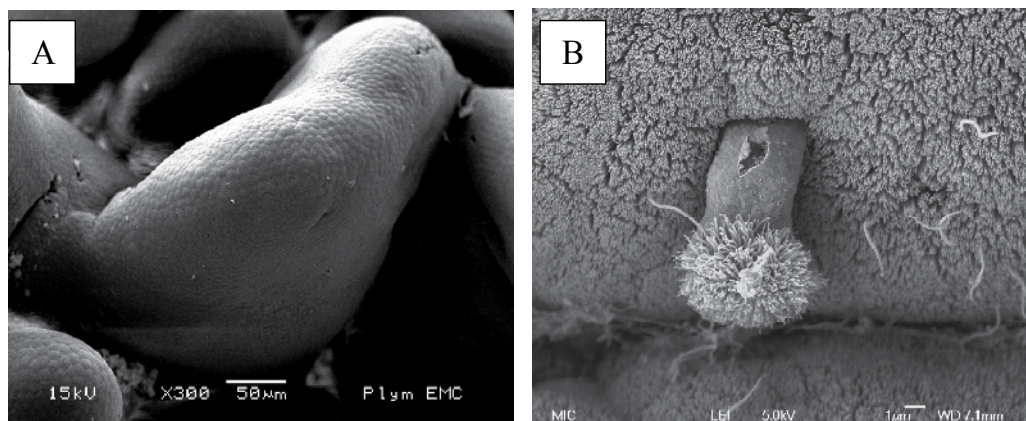
Fig. 1. A) Scanning electron microscopy (SEM) micrograph of mucosal folding (villi) from rainbow trout (Merrifield & Dimitroglou unpublished data). B) SEM micrograph of a dead cell rejected by Atlantic salmon intestine (Myklebust, Olsen & Ringø unpublished data).
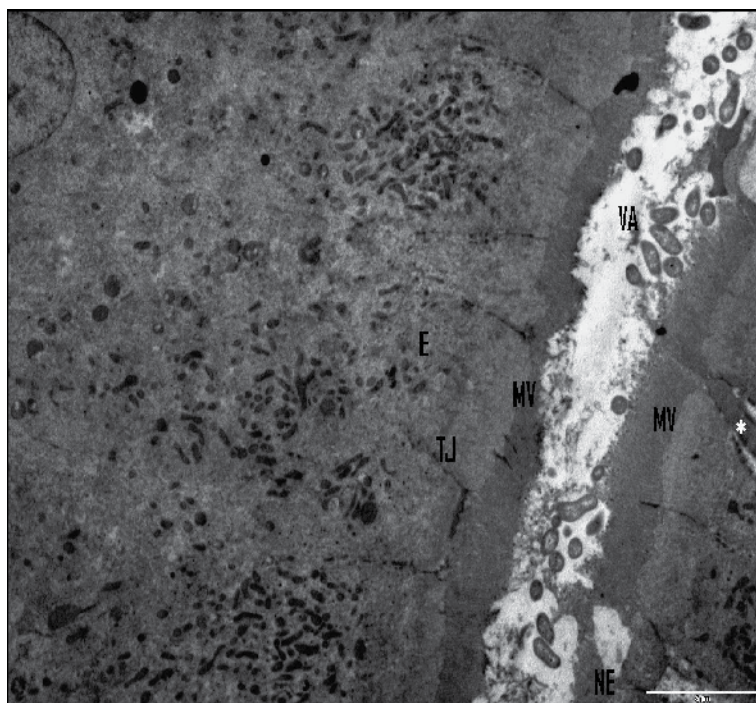
Fig. 2. Transmission electron microscopy (TEM) micrograph of the anterior intestine (mid gut) of rainbow trout exposed to *V.* (*L.*) *anguillarum* (VA). Clear signs of tissue damage are characterised by necrotic enterocytes (NE), disorganised microvilli and irregular and deteriorated tight junctional complexes (TJ) resulting in intracellular spaces (*). E = enterocyte. Scale bar = 5 µm. After Harper et al. (2011).

The GI tract of fish is considered as one of the major infection routes for some pathogens and indigenous intestinal bacteria (Groff & LaPatra, 2000; Ringø et al., 2003; Birkbeck &

Ringø, 2005; Harikrishnan & Balasundaram, 2005; Ringø et al., 2007; 2010a). Indeed, numerous studies have reported that exposure of the epithelium to fish pathogens can result in severe tissue damage, characterised by necrotic enterocytes, deteriorated tight junctions, disorganised and damaged microvilli and damage to the lamina propria (Ringø et al., 2007). An example of the damage that can be visualised at the ultra structural scale with transmission electron microscopy (TEM) is shown in Figure 2, where the midgut of rainbow trout has been exposed to *Vibrio* (*Listonella*) *anguillarum*, the causative agent of vibriosis.

When discussing the inclusion of soybean products in commercial diets, it is of high importance to evaluate the effect on gut histology by both light microscopy (LM) and EM (Ringø et al., 2001; 2003; 2007; Harper et al., 2011) because structural and functional changes in the intestine may explain deleterious effects of SBM on nutrient utilization and disease resistance.

2.1 Soybean meal

SBM is one of the most commonly used protein sources in aquafeeds due to its high protein content and favourable amino acid profile. However, even when heat-treated and supplemented with limiting amino acids, full-fat as well as defatted (standard; hexane-extracted), SBM rich feeds can lead to decreased growth, lower feed intake and reduced energy and lipid digestibility in salmonid species. Using dehulled SBM as the sole protein source has been reported to reduce growth performance and increase mortality in rainbow trout. These SBM products also cause an inflammatory response in the distal intestine (enteritis) of salmonids, characterised by changes in absorptive cells, increased presence of inflammatory cells, endocytic blocking, shortening of villi and disruption of microvilli, which may at least partially explain the effects on growth parameters and feed utilization. It is accepted that such effects are mediated, in part at least, by anti-nutritional factors such as soy saponins which disrupt the intestinal barrier by altering membrane permeability. Subsequently, these histological and morphological changes in the intestine can increase susceptibility to bacterial infection.

Morphological changes evaluated by LM in the distal intestine (DI) of salmonids (Figure 3) are caused by dietary inclusion of full-fat as well as solvent-extracted SBM, with and without hulls (Baeverfjord & Krogdahl, 1996; Aslaksen et al., 2007). These morphological changes in salmonids, known as enteritis, have been described as shortening of the simple and complex mucosal folds with a widening of the central stroma (lamina propria) and submucosa, shortened microvilli of the brush border membrane and increased formation of microvillar vesicles, elevated number of goblet cells and a dramatic decrease or even absence of the normal supranuclear absorptive vacuoles in the enterocytes (van den Ingh et al., 1991; 1996; Baeverfjord & Krogdahl, 1996). Generally, the lamina propria is widened with a profound infiltration of a mixed population of inflammatory cells such as lymphocytes, neutrophilic granulocytes, cells of monocytic lineage, including macrophages, eosinophilic granular cells, and diffuse IgM (Baeverfjord & Krogdahl, 1996; Bakke-McKellep et al., 2000) as well as a mixed population of putative T-cells (Bakke-McKellep et al., 2007 a). Regarding the epithelial cells lining the mucosal folds, the number of cells in early stages of development are significantly increased, as are the number of cells undergoing cellular repair and programmed cell death (apoptosis) (Bakke-McKellep et al., 2007 b).

In their study on Atlantic salmon, Kraugerud et al. (2007) observed morphological changes in the distal intestine of fish fed 10 % dietary defatted SBM for 4 weeks. These changes included shortening and fusion of the simple mucosal folds, widening of the lamina propria with increased cellularity, leucocytic cellular infiltration of the submucosa and lamina
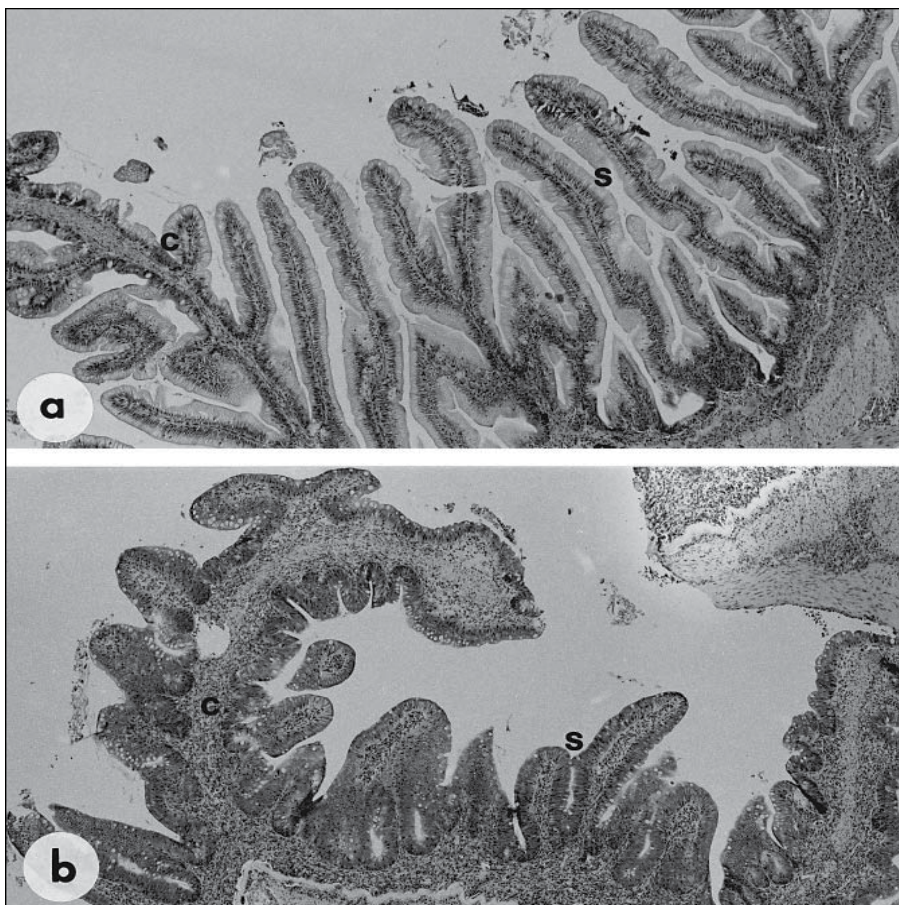
Fig. 3. LM image of the distal intestinal mucosa of Atlantic salmon fed a diet in which FM was the sole protein source (a) or a diet containing 33% extracted SBM (b). In the latter, the lamina propria of the simple (s) and complex (c) intestinal folds are widened and infiltrated with a mixed population of leucocytes. The folds in (b) are also reduced in height compared to (a) (after Baeverfjord & Krogdahl, 1996). X 400 magnification.

propria and reduced supranuclear vacuolisation with apical nuclear displacement within enterocytes. These significant morphological changes may affect the exchange of substances across the barriers including facilitated uptake of pathogenic bacteria. More, recently, Merrifield et al. (2009) observed the effect of replacing 50% of FM with SBM (HiPro soya) on the intestinal epithelium of rainbow trout by using SEM (Figure 4).

After 16 weeks of feeding, EM revealed enteritis - like effects associated with SBM fed fish. Specifically, fish fed the SBM enriched diet displayed missing, damaged, deformed , shorter and thicker microvilli. Additionally, enterocytes appeared to be malformed and irregular. The general result of this study was significantly shorter (distal intestine) and less densely packed (proximal intestine) microvilli on the enterocyte surfaces of fish fed the SBM diet than fish fed the FM diet. This reduction of microvilli density consequently led to increased exposure of enterocyte tight junctions, which combined with necrotic enterocytes is likely to diminish the protective barrier of the intestinal epithelium (Merrifield et al.,
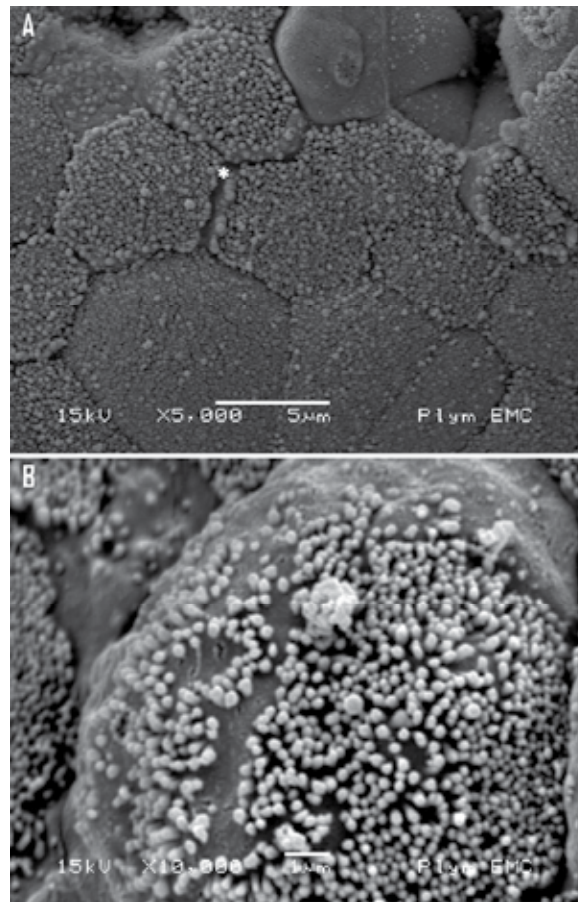
Fig. 4. SEM micrograph of the epithelial brush border of rainbow trout fed a SBM rich diet for 16 weeks. SBM fed fish display irregular enterocyte formations with sparse and irregular microvilli which consequently exposes tight junctions between the enterocytes (*) to luminal contents. Scale bars = 5 µm (A) and 1 µm (B). After Merrifield et al. (2009).

2009). Such enteritis effects have routinely been described in fish with the use of light microscopy (for review see Krogdahl et al., 2010) but considerably fewer studies have utilised electron microscopy to observe ultrastructural changes (Urán et al., 2008 a; Merrifield et al., 2009; Dimitroglou et al., 2010). Such histological and morphological changes which result in damaged enterocytes and exposed intracellular tight junctions may help explain previous observation of increased susceptibility of SBM fed fish to pathogenic infection (Krogdahl et al., 2000).

It is generally accepted that this type of damage is due to the presence of various anti-nutritional factors present in SBM. For example, soy saponins, which disrupt the intestinal barrier by altering membrane permeability which is suggested to trigger the inflammatory process (Knudsen et al., 2007). Recent investigations also suggest that this enteritis effect is related to (either as a cause or effect) the blocking of enterocyte endocytic uptake mechanisms (Urán et al., 2008 b; Rombout et al., 2011). Although the molecular signalling pathways involved in the manifestation of enteritis have not been fully elucidated some

information is available; the expression of pro-inflammatory genes (IL-1β and TNF-α1) have been reported to be up-regulated in the intestinal intraepithelial leucocytes of fish fed dietary SBM (Urán et al., 2008 a).

From the plethora of studies available it has become generally accepted that there are variations in the sensitivity of different fish species to dietary SBM. Due to their carnivorous feeding habits salmonids are less well adapted to plant based ingredients and so species such as Atlantic salmon and rainbow trout (although to a lesser extent than Atlantic salmon) are highly sensitive to dietary SBM. On the contrary, numerous studies have reported no morphological disruptions of the intestinal tract of other fish species, such as Atlantic cod (Refstie et al., 2006), Atlantic halibut (Grisdale-Helland et al., 2002), gilthead seabream *Sparus aurata* (Bonaldo et al., 2008, Dimitroglou et al., 2010), European sea bass *Dicentrarchus labrax* (Bonaldo et al., 2008), cobia *Rachycentron canadum* (Romarheim et al., 2008) and Egyptian sole *Solea aegyptiaca* (Bonaldo et al., 2006), fed dietary SBM. These observations are somewhat strange as some of these species are also highly carnivorous.

However, a recent study by Urán and colleagues (2008 a) reveals important information regarding the time dependent nature of the morphological manifestations of enteritis in fish. This study revealed the onset of histological changes, and related inflammatory molecular markers, in carp after 1-3 weeks feeding on dietary SBM (20% dietary inclusion). However, after 4-5 weeks these morphological changes appeared to subside in tandem with the increase of TGF-β (TGF-β is an anti-inflammatory protein involved in cellular differentiation and proliferation) mRNA transcripts from intraepithelial leucocytes. This indicates that carp display an adaptive period which may have important connotations to the results from other studies which have reported no histological disruptions in the gut of non-salmonid fish because the previous studies on non-salmonid species have assessed the intestinal morphology after ≥6 weeks feeding on SBM diets. Future time dependent studies are necessary to see if such short term enteritis effects occur in other fish species.

2.2 Soybean oil

SBO is extracted from the seeds of the soybean. Regardless of origin, plant oils are deficient in the typical marine long chain highly unsaturated fatty acids (HUFAs) arachidonic acid (ARA, 20:4 *n-6*), eicosapentaenoic acid (EPA, 20:5 *n-3*) and docosahexaenoic acid (DHA, 22:6 *n-3*). The composition of other fatty acids will vary with plant species. One of the predominant plant oils produced and used in aquafeeds, SBO, is rich in 18:2 *n-6* (52 %), 18:1 (24 %) and 16:0 (11 %) but due to its deficiency in long chain HUFAs SBO and other plant oils are typically blended with FO when incorporated into fish diets.

In marine fish, the most predominant effect of increasing plant oils, including SBO, in the diets is the reduction of cellular EFA content and some alteration in membrane composition. In salmonids, including plant oils will not only affect membrane composition, but will also alter the ratio of n-6 / n-3 C_{20} PUFA, which may have pronounced effects on immune functions and eicosanoid production. With the inclusion of plant oils into aquafeeds, fish are faced with several challenges some of which will affect intestinal health and function. One of the most striking effects is the massive accumulation of lipid droplets in enterocytes of fish species such as Arctic charr (Olsen et al., 1999; 2000), gilthead seabream (Caballero et al., 2003), rainbow trout (Caballero et al., 2002; Olsen et al., 2003) and Atlantic salmon (Olsen et al., unpublished data) fed high levels of plant oils. The accumulations may amount to more than 60% of cellular volume which hampers gut functions (Figure 5). Although there is still some controversy with regard to the direct cause of these observations (Oxley et al., 2005;

Caballero et al., 2006; Oxley et al., 2007) the condition appears to be related to the impairment of lipoprotein synthesis within the enterocytes.
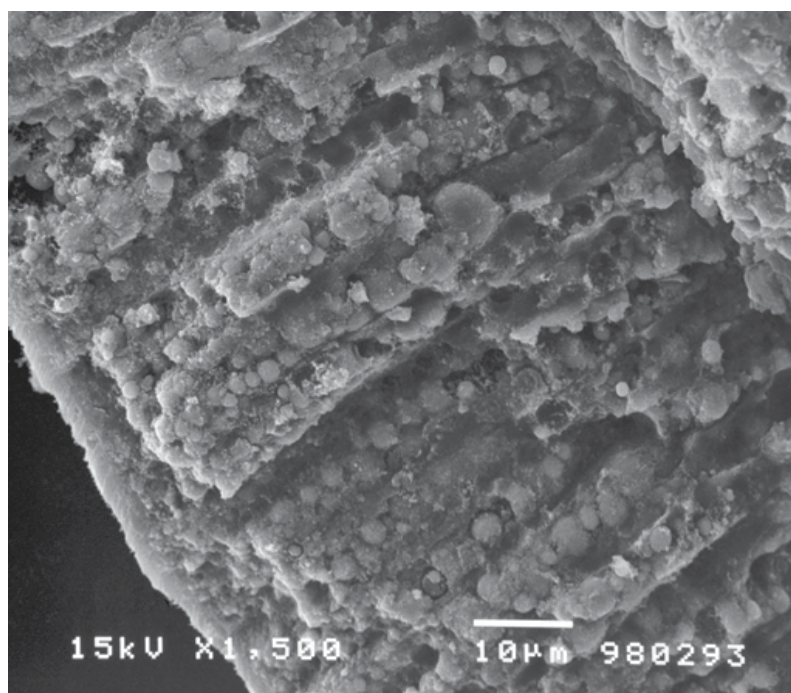
Fig. 5. SEM micrograph depicting the cross section of midgut enterocytes from Arctic charr fed an experimental diet containing SBO as the sole lipid source. Note the significant accumulation of lipid droplets in the enterocytes. After Ringø & Olsen (2008).

In Atlantic salmon substituting up to 100% of the added FO (note some FO is present in FM) with SBO did not increase leukotriene B_4 (LTB_4) or type E_2 prostaglandin (PGE_2) (pro-inflammatory mediators) production of head kidney macrophages during *in vitro* culture with 20:4 *n-6* added to the culture medium, except at 5°C rearing temperature (Gjøen et al., 2004). Phagocytic activity was also unaffected and based on these results the authors concluded that Atlantic salmon seems to tolerate a diet with only SBO as lipid source, without any detrimental effects on growth and immune functions. In a feeding trial with rainbow trout fed purified diets supplemented with only SBO, linseed oil or FO for 9 weeks no differences in serum complement or lysozyme activity were observed (Kiron et al., 2004).

The effect of SBO on disease resistance in challenge experiments is less well studied and the results are somewhat conflicting. In a long-term feeding study using SBO and FOs, Waagbø et al. (1993) showed that Atlantic salmon fed high levels of FOs had better survival when infected with *Aeromonas salmonicida* (the causative agent of furunculosis), despite the fact that head kidney macrophage phagocytosis and bacterial killing was reduced in these fish (IL-1 and serum hemolytic activity was unaffected). In contrast to these results, Gjøen et al. (2004) reported no differences in Atlantic salmon susceptibility to infection by *A. salmonicida*, while Lødemel et al. (2001) reported that Arctic charr fed diets supplemented with SBO and challenged with *A. salmonicida* showed elevated survivability compared to fish fed a FO based diet (SBO = 20% mortality *vs* FO = 48% mortality).

3. Effects of soybean products on gut microbiota

The gut microbiota of fish constitutes a great number of cells and these cells and their metabolites play important roles in host digestive function, gastric development, mucosal tolerance, immunity and disease resistance. In fact, in the absence of gut microbiota the digestive tract fails to differentiate fully, lacks brush border intestinal alkaline phosphatase activity, presents immature patterns of glycan expression, displays reduced epithelial proliferation and a reduction of goblet and enteroendocrine cells (Rawls et al., 2004; Bates et al., 2006). It is of utmost importance for the health of the animal that microbes that improve gut function and the immune apparatus dominate these microbial communities. Therefore, great efforts have been made to find dietary supplements that ensure that benign or beneficial microbes dominate the gut microbiota of fish (Merrifield et al., 2010; Ringø et al., 2010 b; Dimitroglou et al., 2011).

As is the case with terrestrial animals, the gut microbiota of fish is classified as autochthonous or indigenous, when they are able to adhere and colonize the host's gut epithelial surface, or allochthonous, when they are incidental visitors in the GI tract, and are expelled after some time without colonizing (Ringø & Birkbeck, 1999; Ringø et al., 2003; Kim et al., 2007). Compared to the numerous studies describing the dietary effect on fish gut microbiota (Ringø et al., 1995; Austin, 2006; Merrifield et al., 2010) relatively little information is available about the effects of SBM and SBO (Ringø et al., 2002; Hekkinen et al., 2006; Ringø et al., 2006; Bakke-McKellep et al., 2007 b; Ringø et al., 2008; Merrifield et al., 2009; Cai et al., 2011).

3.1 Soybean meal

While much effort has focused on evaluating the extent of SBM induced histological damage the effect on the gut microbiota of fish is not so well documented. This is partly because of our lack of understanding of the importance of the gut microbiota in the past but as our awareness of this complex microbial ecosystem has grown so too has our interest in the factors that might impact or disrupt these communities.

As a result there are now several studies which have sought to assess the impact of dietary SBM on fish gut microbiota. To our knowledge, the first is that of Hekkinen et al. (2006), who utilised both culture-dependent and molecular methods (culture-independent) to observe the effect of dietary SBM (hexane extracted; included at a dietary level of 45%) on the gut microbiota of rainbow trout in comparison to a FM control group. After 8 weeks feeding on the experimental diets the total culturable aerobic, aerotolerant culturable lactic acid and total culturable anaerobic bacterial levels (CFU g^{-1}) were at least one log scale lower in the SBM fed fish than the FM fed fish. Some tentative identifications of bacterial isolates revealed that some bacterial genera/groups may have been specifically affected; compared to the FM group a clear reduction of *Lactobacillus* spp. (FM = 8.57%, SBM = 0%; as a percentage of isolates identified) and *Sphingomonas* spp. (FM = 31.4%, SBM = 3.7%) isolates were observed in the SBM group. Conversely, an increase of *Bacillus* spp. (FM = 0%, SBM = 7.4%) and *Chryseomonas* spp. (FM = 5.7%, SBM = 22.2%) were isolated from the SBM group. However, as this was not a comprehensive study (only 62 isolates were identified during the trial) and identification was based on phenotypic and biochemical characteristics this data should be viewed with caution. Culture-independent analysis, length heterogeneity analysis of PCR amplified 16S rRNA (LH-PCR), from a secondary 18 week feeding trial also suggested that dietary SBM induced qualitative changes in the microbial communities.

Contrary to the findings of Heikkinen et al. (2006), a more recent study by Merrifield et al. (2009), did not reveal any differences in the in total culturable aerobic levels when comparing the autochthonous and allochthonous gut populations of rainbow trout fed a FM control diet with those fed a SBM diet (SBM inclusion at 32%) for 16 weeks. The reason for the differing outcomes is somewhat unclear but it may be related to 1] the different feeding duration (8 weeks vs 16 weeks), 2] the different SBM inclusion level (45% vs 32%), 3] the different SBM characteristics (SBM with hulls vs dehulled HiPro with lower fibre and higher protein content), 4] the different culture conditions (plate count agar, 15°C, 21 days vs trytone soya agar, 20°C, 7 days) and/or 5] other differences in the experimental rearing trial. Despite this however, using partial 16S rRNA sequence analysis, Merrifield and co-authors observed differences in microbial composition comprising the total viable populations (as illustrated in Figure 6). This could be generally characterised as lower levels of *Aeromonas* spp. and *Vibrio* spp., marginally higher levels *Micrococcus* spp. and a substantial increase in the allochthonous levels of a group identified as belonging to the order Actinomycetales (isolates could not be differentiated from *Arthrobacter aurescens*, *Janibacter* spp. and *Streptomyces coelicolor*) observed in the SBM group compared to the FM group. Additionally, a population of *Psychorbacter* spp. (*Psychrobacter* spp. PRwf-1, *P. cryohalolentis* or *P. arcticus*) was present only in the SBM fed group. It was also observed that dietary SBM could affect the levels of indigenous yeast in the gut. Yeast levels, tentatively identified as *Saccharomyces* spp., increased from 8.7% to 39.3% (FM vs SBM; as a proportion of the culturable community), 17.3% to 19.3%, 12.7% to 52.7% and 14.0% to 48.7% in the anterior mucosa, posterior mucosa, anterior digesta and posterior digesta, respectively. It was hypothesised that this might be a direct result of fermentable carbohydrates provided by SBM. Oligosaccharides, such as stachyose and raffinose, typically constitute about 4–5% of SBM by dry weight. Raffinose and stachyose consist of fructose, glucose and galactose and many genera of yeast, including *Saccharomyces*, are able to ferment various sugars, including glucose and galactose; hence, an increase in yeast numbers may be a result of increased available sugars.
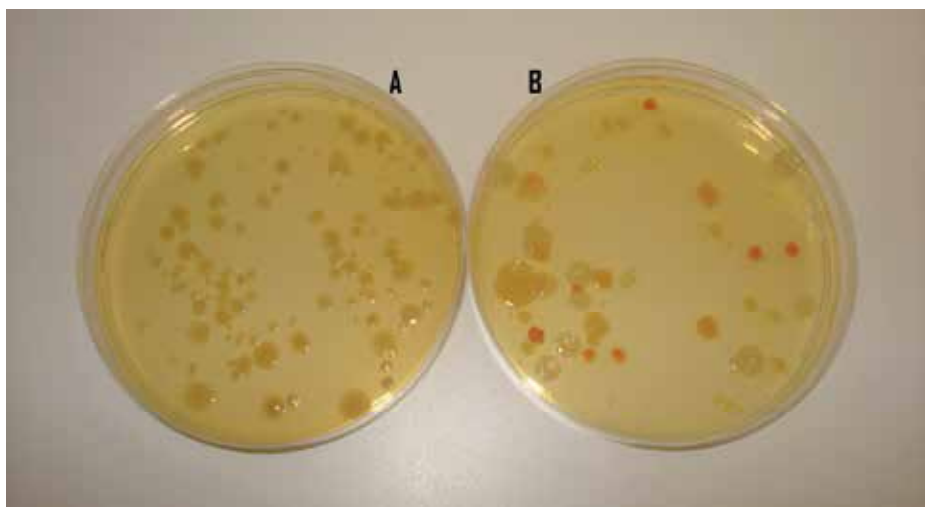
Fig. 6. Tryptic soya agar (TSA) plates showing culturable allochthonous bacteria isolated from the posterior intestine of rainbow trout fed FM (A) and SBM (B) diets.

EM confirmed the presence of complex autochthonous bacterial populations in close association with of the mucosal brushborder of both FM and SBM fed rainbow trout but no distinct colonisation patterns or differences between dietary groups was observed. Due to the reduced density of enterocyte microvilli caused by dietary SBM the exposure of tight junctions to brushborder bacterial populations was increased which may have negative connotations towards defensive barrier function to opportunistic bacterial populations (Figure 4).

These findings are somewhat similar to those of Bakke-McKellep et al. (2007 b) who also reported higher levels of yeast in SBM fed fish. Bakke-McKellep et al. (2007 b) investigated the microbiota of seawater-adapted Atlantic salmon fed diets containing FM as the sole protein source or diets containing 25% dehulled and extracted and toasted SBM. After 3 weeks the culturable aerobic autochthonous mid intestine, autochthonous distal intestine and allochthonous distal intestine bacterial levels were higher in the SBM fed salmon compared to the FM fed salmon. Diet dependent differences in the diversity of bacterial strains identified were also observed. The number of different genera and strains identified were similar in the SBM fed (26) and FM fed (24) fish but the number of some isolated lactic acid bacteria (*Marinilactibacillus psychrotolerans* and *Carnobacterium* (*piscicola*) *maltaromaticum*) was higher in the FM fed salmon. However, *Brevibacterium* and *Enterococcus* spp. were detected in the SBM group but not in the FM group.

In a later experiment post-smolt Atlantic salmon were fed a FM diet or diets containing 14.7% soy fibre (N100), 14.7% extruded soy fibre (E100) or 43.6% SBM (with hull, hexane extracted and toasted) for 4 weeks (Ringø et al., 2008). Culturable allochthonous and autochthonous bacterial levels within the mid and distal intestine were determined and 16S rRNA sequence analysis was used to identify isolates. Contrary to the findings of Bakke-McKellep et al. (2007 b) no significant differences were observed between viable counts from the intestinal samples of the respective FM and soy fed groups. Differences between the bacterial groups were observed which appear to verify some of the findings observed by Bakke-McKellep et al. (2007 b). Specifically, *Carnobacterium* spp. were sensitive to the inclusion of dietary SBM; high levels of *Carnobacterium* spp. were identified as both autochthonous and allochthonous populations with the distal intestine of fish fed the FM diet but were completely absent from both sampling points of the mid and distal intestine of the SBM fed fish. In contrast, *Bacillus* spp. were the greatest genera isolated from the digesta (allochthonous) of the FM fed fish (log 5.8 CFU g^{-1}) but were completely absent from both the mid and distal digesta of the SBM fed fish. Several other bacterial species, present at lower levels, were identified as present in one of the dietary groups but absent in the other.

Some information is also available on the effect of dietary SBM on the culturable intestinal bacterial levels (Refstie et al., 2006; Ringø et al., 2006) and composition (Ringø et al., 2006) of Atlantic cod. These studies compared the allochthonous and autochthonous bacterial populations within the foregut, mid gut and hindgut chamber of cod fed a FM diet with cod fed a diet containing 24.6% SBM (dehulled and solvent extracted) or 21.4% bioprocessed extracted SBM (BPSBM; the bioprocessing reduces the oligosaccharide and phytic acid content, concentrates the protein, and eliminates anti-nutritional factors). Five of the six intestinal bacterial populations investigated were significantly affected by dietary SBM but only three were affected by dietary BPSBM (Refstie et al., 2006). Compared to the FM group, the autochthonous culturable levels were higher in all three intestinal regions whereas the allochthonous levels were significantly lower in the foregut and midgut of the SBM fed group. The autochthonous foregut, allochthonous foregut and allochthonous mid gut levels

were all significantly higher in the BPSBM group than the FM group. The allochthonous distal population remained unaffected by either dietary treatment.

Ringø et al. (2006) used standard biochemical methods and 16S rRNA sequencing analysis (425 isolates) to identify a total of 944 of the isolates derived from these intestinal sampling regions. The authors concluded that the intestinal tract of fish fed FM was dominated by Gram-positive bacterial genera (most notably *Brochothrix* spp., *Carnobacterium* spp. and unknown Gram-positive cocci) whereas Gram-negative bacteria (such as *Chryseobacterium* spp., unknown Gram-negative rods and a number of *Psychrobacter* and *Psychrobacter* - like spp.) were more frequently isolated from the fish fed the SBM and BPSBM diets. The diversity of *Psychrobacter* spp. was also greater in the SBM and BPSBM groups and several other strains (e.g. *Acinetobacter johnsoni*, *Jeotgailbacillus psychrophilus* and *Jeotgalibacillus* - like) were found to be uniquely present in the SBM group as a minor component of the community. Although no clear differences in carnobacterial levels were apparent (there were marginal differences which hinted at a lower frequency of isolation from the BPSBM fed cod) *in vitro* antagonism assays against *A. salmonicida* and *V. (L.) anguillarum* suggest that there might be differences in the carnobacteria antagonistic properties. For example, 57.8% of *Carnobacterium* isolates from the FM group demonstrated antagonistic activity against one or both of the pathogens but only 33.3% of the SBM isolates were able to antagonise one or both of the pathogens and 38.5% of isolates from the BPSBM group inhibited the growth of *A. salmonicida* (none were effective against *V. (L.) anguillarum*). Although this was only a small scale preliminary investigation it may have relevance to previous observations of elevated disease susceptibility of fish fed SBM.

In contrast to the growing literature available on the effect of SBM on the gut microbiota of salmonid fish species relatively little is known about other fish species. This is likely because it has been generally accepted that non-salmonid fish species are less susceptible to enteritis and histological damage of the intestine as a result of dietary SBM inclusion. However, some information is available.

Dimitroglou et al. (2010) assessed the gut microbiota of gilthead seabream fed diets with or without mannan oligosaccharides (MOS) in both FM and SBM (solvent extracted, included at 31.3%) diets. PCR-DGGE analysis revealed clear differences in the allochthonous microbial communities, reflected by cluster analysis from dendrograms (depicting low similarity between treatments) and by higher species richness (increased observable phylotypes; FM = 14.5 ± 0.7 vs SBM = 25.0 ± 0.0), microbial diversity (shannon weaver index; FM = 2.65 ± 0.05 vs SBM = 3.17 ± 0.01) and higher similarity between replicates (SIMPER; FM = 84.79% vs SBM = 92.25%). Unfortunately, no phylotypes were sequenced so identification of sensitive species in the study remains unknown. However, the study revealed that dietary SBM had a greater effect on the gut microbiota than dietary MOS as MOS was shown to affect the microbial communities in the FM fed group but any potential for modulation in the SBM group was masked by the effect of SBM.

In contrast to the clear changes observed in gilthead seabream (Dimitroglou et al., 2010), Cai et al. (2011) observed no significant affect of dietary SBM (30% inclusion) on the gut microbiota of silver crucian carp (*Carassius auratus gibelio* x *Cyprinus carpio*) after 3 weeks feeding. Total culturable aerobic, anaerobic, presumptive *Aeromonas* spp., presumptive *E. coli*, presumptive *Clostridium perfringens* and presumptive bifidobacteria levels remained unaffected by dietary treatment. Further molecular studies and identification of isolates are required to validate these findings.

Despite the growing number of studies beginning to shed light on the effects of dietary SBM on the gut microbiota of fish, present information is largely based on cultured isolates. As is typically the case with microbial communities from the aquatic environment, the culturability of the gut microbiota of fish is inherently low; culturability of the gut microbiota of rainbow trout has typically been reported to be 3-50% (when using the following culture conditions - TSA, 7 days, 15ºC) (Spanggaard et al., 2000; Huber et al., 2004) or 18% (TSA, 10 days, 17ºC) (Navarrete et al., 2010), ca. 1% in coho salmon *Oncorhynchus kisutch* (TSA, 10 days, 17ºC) (Navarrete & Romero, 2006) and <1% in Atlantic salmon (TSA, 10 days, 17ºC) (Navarrete et al., 2009). Therefore it is essential that culture-independent techniques be employed in future studies. As there is limited knowledge about the effect of soybean products on the largely unculturable proportion of gut microbiota, particularly anaerobic microbial communities which have yet to be studied, in the GI tract of various fish species, important questions are raised: is it possible to increase the population level of beneficial gut bacteria by inclusion of dietary soybean products and will these beneficial gut bacteria improve fish welfare? Indeed, it has been suggested that SBM-oligosaccharides may have potential to be utilised as prebiotics to fortify microbial balance and improve host health (Gibson et al., 2004).

Additional investigations need to extend to other important aquatic species as SBM is one of the commonly used plant proteins incorporated into aquafeeds (Gatlin et al., 2007). Furthermore, studies should focus on the autochthonous populations as these populations are in intimate contact with the host epithelium and are of high importance in terms of stimulating host inflammatory responses.

3.2 Soybean oil

It is generally accepted that dietary manipulation modulates the gut microbiota (e.g. Bakke-McKellep et al. 2007b; Merrifield et al., 2009; Dimitroglou et al. 2010) and several studies have observed sensitivity of the gut microbiota of fish to different dietary lipid levels (Lesel et al., 1989; Ringø & Olsen, 1999) and different dietary plant oils (Ringø et al., 2002; Montero et al., 2006). However, to our knowledge only one study has assessed the effect of dietary SBO on the gut microbiome of fish. In their study with Arctic charr, Ringø et al. (2002) evaluated the effect of SBO on the autochthonous gut microbiota. The presence of autochthonous bacteria associated with the microvilli of fish fed the SBO enriched diet is demonstrated in Figure 7.

The bacteriological results of the study showed modulation of the gut microbiota by SBO as well as inter individual fish variations. Specific differences between the groups were the marginal elevation of the total culturable population (by over log 0.5 CFU g^{-1}) in the SBO group and differences of the bacterial components comprising this community. For example, *Acinetobacter* spp. (log 4.35 CFU g^{-1}), *Carnobacterium* spp. (log 4.41 CFU g^{-1}), coryneforms (log 4.59 CFU g^{-1}) and *Kurthia* spp. (log 4.29 CFU g^{-1}) were key components in all 5 fish replicates from the SBO group but were completely absent in all FO fed fish. Conversely, *Aeromonas hydrohpila* (log 3.85 CFU g^{-1}, present in 5 fish) and *Moraxella* spp. (log 3.35 CFU g^{-1}, present in 3 fish) were present in the FO fed fish but were absent in the SBO fed fish. The authors further evaluated the microbial communities after a challenge with *Aeromonas salmonicida* ssp. *salmonicida* (furunculosis). Post challenge the total viable bacterial levels in both dietary groups was reduced, however, the counts were reduced to ca. one log CFU g^{-1} lower in the SBO group than the FO group. Differences in the levels of certain microbial groups persisted after the challenge: *Acinetobacter* spp. and *Carnobacterium* spp. were present in the SBO fed charr but absent in the FO fed charr and *Micrococcus* spp. were present in the FO fed charr but absent in the SBO fed charr.
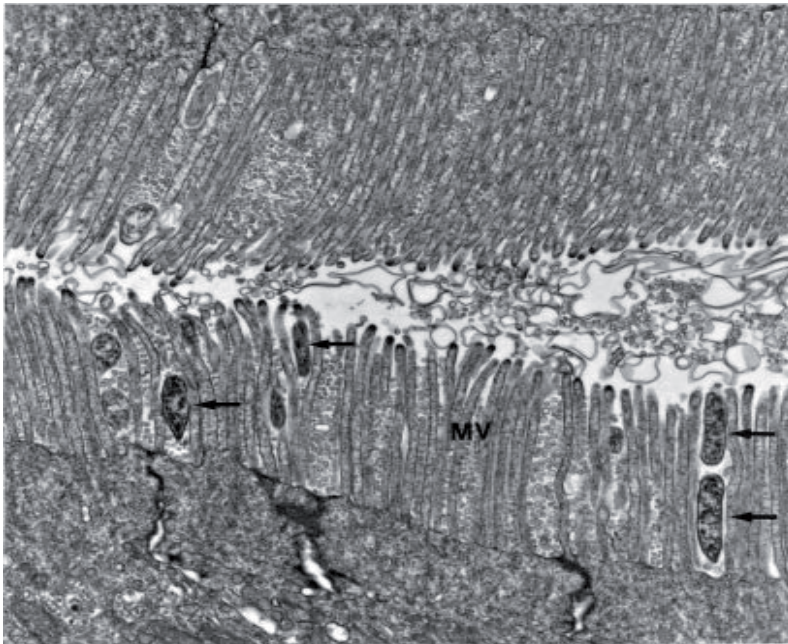
Fig. 7. TEM micrograph of the midgut of Arctic charr fed a diet containing SBO. Note the substantial numbers of bacteria (arrows) associated with the microvilli (MV) of the enterocytes. X 3,750 magnification. After Lødemel et al. (2001).

4. Conclusion

Even though numerous papers regarding the effect of dietary soybean products on the gut histology and gut microbiota of fish have been published in peer reviewed journals further investigations are needed. Using plant-based raw materials may have both advantages and disadvantages. Firstly, imbalanced plant based diets and noxious compounds such as anti-nutrients may impair fish immunity, maturation and functionality of the intestinal mucosa, the first line of defence and in particular damage the GI tract which is a port of entry for many pathogenic agents. Furthermore, endothermic and fish studies have shown atrophy of intestinal mucosa and a reduction in its absorptive and immunological capacity in response to high dietary SBM inclusion. Secondly, using the correct mixture of plant based additives, provides not only the option of limiting harm, but there is also an interesting possibility to enhance GI immunity and disease resistance. Current knowledge is however relatively limited in this regard. However, based on the likelihood that future aquaculture will have to rely on plant-based raw materials, it is strongly recommended that these topics should be given high priority in the years to come. Readers with special interest in the use of plant products in aquaculture are referred to the reviews of Gatlin et al. (2007), Barrows et al. (2008) and Krogdahl et al. (2010).

5. Acknowledgment

6. References

Aslaksen, M.A., Kraugerud, O.F., Penn, M., Svihus, B., Denstadli, V., Jørgensen, H.Y., Hillestad, M., Krogdahl, Å., Storebakken, T. (2007). Screening of nutrient digestibilities and intestinal pathologies in Atlantic salmon, *Salmo salar*, fed diets with legumes, oilseeds, or cereals. *Aquaculture* 272, 541-555.

Austin, B. (2006) The bacterial microflora of fish, revised. *The Scientific World Journal* 6, 931-945.

Baeverfjord, G., Krogdahl, A. (1996) Development and regression of soybean meal induced enteritis in Atlantic salmon, *Salmo salar* L., distal intestine: a comparison with the intestines of fasted fish. *Journal of Fish Diseases* 19, 375-387.

Bakke-McKellep, A.M., Press, C.M., Krogdahl, A., Landsverk, T. (2000) Changes in immune and enzyme histochemical phenotypes of cells of the intestinal mucosa of Atlantic salmon (*Salmo salar* L.) with soybean-meal induced enteritis. *Journal of Fish Diseases* 23, 115-127.

Bakke-McKellep, A.M., Froystad, M.K., Lilleeng, E., Dapra, F., Refstie, S., Krogdahl, A., Landsverk, T. (2007 a) Response to soy: T-cell-like reactivity in the intestine of Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* 30, 13-25.

Bakke-McKellep, AM., Penn, M.H., Salas, P.M., Refstie, S., Sperstad, S., Landsverk, T., Ringø, E., Krogdahl, Å. (2007 b) Effects of dietary soybean meal, inulin and oxytetracycline on gastrointestinal histological characteristics, distal intestine cell proliferation and intestinal microbiota in Atlantic salmon (*Salmo salar* L.). *British Journal of Nutrition* 97, 699-713.

Barrows, F.T., Bellis, Krogdahl. Å., Silverstain, J.F., Herman, E., Sealey, W.M., Rust, M.B., Gatlin III, D.M. (2008) Report of the plant products in aquafeed strategic planning workshop1: An integrated, interdisciplinary research roadmap for increasing utilization of plant feedstuffs in diets for carnivorous fish. *Reviews in Fishery Science* 16, 449-455.

Bates, J.M., Mittge, E., Kuhlman, J., Baden, K.N., Cheesman, S.E., Guillemin, K. (2006) Distinct signals from the microbiota promote different aspects of zebrafish gut differentiation. Developmental Biology 297, 374–386.

Birkbeck, T.H., Ringø, E. (2005) Pathogenesis and the gastrointestinal tract of growing fish. In: Holzapfel, W., Naughton, P. (Eds.), *Microbial Ecology in Growing Animals*. Elsevier, Edinburgh, UK, pp. 208-234.

Bonaldo A., Roem A.J., Pecchini A., Grilli E., Gatta P.P. (2006) Influence of dietary soybean meal levels on growth, feed utilization and gut histology of Egyptian sole (*Solea aegyptiaca*) juveniles. *Aquaculture* 261, 580-586.

Bonaldo, A., Roem, A.J., Fagioli, P., Pecchini, A., Cipollini, I., Gatta, P.P. (2008) Influence of dietary levels of soybean meal on the performance and gut histology of gilthead sea bream (*Sparus aurata* L.) and European sea bass (*Dicentrarchus labrax* L.). *Aquaculture Research* 39, 970–978.

Caballero, M.J., Obach, A., Rosenlund, G., Montero, D., Gisvold, M., Izquierdo, M.S. (2002) Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* 214, 253-271.

Caballero, M.J., Izquierdo, M.S., Kjørsvik, E., Montero, D., Socorro, J., Fernandez, A.J., Rosenlund, G. (2003) Morphological aspects of intestinal cells from gilthead

seabream (Sparus aurata) fed diets containing different lipid sources. *Aquaculture* 225, 325-340.

Caballero, M.J., Gallardo, G., Robaina, L., Montero, D., Fernandez, A., Izquierdo, M. (2006) Vegetable lípid sources affect *in vitro* biosynthesis of triacylglycerols and phospholipids in the intestine of sea bream (*Sparus aurata*). *British Journal of Nutrition* 95, 448-454.

Cai, C-F., Wang, W-J., Ye, Y-T., Krogdahl, Å., Wang, Y-L., Xia, Y-M., Yang, C.-G. (2011) Effect of soybean meal, raffinose and stachyose on the growth, body composition, intestinal morphology and intestinal microflora of juvenile allogynogenetic silver crucian carp (*Carassius auratus* ♀ x *Cyprinus carpio* ♂). *Aquaculture Research* early view, doi: 10.1111/j.1365-2109.2011.02811.x

Davies, S.J., Morris, P.C. (1997) Influence of multiple amino acid supplementation on the performance of rainbow trout, *Oncorhynchus mykiss* (Walbaum), fed soya based diets. *Aquaculture Research* 28, 65-74.

Dimitroglou, A., Merrifield, D. L., Spring, P., Sweetman, J., Moate, R., Davies, S. J., (2010) Effects of dietary mannan oligosaccharides (MOS) and soybean meal on growth performance, feed utilisation, intestinal histology and gut microbiota of gilthead sea bream (*Sparus aurata*). *Aquaculture,* 300, 182-188

Dimitroglou, A., Merrifield, D. L., Carnevali, O., Picchietti, S., Avella, M., Daniels, C. L., Güroy, D., Davies, S. J. (2011) Microbial manipulations to improve fish health and production - a Mediterranean perspective. *Fish & Shellfish Immunology,* 30, 1-16.

FAO (2010) Fisheries Global Information System (FAO-FIGIS) - Web site. Fisheries Global Information System (FIGIS). FI Institutional Websites. In: *FAO Fisheries and Aquaculture Department* [online]. Rome. Updated. http://www.fao.org/fishery/figis/en"

Gatlin III, D.M., Barrows, F., Brown, P., Dabrowski, K., Gaylord, T.G., Hardy, R.W., Herman, E., Hu, G., Krogdahl, Å., Nelson, R., Overturf, K., Rust, M., Sealey, W., Skonberg, D., Souza, E.J., Stone, D., Wilson, R., Wurtele, E. (2007) Expanding the utilization of sustainable plant products in aquafeeds: a review. *Aquaculture Research* 38, 551-579.

Gibson, G.R., Probert, H.M., Loo, J.V., Rastall, R.A., Roberfroid, M.B. (2004) Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutrition Research Reviews* 17, 259-275.

Gjøen, T., Obach, A., Røsjø, C., Helland, B.G., Rosenlund, G., Hvattum, E., Ruyter, B. (2004) Effect of dietary lipids on macrophage function, stress susceptibility and disease resistance in Atlantic salmon (*Salmo salar*). *Fish Physiology and Biochemistry* 30, 149-161.

Grisdale-Helland, B., Helland, S.J., Baeverfjord, G., Berge, G.M. (2002) Full-fat soybean meal in diets for Atlantic halibut: growth, metabolism and intestinal histology. *Aquaculture Nutrition*, 8, 265–270.

Groff, J., LaPatra, S. (2000) Infectious diseases impacting the commercial culture of salmonids. *Journal of Applied Aquaculture* 10, 17-90.

Harikrishnan, R., Balasundaram C. (2005) Modern trends in *Aeromonas hydrophila* disease management with fish. *Reviews in Fisheries Science* 13, 281-320.

Harper, G.M., Dimitroglou, A., Ringø, E., Merrifield, D.L. (2011) Electron microscopy: an important tool to assess the effects of dietary components on the gastrointestinal tract of fish. *Asian Aquaculture Network, The Practical,* 2, (5), 22-24.

Heikkinen, J., Vielma, J., Kemilainen, O., Tiirola, M., Eskelinen, P., Kiuru, T., Navia-Paldanius, D., von Wright, A. (2006) Effects of soybean meal based diet on growth performance, gut histopathology and intestinal microbiota of juvenile rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 261, 259-268.

Huber I., Spanggaard B., Appel K.F., Rossen L., Nielsen T., Gram L. (2004) Phylogenetic analysis and in situ identification of the intestinal microbial community of rainbow trout (*Oncorhynchus mykiss*, Walbaum). *Journal of Applied Microbiology* 96, 117-132.

Huntington, T., Frid, C., Banks, R., Scott, C., Paramor O. (2004) Assessment of the sustainability of industrial fisheries producing fish meal and fish oil. Report to the Royal Society for the Protection of Birds (RSPB). Poseidon Aquatic Resource Management Ltd., Lymington, Hampshire, UK. June 2004. (http://www.rspb.org.uk/Images/fishmeal_tcm5-58613.pdf).

Kim, D., Brunt. J., Austin, B. (2007) Microbial diversity of intestinal contents and mucus in rainbow trout (*Oncorhynchus mykiss*). *Journal of Applied Microbiology* 102, 1654 – 1664.

Kiron, V., Puangkaew, J., Ishizaka, K., Satoh, S., Watanabe, T. (2004) Antioxidant status and nonspecific immune responses in rainbow trout (*Oncorhynchus mykiss*) fed two levels of vitamin E along with three lipid sources. *Aquaculture* 234, 361-379.

Knudsen, D., Urán P., Arnous, A., Koppe, W., Frøkiær H. (2007) Saponin containing subfractions of soybean molasses induce enteritis in the distal intestine of Atlantic salmon. *Journal of Agricultural and Food Chemistry* 55, 2261-7.

Kraugerud, O.F., Penn, M., Storebakken, T., Refstie, S., Krogdahl, Å., Svihus, B. (2007) Nutrient digestibilies, gut function and morphology in Atlantic salmon (*Salmo salar*) fed diets where non-starch polysaccharides from soy replace cellulose. *Aquaculture* 273, 96-107.

Krogdahl, Å., Bakke-McKellep, A.M., Røed, K.H., Baeverfjord, G. (2000) Feeding Atlantic salmon *Salmo salar* L. soybean products: effects on disease resistance (furunculosis), and lysozyme and IgM levels in the intestinal mucosa. *Aquaculture Nutrition* 6, 77–84.

Krogdahl, Å., Bakke-McKellep, A.M., Baeverfjord, G. (2003) Effects of graded levels of standard soybean meal on intestinal structure, mucosal enzyme activities, and pancreatic response in Atlantic salmon (*Salmo salar* L.). *Aquaculture Nutrition* 9, 361-371.

Krogdahl Å., Penn, M., Thorsen, J., Refstie, S., Bakke, A.M. (2010) Important antinutrients in plant feedstuffs for aquaculture: an update on recent findings regarding response in salmonids. *Aquaculture Research* 41, 333-344.

Lesel, R., de la Noüe, J., Choubert, G. (1989) Fecal bacterial flora of rainbow trout under antibiotic treatment: effect of the number of pyloric caeca and the lipid content of food. In: Aquaculture – A Biotechnology in Progress. Vol. 2. pp. 897-903: Eds. De Pauw, N., jaspers, E., Ackerfors, H., Wilkins, N. European Aquaculture Society, Bredane, Belgium.

Lødemel, J.B., Mayhew, T.M., Myklebust, R., Olsen, R.E., Espelid, S., Ringø, E. (2001) Effect of three dietary oils on disease susceptibility in Arctic charr (*Salvelinus alpinus* L.) during cohabitant challenge with *Aeromonas salmonicida* ssp. *salmonicida*. *Aquaculture Research* 32, 935-945.

Merrifield, D. L., Bradley, G., Baker, R. T. M., Dimitroglou, A., Davies, S. J. (2009) Soybean meal alters autochthonous microbial populations, microvilli morphology and compromises intestinal enterocyte integrity of rainbow trout (*Oncorhynchus mykiss* Walbaum). *Journal Fish Diseases* 32, 755-766.

Merrifield, D.L., Dimitroglou, A., Foey, A., Davies, S.J., Baker, R.R., Bøgwald, J., Castex, M., Ringø, E. (2010) The current status and future focus of probiotic and prebiotic applications for salmonids. *Aquaculture* 302, 1-18.

Montero, D., Caballero, M.J., Gijon, D., Real, F., Robaina, L. E. & Izquierdo, M.S. (2006) Influence of dietary blend of vegetable oils on disease resistance and intestinal microbiota of gilthead seabream (*Sarus aurata*). *XII International Symposium on Fish Nutrition and Feeding, May 28th – June 1st, 2006, Biarritz, France*

Montero, D., Mathlouthi, F., Tort, L., Afonso, J.M., Torrecillas, S., Fernandez-Vaquero, A., Negrin, D., Izquierdo, M.S. (2010) Replacement of dietary fish oil by vegetable oils affects humoral immunity and expression of pro-inflammatory cytokines genes in gilthead sea bream *Sparus aurata*. *Fish & Shellfish Immunology* 29, 1073-1081.

Romero, J., Navarrete, P. (2006) 16S rDNA-based analysis of dominant bacterial populations associated with early life stages of coho salmon (*Oncorhynchus kisutch*). *FEMS Microbial Ecology* 51, 422-430.

Navarrete, P., Espejo, R., Romero, J. (2009) Molecular analysis of microbiota along the digestive tract of juvenile Atlantic salmon (*Salmo salar* L.). *FEMS Microbial Ecology* 57, 550-561.

Navarrete, P., Magne, F., Mardones, P., Riveros, M., Opazo, R., Suau, A., Pochart, P., Romero,J. (2010) Molecular analysis of intestinal microbiota of rainbow trout (*Oncorhynchus mykiss*). *FEMS Microbial Ecology* 71, 148-156.

Olli, J.J., Krogdahl, Å. (1994) Nutritive value of four soybean products as protein sources in diets for rainbow trout (*Oncorhynchus mykiss*, Walbaum) reared in fresh water. *Acta Agriculturae Scandinavica Section A Animal Science* 44, 185-192.

Olli, J.J., Krogdahl, Å., van den Ingh, T.S.G.A.M., Brattås, L.E. (1994) Nutritive value of four soybean products in diets for Atlantic salmon (*Salmo salar*, L.). *Acta Agriculturae Scandinavica Section A Animal Science* 44, 50-60.

Olsen, R.E., Ringø, E. (1997) Lipid digestibility in fish: A review. *Recent Research Developments in Lipid Research* 1, 199-265.

Olsen, R.E., Myklebust, R., Kaino, T., Ringø, E. (1999) Lipid digestibility and ultrastructural changes in the enterocytes of Arctic char (*Salvelinus alpinus* L.) fed linseed oil and soybean lecithin. *Fish Physiology and Biochemistry* 21, 35-44.

Olsen, R.E., Myklebust, R., Ringø, E., Mayhew, T.M. (2000) The influences of dietary linseed oil and saturated fatty acids on caecal enterocytes in Arctic char (*Salvelinus alpinus* L.): a quantitative ultrastructural study. *Fish Physiology and Biochemistry* 22, 207-216.

Olsen, R.E., Dragnes, B.T., Myklebust, R., Ringø, E, (2003) Effect of soybean oil and soybean lecithin on intestinal lipid composition and lipid droplet accumulation of rainbow trout, *Oncorhynchus mykiss* Walbaum. *Fish Physiology and Biochemistry* 29, 181-192.

Oxley, A., Torstensen, B.E., Rustan, A.C., Olsen, R,E. (2005) Enzyme activities of intestinal triacylglycerol and phosphatidylcholine biosynthesis in Atlantic salmon (*Salmo salar* L.). *Comparative Biochemistry and Physiology* B 141, 77-87.

Oxley, A., Jutfelt, F., Sundell, K., Olsen, R.E. (2007) Sn-2-monoacylglycerol, not glycerol, is preferentially utilised for triacylglycerol and phosphatidylcholine biosynthesis in

Atlantic salmon (*Salmo salar* L.) intestine. *Comparative Biochemistry and Physiology* B 146, 115-123.

Rawls, J.F., Samuel, B.S., Gordon, J.I. (2004) Gnotobiotic zebrafish reveal evolutionarily conserved responses to the gut microbiota. *Proceedings of the National Acadamey of Science* USA 101, 4596–4601.

Refstie, S., Sahlstrom, S., Brathen, E., Baeverfjord, G., Krogedal, P. (2005) Lactic acid fermentation eliminates indigestible carbohydrates and antinutritional factors in soybean meal for Atlantic salmon (*Salmo salar*). *Aquaculture* 246, 331-345.

Refstie, S., Landsverk, T., Ringø, E., Sundby, A., Shearer, K.D., Kroghdahl, Å. (2006) Digestive responses of 1- and 2-year-old Atlantic cod (*Gadus morhua*) fed standard or bioprocessed soybean meal. *Aquaculture* 261, 269-284.

Ringø, E., Birkbeck, T.H. (1999) Intestinal microflora of fish larvae and fry. *Aquaculture Research* 30, 773-789.

Ringø, E., Olsen, R.E. (1999) The effect of diet on aerobic bacterial flora associated with intestine of Arctic charr (*Salvelinus alpinus* L.). *Journal of Applied Microbiology* 86, 22–28.

Ringø, E., Olsen, R.E. (2008) Gut health in aquatic species, In: *World Nutrition Forum. The Future of Animal Production*, Binder, E.M., Schatzmayr, G (eds), 79-105, Nottingham University Press, ISBN: 978-1-904761-80-8, Nottingham, UK.

Ringø, E., Strøm, E., Tabachek, J-A. (1995) Intestinal microflora of salmonids: a review. *Aquaculture Research* 26, 773-789

Ringø, E., Lødemel, J.B., Myklebust, R., Kaino, T., Mayhew, T.M., Olsen, R.E. (2001) Epithelium associated microbes in the gastrointestinal tract of Arctic charr (*Salvelinus alpinus* L.). An electron microscopical study. *Journal of Applied Microbiology* 90, 294-300.

Ringø, E., Lødemel, J.B., Myklebust, R. Jensen, L., Lund, V., Mayhew, T.M., Olsen, R.E. (2002) Effect of soybean, linseed and marine oils on aerobic gut microbiota of Arctic charr *Salvelinus alpinus* L. before and after challenge with *Aeromonas salmonicida* ssp. *salmonicida*. *Aquaculture Research* 33, 591-606.

Ringø, E., Olsen, R.E., Mayhew, T.M., Myklebust, R. (2003) Electron microscopy of the intestinal microflora of fish. *Aquaculture* 227, 395-415.

Ringø, E., Sperstad, S., Myklebust, R.., Refstie, S., Krogdahl, Å. (2006) Characterisation of the microbiota associated with the intestine of Atlantic cod (*Gadus morhua* L.). The effect of fish meal, soybean meal and a bioprocessed soybean meal. *Aquaculture* 261, 829-841.

Ringø, E., Myklebust, R., Mayhew, T.M., Olsen, R.E. (2007) Bacterial translocation and pathogenesis in the digestive tract of larvae and fry. *Aquaculture* 268, 251-264.

Ringø, E., Sperstad, S., Kraugerud, O.F., Krogdahl, Å (2008) Use of 16S rRNA gene sequencing analysis to characterise culturable intestinal bacteria in Atlantic salmon (*Salmo salar*) fed diets with cellulose or non-starch polysaccharides from soy. *Aquaculture Research* 39, 1087-1100.

Ringø, E., Løvmo, L., Kristiansen, M., Bakken, M., Salinas, I., Myklebust, R., Olsen, R.E., Mayhew, T.M. (2010 a) Lactic acid bacteria *vs*. pathogens in the gastrointestinal tract of fish: a review. *Aquaculture Research* 41, 451-467.

Ringø E., Olsen, R.E., Gifstad, T.Ø., Dalmo, R.A., Amlund, H., Hemre, G.-I., Bakke, A.M. (2010 b) Prebiotics in aquaculture: a review. *Aquaculture Nutrition* 16, 117-136.

Rombout J.H.W.M., Abelli, L., Picchietti, S., Scapigliati, G., Kiron, V. (2011) Teleost intestinal immunology early view. *Fish & Shellfish Immunology* (2010), doi:10.1016/ j.fsi.2010.09.001

Romarheim, O.H., Zhang, C., Penn, M., Liu, Y.-L., Tian, L.-X., Skrede, A., Krogdahl, Å., Storebakken, T. (2007) Growth and intestinal morphology in cobia (*Rachycentron canadum*) fed extruded diets with two types of soybean meal partly replacing fish meal. *Aquaculture Nutrition* 13, 1-7.

Rumsey, G.L., Siwicki, A.K., Anderson, D.P., Bowser, P.R. (1994) Effect of soybean protein on serological response, non-specific defense mechanisms, growth, and protein utilization in rainbow trout. *Veterinary Immunology and Immunopathology* 41, 323-339.

Spanggaard, B., Huber, I., Nielsen, J., Nielsen, T., Appel, K.F., Gram L. (2000) The microflora of rainbow trout intestine: a comparison of traditional and molecular identification. Aquaculture 182, 1-15.

Suyehiro, Y. (1942) A study on the digestive system and feeding habits of fish. *Japanese Journal of Zoology* 10, 1-303.

Tacon, A. (2010) Trends in global Aquaculture and Production. Asian Aquaculture, The Practical. Vol. 2, Issue 1, Jan-March 2011, pp 11-12. *Asian Aquaculture Network, The Practical* 2(1), 11-12.

Urán, P.A., Gonçalves, A., Taverne-Thiele, J.J., Schrama, J.W., Verreth, J.A.J., Rombout, J.H.W.M. (2008 a) Soybean meal-induced enteritis in common carp (*Cyprinus carpio* L.) and the gene expression of inflammatory mediators in intestinal leukocytes. *Fish & Shellfish Immunology* 25, 751-60.

Urán, P.A., Aydin, R., Schrama, J.W., Verreth, J.A.J., Rombout, J.H.W.M. (2008 b) Soy bean meal induced uptake block in the distal enterocytes of Atlantic salmon (*Salmo salar* L.). *Journal of Fish Biology* 73, 2571-9.

van den Ingh, T.S.G.A.M., Krogdahl, Å., Olli, J., Hendricks, H.G.C.J.M., Koninkx, J.F.J.G. (1991) Effects of soybean-containing diets on the proximal and distal intestine in Atlantic salmon (*Salmo salar*): a morphological study. *Aquaculture* 94, 297-305

van den Ingh, T.S.G.A.M., Olli, J.J., Krogdahl, Å. (1996) Alcohol-soluble components in soybeans cause morphological changes in the distal intestine of Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* 19, 47-53.

Waagbø, R., Sandnes, K., Jørgensen, J., Engstad, R., Glette, J., Lie, Ø. (1993) Health aspects of dietary lipid sources and vitamin E in Atlantic salmon (*Salmo salar*). II. Spleen and erythrocyte phospholipid fatty acid composition, nonspecific immunity and disease resistance. *Fiskeri Direktoratets Skrifter Serie Ernæring* 6, 63-80.

Soybean Oil in Horses' Diets

Fernando Queiroz de Almeida and Fernanda Nascimento de Godoi
Veterinary Institute, Universidade Federal Rural do Rio de Janeiro, Seropédica,
Rio de Janeiro,
Brazil

1. Introduction

There is interest in the use of oils and fat in horses' diets aiming to increase energy consumption by animals with high energy requirements, provide essential fatty acids, increase the absorption of fat-soluble vitamins, reduction of the caloric increment, increase energy efficiency and reduce dust from ration avoiding upper respiratory tract diseases (Palmiquist, 1988).

High-performance athletic horses are usually fed diets with high inclusion levels of grain to reach energy requirements, which can cause intestinal acidosis, gastrointestinal mucosal injury and disorders in the microbial ecosystem, causing colic and laminitis. According to Holland et al. (1996), horses fed diets with oil reduced the activity and excitability.

Horses use non-structural carbohydrates such starch, maltose and sucrose as a primary source of energy, being hydrolyzed and absorbed as glucose in the small intestine. However, intestinal amylase activity is limited in the equine species and, because of the low stomach capacity, providing large amounts of starch in the diet compromises digestion in the small intestine, increasing intake of rapidly fermentable carbohydrate in the colon-cecum, which may contribute to metabolic complications such as endotoxemy, colic and laminitis (NRC, 2007). Critical capacity for overload of hydrolysable carbohydrate digestion is approximately 0.4% of horse body weight (Potter et al., 1992).

It is known that the intake of concentrate containing high levels of fats presents some advantages in metabolic point of view and this kind of diet can reduce the risk of gastrointestinal disturbances, because the intake of fat stimulates the flow of digesta in the jejunum and ileum (Meyer et al., 1997). Oils and fats are used in horse diets to replace the hydrolysable and rapidly fermentable carbohydrates that are present in grains and cereals (Frape, 2004).

Oils and fats addition in the diets of high activities sport horses aim to reach the high energy requirements and, according to NRC (2007), the increase on performance of athletic horses fed diets containing oils is due to better the energy / weight relation, with a reduction in dry matter intake and gastrointestinal tract weight; lower metabolic heat production associated to digestion and exercise; greater physical performance resulting from a lower muscle glycogen use, best performance in short distance running energy from anaerobic glycolysis and acidemia reduction during high intensity exercise.

High fat level diets reduce the activity of lipase in adipose tissue and increase their activity in muscle, increase muscle glycogen stores, increasing the energy of the glycolytic pathway,

with fatigue delay during aerobic exercise with large duration, increase or maintain blood glucose concentration during extensive exercise and slow lactic acid accumulation during anaerobic exercise (Frape, 2004), more over improving respiratory and cardiac recovery post-exercise (Mattos et al., 2006), providing athletic horse better conditions for their performance.

2. Effects of oil in horse digestion

The use of oils or fats in horses' diets has been studied for a long time. Bowman et al. (1977) studied the inclusion of corn oil in horses' diets. Oils are easily digestible with the production of 9 Mcal of digestible energy per kg of dry matter, resulting in a readily available source of energy for exercise and digestibility above 90% (Kronfeld et al. 2004; Frape, 2004).

However, there are differences in the absorption of fatty acids and glycerides in the small intestine, emphasizing such factors as the fatty acid chain length- increasing the number of carbons in the fatty acid chain reduces the absorption, the number of instaurations and the presence of a larger number of instaurations in the fatty acid seem to favor its absorption, the distribution order of the fatty acid in the glycerol molecule - a saturated monoglyceride in position 2 has a higher absorption rate, as an example one may cite the free palmitic acid, whose absorption is 12%, and the same fatty acid in the two monopalmitic form would present absorption approximately 55%; animal age - younger animals have lower ability to digest fats than adults, the relationship unsaturated/saturated fatty acids (UFA / SFA) in the diet - experiments show the presence of UFA encourages the absorption of SFA, and the melting point - the digestibility is higher in fats with low melting point, such as vegetable oils, than in saturated animal fat (Meyer, 1995).

Some authors found that high dietary energy density due to the oil addition on horses diets reduces dry matter intake. Marqueze et al. (2001) using diets with 7.8% soybean oil observed the dry matter intake of 1.66% PV, similar to that reported by Kronfeld et al. (2004), from 1.60% BW in several digestibility trials with hiperlypidemic diets. Mattos et al. (2006) observed reduction in dry matter intake in horses fed 3.1 and 6.8% soybean oil diet, 1.74 and 1.6% BW, respectively. Delobel et al. (2008) evaluating diets with 8% linseed oil in adult horses for 90 days, with dry matter intake of 1.2% BW, found that horses remained healthy throughout the period.

Godoi et al. (2009b) evaluating jumping and dressage horses consuming diets without inclusion of soybean oil (control) and with addition of 8.5% and 19.5% soybean oil found that the dietary soybean oil addition increased significantly the diet energy density, with dry matter intake, expressed as a percentage of body weight, 1.80, 1.55 and 1.26% BW, respectively, keeping constant the concentrate:forage ratio in all diets. In these horses the digesta kinetics in the gastrointestinal tract was not affected (P> 0.05), with average values for mean retention time (MRT), rate of passage (RP) and transit time (TT) of the digesta liquid phase of 35.7 hours, 2.8%/ hour and 7.6 hours, respectively (Table 1).

The inclusion of 8.5 and 19.5% of soybean oil in the diets of horses did not influence the daily fecal production based on natural matter, the water content and feces characteristics, with average values of 2.18% of BW and 71.4% moisture (Godoi et al. 2009a). Results similar to those observed in healthy horses and with varied diet, with daily fecal production from 1 to 3% of BW on natural matter and 75% moisture (Meyer, 1995). Soybean oil inclusion until 19.5% does not alter the feces characteristics (Godoi et al. 2009a).

Item	Soybean oil inclusion (%)			
	0%	8.5%	19.5%	CV (%)
Dry matter intake (Kg DM/day)	9.0[a]	7.1[b]	6.2[c]	7.4
Dry matter intake (% BW)	1.88[a]	1.55[b]	1.26[c]	9.8
Fecal production (Kg DM/day)	3.4[a]	2.7[b]	2.3[b]	17.2
Fecal production - natural matter (kg/day)	13.3[a]	9.3[a]	8.6[a]	19.6
Fecal production - dry matter (% BW)	0.71[a]	0.58[ab]	0.47[b]	19.6
Moisture (%)	72.5[a]	69.9[a]	71.7[a]	3.1
MRT (hours)	34.3[a]	40.4[a]	32.3[a]	11.8
RP (%/hours)	2.9[a]	2.5[a]	3.1[a]	12.3
TT (hours)	7.3[a]	10.7[a]	4.7[a]	89.1
	Feces characteristics			
Consistency	Standard*	Standard	Standard	
Color	Standard	Standard	Standard	
Strange particle	Absent	Absent	Absent	
Hay	Present	Present	Present	
Grains	Present	Present	Present	

*One horse produced softer feces than others.
Means in line followed by different letter differ by SNK test (P<0.05)

Table 1. Dry matter intake, fecal production, mean retention time (MRT), rate of passage (RP) and transit time (TT) of liquid phase of digesta and feces characteristics of horses fed diets with soybean oil

As for the dry matter digestion in the digestive tract of horses fed diets with oil should be considered that the feed management or the meals supply frequency influences the dry matter digestibility, especially due to the division of oil consumption, because it avoid lipids overloads in the small intestine and transport to the cecum-colon. In studies conducted by Kane et al. (1979), with the addition of up to 10% corn oil, Hughes et al. (1995), which added 10% of animal fat in the concentrate, and Bush et al. (2001), which included up to 15% of corn oil in the horses diets, fractionated 2x/day, no significant difference in dry matter digestibility was found. However, authors observed that soybean oil inclusion reduced the dry matter digestibility significantly when soybean oil supplied 37 to 63% of net energy of the ration (Jansen et al., 2000). Jansen et al. (2007) also found a decrease in dry matter digestibility of 82.4% in diets without the inclusion of soybean oil to 73.2% including 15% of soybean oil in the ponei's diet. However, Delobel et al. (2008), evaluating diets with 8% linseed oil inclusion in the concentrate, found a significant increase in dry matter digestibility, with values of 64.1 to 66.5%, with diet fed two times a day. Godoi et al. (2009b) fed horses with diet with 19.5% of soybean oil, diets were fractionated into five different times, roughage were offered separately of concentrate for at least three hours apart and found no effects on digestibility of dry matter (P> 0.05), whose average value was 62.6% (Table 2). This suggests management adopted to avoid the adherence of soybean oil to the hay and that this will lead to the large intestine, thus preventing digestion. These contradictory results may be related to the oil amount used and the feeding management applied in each experiment.

Another aspect to be considered is the concentrate:forage ratio from fat diets. This relationship differs among authors, ranging from diets exclusively with concentrate (Kane et al., 1979) until the ratio of 30:70 (Jansen et al., 2002). Suggesting that feed management, as well as concentrate:forage ratio, will not influence, directly, the dry matter digestibility coefficient, being necessary to consider other factors such as the amount and oil type used in each diet and horses physical activity.

Protein digestibility varies according to protein source, the ingredients and the concentrate: roughage ratio (NRC, 2007). The soybean meal protein has high digestibility, averaging 92.2% (NRC, 2007). Hughes et al. (1995) and Julen et al. (1995), evaluating dietary inclusion of animal fat and using soybean meal to balance diets, observed a significant increase in the digestibility of crude protein.

According Jansen et al. (2000), Bush et al. (2001), Kronfeld et al. (2004) and Jansen et al. (2007), the inclusion of oils or fats in diets for horses does not affect the digestibility coefficient of crude protein. However, Jansen et al. (2002) evaluating diets for adult horses, varying only the energy source, glucose, starch or soybean oil, found a decrease in crude protein digestibility. In studies by Godoi et al. (2009b), the apparent digestibility of crude protein increased in diets 8.5 and 19.5% soybean oil inclusion, in 9.8 and 12.8 percentage points compared to the control diet, respectively (Table 2). This can be explained by the inclusion of soybean meal as protein source in the balance of fat diets, probably because the protein of soybean meal have a higher digestibility than the protein source used in the commercial concentrate.

In relation to digestibility of dietary energy Kane et al. (1979) feeding horses diets with corn oil inclusion equivalent to 15 and 30% of digestible energy diet did not observe differences in energy digestibility, averaging 73.3%. Even as Bush et al. (2001) that using corn oil found no significant difference in energy digestibility. Jansen et al. (2000) found significant reduction of 7.2 percentage points in energy digestibility in diets containing soybean oil. Godoi et al. (2009b) observed that the energy digestibility was not influenced by the inclusion of soybean oil in the diet, with a small increase of 4.1 percentage points in the diet with 19.5% soybean oil inclusion, increasing energy availability for horses (Table 2).

The effects of oil inclusion on fiber digestion in the equine digestive tract are contradictory. The absence of marked effects on fiber constituent digestibility in fat diets was observed by Kane et al. (1979) that, using corn oil at levels up to 30% of digestible energy of diet observed ADF digestibility of 24.1%. Bush et al. (2001), when added up to 15% of corn oil in the concentrate, observed average digestibility of 23% NDF.

However, several authors (Hughes et al. 1995; Julen et al. 1995; Rammerstorfer et al., 1998) observed a significant increase in NDF digestibility, 7.4, 8.9 and 8.7 percentage points respectively, on a diet with 10% of animal fat inclusion in concentrate in relation to control diet for 28 days. Likewise, Delobel et al. (2008) observed a significant increase in NDF digestibility by 2.3 percentage points during the experimental period of 90 days, with a 50% concentrate:roughage ratio. This author justifies that when carbohydrate is replaced by oil or fat, there is a reduction on deleterious effects of starch fermentation on fiber digestion in the cecum-colon, which could explain the increase in NDF digestibility.

Nevertheless, there are reports of reduction in apparent digestibility of the fiber constituents in horses fed large amounts of oil in diets. Jansen et al. (2000), evaluating diets with and without addition of soybean oil equivalent to 37% of net energy in concentrate, with 70:30 and 60:40 concentrate:roughage ratio, respectively, observed a significant reduction in

digestibility of NDF, ADF and cellulose, with average values of 60.8, 50.5, 57.0 and 54.6%, 42.2, 50.2%, respectively.

Jansen et al. (2002), evaluating diets for horses with three different energy sources: starch, glucose and soybean oil, and approximately 70:30, 50:50 and 30:70 concentrate:forage ratio, respectively, observed that the digestibility of the fiber constituents in diets with starch or glucose showed no significant differences, however, the diet with soybean oil provided a significant reduction in digestibility of NDF, ADF and cellulose, 9.4, 13.3 and 16.9 percentage points when compared to other diets. Jansen et al. (2007), using the kinetics in vitro fermentation technique observed that cecum, colon and feces inoculum of horses fed diets with soybean oil inclusion had lower gas production with incubated cellulose and justified by cellulolytic microflora inhibition, because there was a reduction of 4.1 x 106 cfu / mL to 3.6 x 106 cfu / mL in the bacteria amount in diet with soybean oil. The amount of hay used by Jansen et al. (2000, 2002, 2007) was not similar among diets with and without soybean oil, which produced alterations in concentrate:forage ratio. In addition, the silage was fed with the concentrate and oil. This management may promoted increase of rate of passage in the small intestine carrying fats to large intestine, which could reduce microbial fermentation in the cecum-colon and fiber digestibility of fat diets.

Godoi et al. (2009b) evaluated the digestibility of fiber fractions, and observed that there was significant reduction in cellulose apparent digestibility in horses fed a diet with 19.5% soybean oil inclusion. This reduction was 18.3 and 11.1 percentage points, while the diet with large amounts of soybean oil was compared with the control diet and 8.5% inclusion of soybean oil, respectively (Table 2). Godoi et al. (2009b) maintained the relation of concentrate:forage similar in all diets and coast-cross hay was provided separately, thus reducing the possibility of carrying fats to the cecum-colon.The lack of significant effect of oil inclusion in the hemicelluloses digestibility may be due to the fact that non-ruminant herbivores digest relatively more hemicelluloses than cellulose (Van Soest, 1994).

Morgado et al. (2009) evaluated the apparent digestibility coefficient of total carbohydrates, non-fibrous carbohydrates and their hydrolysable and rapidly fermentable fractions in horses fed diet with higher levels of soybean oil inclusion observed that the higher level of soybean oil inclusion, 19.5% resulted in significant reduction in apparent digestibility of non-fibrous carbohydrates, at 26.6 percentage points. The apparent digestibility coefficient of rapidly fermentable carbohydrate showed the largest significant reduction of 94.9% in the diet without the soybean oil addition, to 53.2% for in the diet with 19.5% soybean oil inclusion. Hydrolysable carbohydrates are composed of fructans, pectins, β-glucans and galactans that are not digested by equine digestive enzymes, but are fermented by microorganisms in the large intestine. The lowest digestibility value of rapidly fermentable carbohydrates associated with a greater level of soybean oil inclusion may be due to the microfloral change, reducing these carbohydrates digestibility. However, there were no significant differences in neutral detergent fiber (NDF) and acid detergent fiber (ADF) digestibility, which becomes important, the fractionation of non-fibrous carbohydrates (Table 2).

The efficiency of utilization of dietary fiber in horses is related to diet composition, especially by structural carbohydrates and non-structural fractions, the rate of fermentation and rate of passage through the digestive system that is influenced by intake (Drougoul et al. 2000).Changes in forage consumption can modify the digesta rate of passage, exposing the microflora in the large intestine to a change in the amount of fermentable substrates and thus may affect the apparent digestibility of fiber constituents (Hallebeek & Beynen, 2002). The influence of associative effects on nutrients digestibility is related to ingredients quality

and quantity in diets (Palmgren Karlsson et al., 2000). The carbohydrates availability varies between different cereals types and, likewise, the fibrous components percentage varies among different forage and concentrate feeds, which may modify the fermentation in the large intestine. According to NRC (2007) the concentrate:forage ratio, ingredients, feed supply at the same time or separately, among other factors, can alter the intake and digestibility of nutrients.

Coefficient of digestibility (%)	Soybean oil inclusion (%)			CV
	0%	8.5%	19.5%	(%)
Dry matter[1]	62.3[a]	62.6[a]	62.8[a]	8.0
Organic matter[1]	65.8[a]	64.1[a]	61.6[a]	7.8
Crude protein[1]	70.5[b]	80.3[a]	83.3[a]	6.3
Gross energy[1]	63.0[a]	66.2[a]	67.1[a]	8.1
Ether extract[1]	71.8[b]	89.7[a]	91.2[a]	7.7
Neutral detergent fiber[1]	53.9[a]	48.8[a]	41.2[a]	13.3
Acid detergent fiber[1]	41.3[a]	35.9[a]	30.7[a]	19.5
Cellulose[1]	50.1[a]	42.9[a]	31.8[b]	16.8
Hemicelluloses[1]	63.2[a]	59.2[a]	51.2[a]	10.6
Non-fibrous carbohydrates[2]	96.5[a]	87.3[a]	69.9[b]	9.5
Hydrolysable carbohydrates[2]	97.7[a]	97.3[a]	91.9[b]	2.7
Rapidly fermentable carbohydrates[2]	94.9[a]	76.3[a]	53.2[b]	17.8
Total carbohydrates[2]	65.0[a]	58.4[a]	48.8[b]	10.0

Means in line followed by similar letter do not differ by SNK test (P>0.05)
[1] Godoi et al. (2009), [2]Morgado et al. (2009).

Table 2. Apparent digestibility coefficient of nutrients in horses fed diet with higher levels of soybean oil inclusion

Sales & Homolka (2011) in a meta-analysis of 22 papers about use of oil in diets for horses observed no significant effects of fat supplementation in protein and NDF digestibility, that can be explained by the anatomy of the gastrointestinal tract of horses with large bowel fermentation opposed to the ruminants.

Contradictory results reported in the literature are probably related to varying levels of fat, differences in relation to the dietary ingredients, especially in relation to the NDF and ADF. In relation to the contradictions in the results of the fiber constituents digestibility observed among various authors, these may be related to inadequate oils and fats adaptation in horse, and also a short period of replacement of rapidly hydrolysable carbohydrates by oils and fat (NRC, 2007).

Horses fed diets with soybean oil increased number of erythrocytes and reduced mean corpuscular volume (Godoi et al., 2009a). Hemoglobin level of evaluated horses differed only among animals fed control diet and 8.5% soybean oil inclusion, with lower value in the control diet, 9.5 g / dL. Including 19.5% of soybean oil in the diet increased serum levels of triglycerides in the horse.

Soybean oil was found to be palatable and its use is common in compound diets or added to diets with grains in equine nutrition (Meyer & Coenen, 2002). The absence of negative changes in hematological, biochemical and feces indicates that the inclusion of soybean oil in the diets of horses can be used to reduce dry matter consumption, leading to reduced

consumption of rapidly fermentable carbohydrates and lighter digestive tract during exercise, what can improve athletic performance in horses (Godoi et al. 2009a). Zeyner et al. (2002) evaluating fed horses with inclusion of 11.5% of soybean oil in the diet during 390 days, also observed no adverse effects.

In a study with three adult horses fistulated at right dorsal colon with 300 kg body weight, soybean oil was included in different ways. Horses were distributed in randomized complete block design with five treatments and three blocks formed by animals, with each block consisting of an experimental unit. Experimental diets were composed of *coastcross* hay (*Cynodon dactylon*), commercial concentrate, soybean meal and soybean oil in a forage: concentrate ratio of 60:40 on a dry matter basis, defined as: diets without soybean oil and diet with soybean oil on the level of 10% of total diet.

Soybean oil supplied with concentrate in four different ways: 1) one time a day at 07 a.m., 2) two times a day (two equal fractions) at 7 a.m and 17 p.m., 3) three times a day (three equal fractions) at 7 a.m., 13 p.m. and 17 p.m , or 4)four times a day (four equal fractions) at 7 a.m., 13 p.m., 17 p.m. and 19 p.m. Roughage supply was always performed in two equal fractions, at 11 a.m and 21 p.m. Diets were formulated according to nutritional requirements (Table 3) for adult horses at maintenance (NRC, 2007), with daily intake of approximately 2.0% BW, on dry matter basis.

Item (%)	Nutritional composition				Diet without soybean oil	Diet with soybean oil
	Concentrate	Soybean oil[1]	Coastcross hay	Soybean meal		
Dry matter	89.9	99.6	87.9	88.0	88.6	89.6
Crude protein	12.5	-	7.4	46.9	9.2	8.2
Gross energy (Mcal/Kg DM)	3.5	9.4	3.8	4.2	3.8	4.3
Ether extract	3.4	100.0	1.4	2.7	2.1	12.2
NDF[2]	32.7	-	63.5	10.7	52.3	48.3
ADF[3]	16.7	-	30.3	9.6	25.4	23.4
HEM[4]	16.0	-	33.2	1.1	26.9	24.9
Celulose	8.8	-	23.0	6.9	21.9	16.7
NFC[5]	44.3	-	19.3	22.1	28.4	24.1
CHO-H[6]	6.3	-	1.7	1.8	3.3	2.8
CHO-RF[7]	38.0	-	17.6	20.3	25.1	21.3
CHO-T[8]	77.0	-	82.8	32.8	80.7	72.4
Control diet	40.0	-	60.0	-	-	-
Diet with soybean oil	26.9	10.4	62.1	0.6	-	-

[1]Rostagno (2005)
[2]Neutral detergent fiber, [3]Acid detergent fiber, [4]HEM – hemicelluloses, [5]Non-fibrous carbohydrate;
[6]Hydrolizable carbohydrates; [7]Rapidly fermentable carbohydrate; [8]Total carbohydrate

Table 3. Nutritional composition and percentage of ingredients in the diet, on a dry matter basis

Animals were previously adapted to soybean oil with inclusion gradually in the diet, during 25 days. First, the trial was conducted in four periods with 17 days, 10 days to diet adaptation, 4 days to feces collection, 1 day to blood collection and 1 day to digesta

collection at right dorsal colon. Next, animals were re-adapted to the diet with decreasing levels of soybean oil during 25 days and then proceeded another trial with a control diet, totaling 135 days. Feces were collected from each animal immediately after defecation, directly from the floor of stalls without bedding, during 24 hours over four collection days.

Blood collections were performed on the 16th day of each experimental period, with the first sample collected before the morning meal, at 7:00 pm and at 30, 60, 120, 180, 240 and 300 minutes of the postprandial period.

Digesta collection from the colon was performed four hours after first meal of the day, obtaining a aliquot of about 1.5 kg of digesta per animal. A 100g digesta aliquot was immediately used for pH measurement and determination of buffer capacity (Zeyner et al., 2004). Another digesta aliquot from the colon was directed to the analyzing process. Hydrolysable carbohydrates were estimated directly, non-fibrous carbohydrates, rapidly fermentable carbohydrates and total carbohydrates were estimated (Hoffman et al., 2001).

The inclusion of 10% soybean oil, in a single or fractionated form did not affect (P> 0.05) dry matter intake (equivalent to 1.73% BW) with fibrous fractions intake of 2.7, 1.3, 1.5 and 1.0 kg to NDF, ADF, hemicelluloses and cellulose, respectively. Average daily intake of hydrolysable carbohydrates observed in this study was 100 g / day, similar to the results observed by Hoffman et al. (2001), in diets with 11% corn oil and intake from 118 to 186 g hydrolysable carbohydrates / day. Soybean oil did not affect the nutrient intake by horses probably due to the large period of adaptation to the diet with soybean oil, and Kronfeld et al. (2004) suggested 4 to 14 days of adaptation, depending on the amount of oil in order to avoid negative effects.

Soybean oil inclusion, either on a single or fractionated did not affect (P>0.05) dry matter digestibility, with a mean value of 69.4%, crude protein with average coefficient of 71.6%, gross energy with an average of 73.2%, and, there was effect (P<0.05) of soybean oil inclusion in the ether extract digestibility coefficient, as well as in intake of 0.1 kg in control diet and 0.7 kg in fat diet, but the fractionation of soybean oil did not influence the digestibility of fat, averaging 94.7% (Table 4). Kronfeld et al. (2004) evaluating different oils and fats sources in diets for horses observed an increase in ether extract digestibility from 55 to 81% when compared to the basal diet.

Digestibility coefficient of NDF, ADF and cellulose were not affected by the inclusion of soybean oil (P>0.05). However, significant increase was observed in hemicelluloses digestibility when horses fed hiperlypidemic diet fractionated into one, two and three times, averaging 63.8, 66.8 and 67.0% respectively. Fractionation of soybean oil did not affect (P>0.05) non-fibrous, hydrolyzable and rapidly fermentable carbohydrates digestibility, with average values of 99.1, 99.0 and 99.1%, respectively. Diet without soybean oil and diet with soybean oil in four fractions, the carbohydrates digestibility was better (P<0.05) than diets with a fractionation of up to three times. In this study, soybean oil inclusion was done four times, to avoid high intake of hydrolysable carbohydrates that can change the microflora of large intestine of horses and consequently decreased digestibility.

According to Hoffman et al. (2001), providing large amounts of hydrolysable carbohydrates in the diets of horses undertakes its digestion in the small intestine, increasing intake of rapidly fermentable carbohydrates in the cecum and colon, and the critical capacity to overload of hydrolysable carbohydrate digestion is approximately 0.4% BW of horses.

Plasma concentrations of glucose ranged during postprandially period in the control diet (P<0.05), showing higher concentrations 30, 60, 120 and 180 minutes after intake. In diets with oil, even single or fractionated forms, glucose levels did not change during this period remained within the reference values (Dukes, 1996), 80 to 120 mg / dL (Table 5). It was

observed lower plasma glucose concentration 300 minutes after intake in horses fed control diet, compared to horses fed diets with soybean oil (P<0.05).

Item (%)	Soybean oil in diets						
	Control diet	One time	Two times	Three times	Four times	Mean (%)	CV (%)
Dry matter	70.3[a]	66.6[a]	68.9a	69.5a	71.8a	69.4	4.4
Crude protein	73.7[a]	70.2[a]	70.7 a	71.2 a	72.2 a	71.6	3.9
Gross energy	69.7[a]	72.1[a]	73.9 a	74.9 a	75.2 a	73.2	3.9
Ether extract	59.3[a]	94.7[b]	95.2 b	95.3 b	93.4 b	---	3.6
NDF[1]	67.8[a]	58.4[a]	61.3a	62.6a	66.2a	63.3	5.5
ADF	62.8[a]	52.7a	55.5a	57.7a	60.4a	57.8	8.8
Hemicellulloses	71.9[a]	63.8b	66.8b	67.0b	70.9a	---	3.4
Celulose	70.8[a]	61.1a	66.2a	67.1a	69.9a	67.0	5.7
NFC [2]	99.2[a]	99.0a	98.9a	99.2a	99.0a	99.1	1.1
CHO-H[3]	98.9[a]	99.0a	99.1a	99.2a	98.7a	99.0	1.3
CHO-RF[4]	99.2[a]	99.0a	99.2a	99.0a	98.9a	99.1	1.3
CHO-T[5]	77.6[a]	70.8b	72.9b	73.6b	77.1a	---	3.3

Means in the line followed by same letter do not differ with Scott Knott test (P>0.05)
[1]Carbohydrates slowly fermentable (CHO-SF) represented by NDF
[2]Non-fibrous carbohydrates; [3]Hydrolizable carbohydrates; [4]Rapidly fermentable carbohydrates; [5]Total carbohydrates

Table 4. Coefficient of digestibility of nutrients and fiber fractions in horses fed fat diet.

The increase in plasma glucose between 30 and 180 minutes after control diet intake may be related to higher hydrolysable carbohydrate concentration in this diet, leading to increased glucose absorption in the small intestine during the first 180 minutes of the postprandial period. Lower plasma glucose concentration 300 minutes after intake of control diet, compared to diets supplemented with soybean oil, even single or fractionated forms, must also be related to higher concentration of starch in this diet. So, the pronounced increase in plasma glucose after ingestion of control diet stimulated greater insulin release, increasing efficiency in the blood glucose uptake by the tissues, resulting in lower plasma glucose concentration 300 minutes postprandial. Taylor et al. (1995) and Orme et al. (1997); Marqueze et al. (2001), Mattos et al. (2006) and Godoi et al. (2009a) also observed no influence of the intake of hiperlipidemic diets on plasma levels of glucose in the horses.
No differences (P>0.05) was observed on triglyceride concentration in horses fed control diet or fed diet with soybean oil fractionated one, two and three times a day. Fractionated soybean oil inclusion into four times reduced plasma triglyceride levels 60 and 120 minutes postprandial (P<0.05).
In several studies contrasting results were observed in triglyceride levels in horses supplemented with vegetable oils. Harking et al. (1992) evaluating diet inclusion of corn oil equivalent to 10% of digestible energy, fed twice a day and Hallebeek & Beynen (2002) evaluating diet with soybean oil inclusion of 15%, also fed twice a day, observed no changes in plasma triglycerides levels. However, Orme et al. (1997) evaluating the soybean oil in diets fed twice a day, Geelen et al. (2001) with inclusion of soybean oil 15% diet, fed twice a day and Sloet van Oldruitenborgh-Oostebaan et al. (2002) evaluating the addition of 11.8% soybean oil, observed reduction in triglyceride levels. But Godoi et al. (2009a) observed an

increase in serum triglycerides of horses fed diet with 19.5% soybean oil inclusion, twice a day compared to diets without and with 8.5% for soybean oil.

Soybean oil in diet	Postprandial (minutes)							Mean	CV (%)
	0	30	60	120	180	240	300		
	Glucose (mg/dL)								
Control	84.0[b]	96,3[a]	105.7[a]	105.0[a]	95.0[a]	85.7[b]	83.7[bB]	-	
One time	99.3	102,1	101.8	94.2	97.5	98.5	94.8[A]	98.3	
Two times	98.7	100,4	102.1	105.1	106.5	106.9	113.9[A]	104.8	1.6
Three times	94.4	105,6	106.5	97.8	102.5	106.1	96.3[A]	101.3	
Four times	98.0	101,7	99.0	88.3	102.0	97.3	93.7[A]	97.1	
Mean	94.9	101,2	103.0	98.1	100.7	98.9	-		
CV (%)	4.4								
	Triglycerides (mg/dL)								
Control	32.0	32.0	33.3	29.7	24.3	22.3	30.0	29.1	
One time	22.1	21.6	21.6	20.3	22.7	22.5	24.8	22.2	
Two times	30.2	30.0	30.0	27.2	25.9	22.3	23.1	26.9	6.0
Three times	26.5	21.7	21.7	24.2	30.9	23.4	22.7	24.4	
Four times	25.0[a]	29.3[a]	19.3[b]	22.0[b]	24.3[a]	23.3[a]	35.0[a]	-	
Mean	272	26.9	25.2	24.7	25.6	22.8	27.1		
CV (%)	19.3								

Means in line followed by same lowercase letters do not differ by the Scott Knott test (P <0.05). Means in columns followed by same uppercase letters do not differ by the Scott Knott test (P <0.05)

Table 5. Mean values of plasma glucose and triglycerides in horses fed fat diets.

According to Orme et al. (1997) the reduction in the triglycerides concentrations in fat diets are associated with increased postprandial lipoprotein lipase activity and postprandial plasma cholesterol. These authors observed a 50% increase in lipoprotein lipase activity in horses, after intake of a diet with inclusion of soybean oil. In the present study, the largest interval between meals with soybean oil, observed in the diet where the oil inclusion was fractionated into four times, may have promoted the lipoprotein lipase activity increase, leading to reduction in plasma triglycerides concentration at 60 and 120 minutes postprandial.

Horses fed diets with soybean oil did not increase (P>0.05) plasma cholesterol, HDL and LDL, compared to control diet. Serum cholesterol levels remained within reference values of 75 to 150 mg / dL (Kaneko et al., 1997) (Figure 1).

Absence of cholesterol concentration changes may be related to the maintenance state of horses used in this study. Orme et al. (1997) evaluated horses that were submitted to aerobic training for 10 weeks and reported cholesterol concentrations increase in horses fed diet with soybean oil. According to these authors, cholesterol concentrations increase in horses may arise as greater feed intake result or as increased cholesterol biosynthesis. Cholesterol dietary content commonly given to horses probably is minimal, since the ingredients were grains, forages, by-products of grains and vegetable oils have low cholesterol contents. Thus, the increase in cholesterol observed by Orme et al. (1997) should be result of increased endogenous synthesis of cholesterol, due to increased acetyl CoA production via triglycerides β-oxidation. Thus, the greatest energy demand needed for muscle activity

during exercise, increased the triglycerides β-oxidation for energy generation. Geelen et al. (2001) and Hallebeek & Beynen (2002) also observed any changes in cholesterol concentrations in horses fed fat diets.
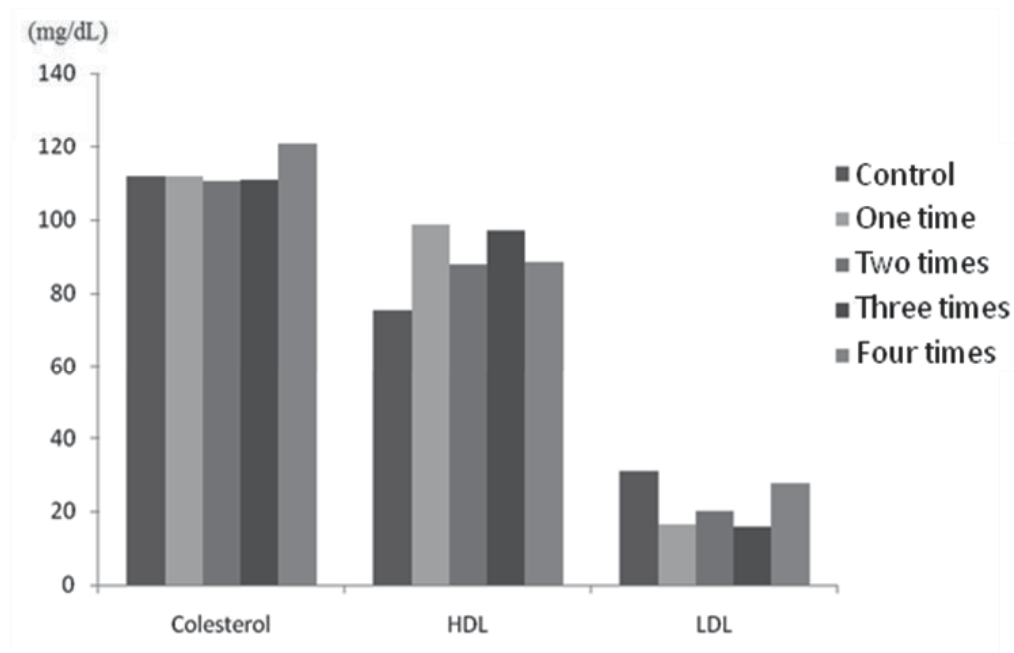
Fig. 1. Mean values of plasma cholesterol, HDL and LDL cholesterol, mg / dL, horses fed diets with soybean oil.

Item (%)	Soybean oil in diets					Mean (%)	CV (%)
	Control diet	One time	Two times	Three times	Four times		
Water	91.9	91.5	89.9	90.5	94.1	91.6	3.3
Dry matter	6.9	8.1	11.4	10.8	6.9	8.8	26.8
NDF[1]	66.8	62.9	67.4	68.6	64.3	66.0	8.6
ADF	35.6	36.7	40.0	40.1	35.9	37.6	7.0
Hemicelulloses	31.2	26.2	27.4	28.5	28.3	28.3	13.4
Celulose	21.2	22.3	24.3	23.8	21.1	22.5	11.1
NFC[2]	14.5	15.7	11.0	9.8	14.3	13.1	30.6
CHO-H[3]	2.0	2.2	2.3	2.6	2.4	2.3	13.7
CHO-RF[4]	12.5	13.4	8.7	7.2	11.9	10.7	37.6
CHO-T[5]	81.3	78.6	78.4	78.3	78.6	79.0	3.5

[1]Carbohydrates slowly fermentable (CHO-SF) represented by NDF; [2]Non-fibrous carbohydrates; [3]Hydrolizable carbohydrates; [4]Rapidly fermentable carbohydrates; [5]Total carbohydrates

Table 6. Mean values of water content and chemical digesta composition from the dorsal colon of horses fed diets with soybean oil.

There there wasn't any effect of soybean oil inclusion in horses' colon pH (P>0.05), averaging 6.46. This is below the value cited by Dukes (1996) of 7.09, but was similar to that reported by Santos et al. (2009), evaluating digesta pH in segments of the gastrointestinal tract of horses fed diets with roughage: concentrate similar to the present study found the average pH in the right dorsal colon of 6.41.

Water in the right dorsal colon contents did not differ (P>0.05) in horses fed control diet and diet with soybean oil, averaging of 91.6% (Table 6). Lopes et al. (2004) observed lower values of water in the right dorsal colon contents, with a value of 89.6%, when horses were fed concentrate diets, compared to horses fed only hay diet, of 94.2%. Santos et al. (2009) observed mean water concentration in horse right dorsal colon content of 93.4%, a value similar to that observed in this study.

3. High fat diets and performance of horses

Fat animal adding to diet of athletes occurred in 1973, aiming to prevent rhabdomyolysis in racing dogs (Kronfeld et al., 1998). From this date, studies with horses were also developed with the same intention. Subsequently, fat inclusion in athletic horses' diets began to be studied in order to reduce muscle fatigue. The possible delay of fatigue, obtained with fat addition in athletic horses' diets may mean the exercise speed maintenance for longer periods or increasing the exercise speed (Meyers et al., 1989).

Horses adapted to physical exercise and fed diets supplemented with oils show a greater ability to oxidize fatty acids as an energy source, saving hepatic glycogen content and providing greater amount of blood glucose, reducing caloric increment and yielding lower respiratory quotients, and producing less CO_2 when compared to diets containing only carbohydrates. With the increase in free triglyceride concentration, horses slow the anaerobic pathway use with consequent delay in lactate production (Pagan, 2001). Horses can efficiently digest diets containing up to 30% of digestible energy as fat (Kane et al., 1979).

The addition of vegetable oils or animal fat is an excellent way of increasing dietary energy without increasing the consumed food volume (Cirelli, 1993). This alternative has improved the performance of athletic horses (Harkins et al., 1992), being especially beneficial for horses exercised under high temperatures conditions, due to the low caloric increment being approximately 3% more heat is produced during ATP formation by glucose oxidation, when compared to free fatty acids oxidation (Kohn et al., 1996). Horses fed diets supplemented with oil had large oxidation capacity of free fatty acids, sparing muscle and hepatic glycogen stores during the exercise (Pagan, 2001).

The main benefit of lipids introduction in the daily horses feeding is providing much energy when you've already reached the maximum rate of dry matter intake (Lawrence, 1990). Oil addition of 250 and 500 g in horses diet with average body weight of 400 kg and submitted to medium intensity exercise, increased performance of athletic horses. Equines that consumed oil in quantities of 500 grams daily showed better recovery with better post-test heart rate and hematocrit values (Mattos et al., 2006).

Godoi et al. (2010) evaluated physiological, hematological and biochemical parameters of Eventing horses during a training period consuming a diet with 10% soybean oil inclusion and subjected to physical effort tests. The trial lasted 82 days, performing three physical effort tests: in the beginning, the 60th and 82th day. There was effect of training duration,

improving horses conditioning, observed in the lactate concentration reduction and increased glucose concentration in the last physical effort test. Soybean oil inclusion only changed the concentration of γ-glutamyl transferase (GGT) and creatinine according to the time of diets consumption (Table 7).

Meyers et al. (1989) and Marquez et al. (2001) found no effect on heart rate before and 20 minutes after exercise in horses receiving diet with or without soybean oil inclusion. However, Mattos et al. (2006), evaluating the performance of horses fed for 30 days with diets containing 0, 3.1 and 6.8% soybean oil inclusion and exercised at a trot for two hours, found that horses consuming a diet with greater soybean oil inclusion had lower levels of heart rate immediately after and 15 minutes after the exercise.

Item	Physical effort test					P
	At rest	Immediately after	10 min after	20 min after	120 min after	
Beginning						
Heart rate (bpm)	33.5[d]	102.5[a]	75.8[b]	54.3[c]	36.4[d]	0.000
Body temperature (°C)	37.3[c]	38.7[a]	38.5[a]	38.3[b]	37.4[c]	0.000
Glycose (mg/dL)	88.5[a]	84.5[a]	88.3[a]	95.5[a]	89.7[a]	NS
Lactate (mmol/L)	0.6[d]	6.2[a]	4.5[b]	3.7[c]	0.9[d]	0.000
γ-Glutamyl transferase (U/L)	11.1[b]	14.2[a]	14.0[a]	13.9[a]	12.3[b]	0.001
Aspartate aminotransferase (U/L)	343.6[a]	399.1[a]	374.5[a]	343.6[a]	355.5[a]	NS
Creatine kinase (U/L)	147.2[a]	209.1[a]	101.4[a]	212.4[a]	196.6[a]	NS
Creatinin (mg/dL)	1.3[a]	1.3[a]	1.4[a]	1.2[ab]	0.9[b]	0.001
60° Day						
Heart rate (bpm)	40.1[d]	132.5[a]	67.0[b]	57.0[c]	40.1[d]	0.000
Body temperature (°C)	37.6[d]	38.9[ab]	38.7[a]	38.4[b]	37.9[c]	0.000
Glucose (mg/dL)	83.5[ab]	75.8[b]	84.2[a]	86.9[a]	88.0[a]	0.043
Lactate (mmol/L)	0.9[e]	6.1[a]	4.0[b]	2.6[c]	1.2[d]	0.000
γ-Glutamyl transferase (U/L)	22.9[a]	29.8[a]	26.6[a]	27.1[a]	22.9[a]	NS
Aspartate aminotransferase (U/L)	127.7[b]	145.7[b]	151.8[b]	169.2[b]	243.7[a]	0.049
Creatine kinase (U/L)	84.3[a]	93.1[a]	127.7[a]	114.0[a]	112.2[a]	NS
Creatinin (mg/dL)	1.0[c]	1.0[bc]	1.0[ab]	1.1[a]	1.1[ab]	0.006
82° Day						
Heart rate (bpm)	40.0[d]	130.7[a]	75.3[b]	59.3[c]	42.0[d]	0.000
Body temperature (°C)	37.6[b]	39.3[a]	39.2[a]	39.1[a]	37.6[b]	0.000
Glucose (mg/dL)	90.8[a]	94.3[a]	96.1[a]	109.1[a]	96.6[a]	NS
Lactate (mmol/L)	0.5[d]	4.1[a]	2.9[b]	1.6[c]	0.4[e]	0.000
γ-Glutamyl transferase (U/L)	13.3[a]	15.9[a]	14.6[a]	14.3[a]	10.7[a]	NS
Creatine kinase (U/L)	136.7[b]	193.2[a]	232.3[a]	180.6[a]	207.1[a]	0.014
Creatinine (mg/dL)	2.1[b]	2.2[a]	2.4[a]	2.4[a]	2.2[a]	NS

Values, within a line, followed different letters are different, in function collect time, by Friedman test (P<0.05).

Table 7. Heart rate, body temperature and blood biochemical of horses fed with soybean oil inclusion in diet and submitted to exercise tests at beginning, 60° and 82° day

According to NRC (2007), many factors might be related to contradictory results observed in literature, such as type and amount of oils and fats used equines diets, experimental duration, intensity variation and duration of physical efforts, besides reduced number of equines used for treatment and difference in physical conditioning of these animals. According to Hodgson & Rose (1994), Boffi (2006), Mattos et al. (2006) and Brandi et al. (2008), the beneficial effect in diet intake with soybean oil in horse performance is more evident when submitted to exercise of low intensity and long duration.

Some of the nutritional strategies to prevent postprandial hyperglycemia and insulin responses include reducing starch intake (Vervuert et al., 2009) or the replacement of starch by fat (Treiber et al., 2005). Vervuert et al. (2010), evaluating horses fed three different diets containing ground corn, ground corn with soybean oil or corn with fish oil, observed that there was no effect of corn oil or fish oil on serum glucose and insulin in the postprandial period, suggesting that, to avoid postprandial hyperglycemia and hyperinsulinemia, a feed strategy aiming the reduction of starch intake would be better than fat intake. However, linseed oil addition (0.5 mL / kg BW) in diet composed by grains did not affect the postprandial glycemic response, but reduced insulinic concentrations by almost 50% (Fayt et al., 2008).

Increasing the level at 7.8% of oil in the diet did not significantly influence ($P>0.05$) heart rate, respiratory rate, glucose and lactate levels before and after exercise in horses of Quarter Horse race in moderate exercise intensity (227m/min). Muscle glycogen concentration was higher ($P<0.025$), before exercise, in horses fed diets with soybean oil. The increasing concentration of glycogen in horses conditioned to consume a diet with soybean oil can mean a greater energy supply for muscle activity during exercise (Marqueze et al., 2001).

Soybean oil inclusion in diets possibility energetic demand supply with decreased of dry matter intake, avoiding gastrointestinal disturbs. These are beneficial factors justified utilization of lipid sources in diets of horses in any sportive activity.

4. Conclusions

Oils and fats addition in horses' diets should be used in order to raise energy dietary concentration by increasing the availability of blood glucose during postprandial period. It is expected the dry matter intake reduction, ether extract digestibility increasing and greater availability of polyunsaturated fatty acids beneficial to horses athletes without occurrence of diarrhea or changes in feces characteristics.

Soybean oil inclusion in diets of horses should be fractionated into at least four schedules during the day, and mustn't exceed amounts greater than 20%, avoiding nutrients digestibility losses, particularly of fiber, such as hemicelluloses and cellulose, and non-fiber carbohydrates and its fractions.

Fractionation of the soybean oil addition in the diet increases β-oxidation triglycerides with reduction in plasma concentration, does not alter plasma cholesterol, HDL and LDL concentrations, and increases buffering capacity of colon digesta pH witch does not influence the liquid phase passage kinetics in the digestive tract.

Further researches should be conducted to assess the lipids interactions with other nutrients in the small intestine and large intestine of horses with the goal of developing safer diets for athletic horses which result in increased athletic performance.

5. References

Boffi, F. (2006). *Fisiología del Ejercicio en Equinos*. (1.ed.) Inter-Médica, ISBN 950-555-314-5, Buenos Aires, Argentina.

Bowman, V.A., Fontenot, J.P. & Webb, K.E. (1977). Digestion of fat by equine. *Proceedings of 5th Equine Nutrition And Physiology Symposium,* pp. 40 St. Louis, 1977.

Brandi, R.A., Furtado, C.E., Martins, E.N., Freitas, E.V.V., Lacerda-Neto, J.C. & Queiroz-Neto, A. (2008). Efeito de dietas com adição de óleo e do treinamento sobre a atividade muscular de equinos submetidos à prova de resistência. *Acta Scientiarum Animal Science*, Vol.30, No.3, (2008), pp.307-315, ISSN 1806-2636.

Bush, J.A.; Freeman, D.E., Kline, K.H., Merchen, N.R. & Fahey Jr., G.C. (2001). Dietary fat supplementation effects on in vitro nutrient disappearance and in vivo nutrient intake and total tract digestibility by horses. *Journal of Animal Science*, Vol.79, (2001), pp.232-239, ISSN 1525-3163.

Cirelli, A.A.Jr. (1993). Principles of nutrition for the performance horse. *Equine Athlete*, Vol.6, No.5, (1993), pp.13-14.

Delobel, A., Fabry, C., Schoonheere, N., Fabry, C., Schoonheere, N., Istasse, L. & Hornick, J.L. (2008). Linseed oil supplementation in diet for horses: Effects on palatability and digestibility. *Livestock Science*, Vol.116, (2008), pp.15-21, ISSN 1871-1413.

Drougoul, C., Poncet, C. & Tisserand, J.L. (2000). Feeding ground and pelleted hay rather than chopped hay to ponies. Consequences for in vivo digestibility and of passage de digesta. *Animal Feed Science and Technology*, Vol.87, (2000), pp.117-130, ISSN 0377-8401.

Dukes, H.H. (1996). *Fisiologia dos animais domésticos*, (11. Ed) Guanabara Koogan, ISBN 8527703300, Rio de Janeiro, Brasil.

Fayt, J., Dotreppe, O., Hornick, J.L. & Istasse, L. (2008). Spelt, an ancient cereal and first pressure linseed oil as ingredients of compound feedstuffs for modern horse feeding. *Journal of Animal Physiology and Animal Nutrition*, Vol.92, (2008), pp.303-309, ISSN 1139-0396.

Frape, D. (2004). *Equine nutrition and feeding,* (3.Ed.), Blackwell Publishing, ISBN 1-4051-0598-4, Ames, Iowa, USA.

Geelen, S.N.J., Blaâzquez, C., Geelen, M.J.H., Sloet van Oldruitenborgh-Oosterbaan, M.M. & Beynen, A.C. (2001). High fat intake lowers hepatic fatty acid synthesis and raises fatty acid oxidation in aerobic muscle in Shetland ponies. *British Journal of Nutrition*, Vol.86, (2001), pp.31-36, ISSN 0007-1145.

Godoi, F.N., Almeida, F.Q., Migon, E.X.F, Almeida, H.F.M., Monteiro, A.B.F. & Santos, T.M. 2010. Performance of eventing horses fed high fat diet. *Revista Brasileira de Zootecnia*, Vol.39, No.2, (2010), pp.335-343, ISSN 1806-9290.

Godoi, F.N., Almeida, F.Q., Guarienti, G.A., Santiago, J.M., Guedes Jr, D., Nogueira, Y.C. & Brasileiro, L.S. (2009a). Perfil hematológico e características das fezes de equinos consumindo dietas hiperlipidêmicas. *Revista Ciência Rural*, Vol.39, No.9, (2009), pp.2571-2577, ISSN 0103-8478.

Godoi, F.N., Almeida, F.Q., Saliba, E.O.S., Ventura, H.T., França, A.B. & Rodrigues, L.M. (2009b). Consumo, cinética digestiva e digestibilidade de nutrientes em equinos atletas alimentados com dietas contendo óleo de soja. *Revista Brasileira de Zootecnia*, Vol. 38, (2009b)pp. 1928-1937, ISSN 1806-9290.

Hallebeek, J.M. & Beynen, A.C. (2002). The plasma level of triacylglycerols in horses fed high-fat diets containing either soybean oil or palm oil. *Journal of Animal Physiology and Animal Nutrition,* Vol.86, (2002), pp.111-116, ISSN 1139-0396.

Harkins, J.D., Morris, G.S., Tulley, R.T., Nelson, A.G. & Kamerling S.G. (1992). Effect of added dietary fat on racing performance in thoroughbred horses. *Journal of Equine Veterinary Science*, Vol.12, No.2, (1992), pp.123-129, ISSN 0737-0806.

Hodgson, D.R. & Rose, R.J. (1994). *The athletic horse: principles and practice of equine sports medicine,* (1.ed.) W.B. Saunders Co, ISBN 0721637590, Philadelphia, USA.

Hoffman, R.M., Wilson, J.A., Kronfeld, D.S., Cooper, W.L., Lawrence, L.A., Sklan, D. & Harris, A. (2001). Hydrolyzable carbohydrates in pasture, hay, and horse feeds: direct assay and seasonal variation. *Journal of Animal Science*, Vol.79, (2001), pp.500-506, ISSN 0021-8812.

Holland, J.L., Kronfeld, D.S. & Meacham, T.N. (1996). Behavior of horses is affected by soy lecithin and corn oil in the diet. *Journal of Animal Science*, Vol.74, (1996), pp.1252-1255, ISSN 0021-8812.

Hughes, S.J., Potter, G.D., Greene, L.W., Odom, T.W. & Murray-Gerzik, M. (1995). Adaptation of Thoroughbred horses in training to a fat supplemented diet. *Equine Veterinary Journal*, Vol.18, (1995), pp.349-352, ISSN 2042-3306.

Jansen, W.L. Geelen, S.N., van der Kuilen J. & Beynen, A.C. (2002). Dietary soybean oil depresses the apparent digestibility of fiber in trotters when substituted for an iso-energetic amount of corn starch or glucose. *Equine Veterinary Journal*, Vol.34, No.3, (2002), pp.302-305, ISSN 2042-3306.

Jansen, W.L., Cone, J.W., Geelen, S.N.J., Sloet van Oldruitenborgh-Oosterbaan, M.M., Van Gelder, A.H., Oude, S.J.W.H. & Beynen, A.C. (2007). High fat intake by ponies reduces both apparent digestibility of dietary cellulose and cellulose fermentation by faeces and isolated caecal and colonic contents. *Animal Feed Science and Technology*, Vol.133, (2007), pp.298-308, ISSN 0377-8401.

Jansen, W.L., Van der Kuilen, J., Geelen, S.N.J. & Beynen, A.C. (2000). The effect of replacing nonstructural carbohydrates with soybean oil on the digestibility of fiber in trotting horses. *Equine Veterinary Journal*, Vol.31, No.1, (2000), pp.27-30, ISSN 2042-3306.

Julen, T.R., Potter, G.D., Greene, L.W. & Stott, G.G. (1995). Adaptation to a fat-supplemented diet by cutting horses. *Journal of Equine Veterinary Science,* Vol.15, No.10, (1995), pp.436-440, ISSN 0737-0806.

Kane, E., Baker, J.P. & Bull, L.S. (1979). Utilization of corn oil supplemented diet by the pony. *Journal of Animal Science*, Vol.48, (1979), pp.1379- 1384, ISSN 0021-8812.

Kaneko, J.J, Harvey, J.W. & Bluss, M.L. (1997). *Clinical biochemistry of domestic animals*. (5. Ed.) Academic Press, ISBN 9780123963055, New York, USA.

Kohn, C., Allen, A.K. & Harris, P. (1996). Nutrition for the equine athlete. *Equine Athlete*, Vol.9, No.4, (1996), pp.12-17.

Kronfeld, D.S., Holland, J.L., Rich, G.A., Meacham, T.N., Fontenot, J.P., Sklan D.J. & Harris P.A. (2004). Fat digestibility in *Equus caballus* follows increasing first-order kinetics. *Journal of Animal Science,* Vol.82, (2004), pp.1773-1780, ISSN 0021-8812.

Kronfeld, D.S., Crandel, K.M., Custalow, S.E, Ferrante, P.L., Gay, L.A., Graham-Thiers, P., Kline, K.A., Taylor, L.E. Holland, J. L., Wilson, J.A., Sklan, D.J., Harris P.A. & Tiegs W. (1998). Studies of fat adaptation and exercise. *Proceedings of Recent Advances In*

Equine Nutrition - Kentucky Equine Research, pp.37-39, ISBN 978-1-904761-28-0 Kentucky, USA, 1998.

Lawrence, L.M. (1990). Nutrition and fuel utilization in the athletic horse. *Veterinary Clinics of North America: Equine Practice,* Vol.6, (1990), pp.393-418, ISSN 0749-0739.

Lopes, M.A.F.; White, M.A.; Crisman, M.V. & Ward, D.L. (2004). Effects of feeding large counts of grain on colonic contents and feces in horses. *American Journal of Veterinary Research*, Vol.65, No.5, (2004), pp.687-694, ISSN 0002-9645.

Marqueze, A. Kessler, A.M. & Bernardi, M.L. (2001). Aumento do nível de óleo em dietas isoenergéticas para cavalos submetidos a exercício. *Ciência Rural,* Vol.31, No.3, (2001), pp.491-496, ISSN 0103-8478.

Mattos, F., Araújo, K.V., Leite, G.G. & Goulart, H.M. (2006). Uso de óleo na dieta de equinos submetidos ao exercício. *Revista Brasileira de Zootecnia,* Vol.35, No.4, (2006), pp.1373-1380, ISSN 1806-9290.

Meyer, H. (1995). *Alimentação de cavalos*. (1.Ed.), Varela, ISBN 8585519096 São Paulo, Brasil.

Meyer, H., Flothow, C. & Radicke, S. (1997). Pre-ileal digestibility of coconut fat and soybean oil in horses and their influence on metabolites of microbial origin of the proximal digestive tract. *Archives of Animal Nutrition,* Vol. 50, (1997), pp.63-74, ISSN 1745-039X.

Meyer, H. & Coenen, M. (2002). Ernährungsbedingte Krankheiten und Störungen. In: *Pferdefütterung.* Meyer, H. & Coenen, M., pp.161–187, ISBN 3830440219, Parey Buchverlag, Berlin, Alemanha.

Meyers, M.C., Potter, G.D., Evans, J.W., Greene, L.W. & Crouse S.F. (1989). Physiologic and metabolic response of exercising horses to added dietary fat. *Journal of Equine Veterinary Science,* Vol.9, (1989), pp.218-223, ISSN 0737-0806.

Morgado, E.S., Almeida, F.Q., Godoi, F.N., Gomes, A.V.C., Galzerano, L., França, A.B. & Brasileiro L.S. (2009). Digestão de carboidratos em equinos alimentados com dietas suplementadas com concentrado ou óleo de soja. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia,* Vol. 6, (2009), pp. 1112-1119, ISSN 0102-0935.

National Research Council – NRC (2007). *Nutrient requirements of horses*. 6.ed.Rev. National Academies Press, ISSN 978-0-309-10212-4, Washington, D.C., USA.

Orme, C.E., Harris, R.C., Marlin, D.J. & Hurley, J. (1997). Metabolic adaptations to a fat supplemented diet by the thoroughbred horse. *British Journal of Nutrition*, Vol.78, (1997), pp.443-458, ISSN 0007-1145.

Pagan, J.D. (2001). Recent developments in equine nutrition research. In: *2^{sd} Advance On Equine Nutrition*, Pagan, J.D., pp.251-258, ISBN 978-1-904761-87-7, Kentucky Equine Research Inc. Kentucky, USA.

Palmgren Karlsson, C., Lindberg, E.J. & Rundgren, M. (2000). Associative effects on total tract digestibility in horses fed different ratios of grass hay and whole oats. *Livestock Production Science,* Vol.65, (2000), pp.143-153, ISSN 0301-6226.

Palmiquist, D.L. (1988). The feeding value of fats. In: *Feed Science*. Orskov, E.R., Elsevier Science Publishers, ISBN 0444426620.

Potter, G.D., Arnold, F.F., Householder, D.D., Hansen D.H., & Brown, K.M. (1992). Digestion of starch in the small or large intestine of the equine. *Pferdeheilkunde.* Vol.1, (1992), pp.107−111, ISSN 978-1-904761-87-7.

Rammerstorfer, C., Potter, G.D., Cudd, T.A., Gibbs, P.G., Varner, D.D. & Householder, D.D. (1998). Physiological responses of mature quarter horses to reining training when

fed conventional and fat-supplemented diets. *Journal of Equine Veterinary Science,* Vol.18, No.3, (1998), pp.175-183, ISSN 0737-0806.

Rostagno, H.S. (2005). *Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais.* (2.ed) Universidade Federal de Viçosa, Viçosa, Brasil.

Sales, J. & Homolka, P. (2011). A meta-analysis of the effects of supplemental dietary fat on protein a fibre digestibility in the horse. *Livestock Science*, Vol.136, (2011), pp.55–63, ISSN 1871-1413.

Santos, T.M., Almeida, F.Q., Godoi, F.N., Silva, V.P., França, A.B., Santiago, J.M., Santos, C.S. (2009). Capacidade tamponante, pH e consistência das fezes em equinos submetidos à sobrecarga dietética com amido. *Ciência Rural*, Vol.39, No.6, (2009), pp.1782-1788, ISSN 0103-8478.

Sloet van Oldruitenborgh-Oostebaan, M.M., Annee, M.P., Verdegaal, J.M.M., Lemmens, A.G. & Beynen, A.C. (2002). Exercise-and metabolism-associated blood variables in Standardbreds fed either a low-or a highfat diet. *Equine Veterinary Journal*, Vol.34, (2002), pp.23-32, ISSN 2042-3306.

Taylor, L.E.; Ferrante, P.L.; Kronfeld, D.S. & Meacham, T.N. (1995). Acid-base variables during incremental exercise in sprint-trained horses fed a high-fat diet. *Journal of Animal Science*, Vol.73, (1995), pp.2009-2018, ISSN 0021-8812.

Treiber, K.H., Boston, R.C. Kronfeld, D.S. Staniar W.B. & Harris P.A. (2005). Insulin resistance and compensation in Thoroughbred weanlings adapted to highglycemic meals. *Journal of Animal Science*, Vol. 83, (2005), pp. 2357–2364, ISSN 0021-8812.

Van Soest, P.J. (1994). Carbohydrates. In: *Nutritional ecology of the ruminant*. Van Soest, P.J. (2. Ed.) Cornell University Press, p.156-176, ISBN 9780801427725, Ithaca, USA.

Vervuert, I., Voigt, K., Hollands, T., Cuddeford, D. & Coenen, M. (2009). Effect of feeding increasing quantities of starch on glycaemic and insulinaemic responses in healthy horses. *The Veterinary Journal*, Vol.182, (2009), pp. 67–72, ISSN 1090-0233.

Vervuert, I., Klein, S. & Manfred, C. (2010). Short-term effects of a moderate fish oil or soybean oil supplementation to a starchy meal on postprandial glucose and insulin responses in healthy horses. *The Veterinary Journal*, Vol.184, (2010), pp.162-166, ISSN 1090-0233.

Zeyner, A., Geibler, C. & Dittrich, A. (2004). Effects of hay intake and feeding sequence on variables in faeces and faecal water (dry matter, pH value, organic acids, ammonia, buffering capacity) of horses. *Journal Animal Physiologic and Animal Nutrition,* Vol.88, (2004), pp.7-19, ISSN 1139-0396.

Zeyner, A., Bessert, J. & Gropp, J.M. (2002). Effect of feeding exercised horses on high-starch or high-fat diets for 390 days. *Equine Veterinary Journal*, Vol.34, (2002), pp.50-57, ISSN 2042-3306.

Effect of Maternal Selenium and Methionine on Poultry Products (Egg and Meat) Qualities and Oxidative Stability

D. J. Wu[1], X. J. Pan[2], Z. G. Wang[3], Z. Q. Peng[1],
L. Y. Zhao[4] and Y. W. Zhang[5]
[1]College of Food Science and Technology
Nanjing Agricultural University, Nanjing
[2]Department of Biotechnology and Food Engineering
Bengbu College, Bengbu, 233030
P.R. China

1. Introduction

As the events during critical development periods may influence long-term or life-time structure and function of the body (Angelbeck and Du Bru 1983; Lucasand others 1990, 1996; Snoeck and others 1990; Desaiand others 1995, 1996), the impact of breeders nutrition on nutritional status of off springs has received considerable attention.

Selenium (Se) and methionine (Met) are 2 essential substances for poultry nutrition. Se is an essential component of a variety of selenoproteins, the best known of which is glutathione peroxidase (GSH-Px). The GSH-Px family of enzymes is a crucial player in the integrated antioxidant system, neutralizing potential threats to the integrity of cellular macromolecules by eliminating hydrogen peroxide and detoxifying lipid hydro peroxides (Brigelius-Flohe1999). Se derived from the diet of the female bird is deposited in the egg and is distributed among the developing tissues during embryo genesis (Ga´alandothers1995; Surai2000; Paton and others2002). Consequently, GSH-Px is expressed in the chicken embryo in a tissue and stage-specific manner(Wilson and others1992; Ga´aland others1995; Surai1999). Supplementary Se in the diet of the hen was shown to increase the concentration of this element in the egg and in the tissues of the chick at hatch, and to elevate the expression of GSH-Px, while reducing the generation of lipid peroxides in the liver of the day-old chick(Surai2000; Paton and others2002). Pappas and others(2005) have shown that dietary supplementation of the female chicken with Se increased Se concentrations and GSH-Px activity in blood, liver, and breast of chicks for 2 to 4 wk post hatch. Surai(2000) also revealed that the effects of maternal Se supplementation remained significantly after10 d of hatching.

Methionine is considered to be the 1st limiting factor in classical diets used for growing chickens that plays unique roles, both in protein structure and in metabolism (Baker 2006). Methionine, an essential dietary amino acid, is used to synthesize proteins and other amino acids. Cysteine and homocysteine are produced during Met metabolism. In most cells,

especially liver cells, about half of cysteine come from Met by the transsulfuration pathway (M`etayer and others 2007). Met plays a particularly important role in providing cysteine for glutathione (GSH) synthesis (Beatty and Reed 1980). These common S-containing amino acids and GSH are antioxidants (Mosharov et al., 2000). All of the elements of the antioxidant system interact with each other and form an efficient antioxidant defense. This interaction probably starts at the level of nutrient absorption and continues during metabolism (Surai, 2000). All these common sulfur-containing amino acids and GSH are antioxidants(Mosharov and others 2000).

In order to study the effects of selenium **(Se)** and methionine **(Met)** supplementation of breeder hens diets on their eggs qualities and offspring's meat quality, a total of four hundred fifty 52-week-old Lang-shan hens (dual-purpose type, an indigenous poultry breed of China) were randomly divided into 9 treatments with 5 replicates each treatment. Birds were fed corn and soybean-based diets (0.13mg Se/kg) supplemented with 0, 0.30 and 0.60 mg/kg Se from Se yeast and 3.2, 4.0 and 5.4 g of DL-Met/kg, respectively. A 30-d adapting period and 70-d experiment period were used for collecting eggs. After incubation for 21 d, 160 healthy chicks from each treatment group were randomly divided into 5 replicates and fed with the same corn and soybean-based diet similar to industry recommendations for another 3-phase feeding program. The starting phase was fed 1.20% total Lys and 2.80 Mcal/kg of ME

from 0 to 21 d, the growing phase from 22 to 42 d with 1.08% total Lys and 2.70 Mcal/kg of ME, and the finishing phase from 43 to 91 d with 0.90% total Lys and 2.60 Mcal/kg of ME. Then the effects of Se and Met supplementation of breeder hen diets on physical qualities and antioxidant capacity of the breeding eggs, meat quality and antioxidant capacity of their male offspring and Se concentration and oxidative stability of lipids in the thigh muscles of progeny were examined.

1.1 Se concentration and oxidative stability of lipids in the thigh muscles of progeny

With precooked and chilled storage, poultry meat is particularly prone to oxidation due to its high polyunsaturated fatty acid (PUFA) content(Mercier and others 1998; Racanicci and others 2004), which results in rancid flavor development and decreased quality. Se and Met to diets can increase anti oxidative capacity in animals. Unfortunately, there is little information available on the effects of Se and Met supplementation of breeders on lipid oxidation of the meat of progeny during growth. In order to study such effect, Lang-shan breeding hens (450) were obtained at 52 wk of age and randomly allotted to 9 treatments; 5 replicates of each treatment were carried out. The breeders were fed a basal corn–soybean meal diet (0.13 mg Se/kg) supplemented with 0, 0.30, or 0.60 mg/kg Se from Sel-Plex and 0.32%, 0.40%, or 0.54% Met for the 30-d adapting period and 70-d experiment period. Se and glutathione (GSH) concentrations, glutathione peroxidase (GSH-Px) activity, and the oxidative stability of muscular lipids of 90-d progeny were determined by testing the TBARS values to evaluate the effects of Se and Met supplementation of breeders on lipid oxidation of the meat of progeny during growth.

2. Experiment parameters measured

2.1 Se concentration

The Se content in progeny thigh was generally between approximately 0.075 and 0.093 mg/kg (Table 1). The main effect of either dietary Se or Met supplementation of breeders

Effect of Maternal Selenium and Methionine on Poultry
Products (Egg and Meat) Qualities and Oxidative Stability
271

was not significant, but a significant interaction was found between them ($P < 0.01$). Compared to the control treatment, Se concentration in progeny thigh was reduced with the supplementation of 0.60 mg Se/kg diet of breeders($P < 0.05$), but the supplementation effect was significant only when the breeders were supplemented with 0.32% Met (Table 1). Se content was increased in addition to 0.54% Met supplementation compared to 0.32% ($P < 0.05$), when breeders were supplemented with 0.6 mg Se/kg diet (Table 1).

Se mg/kg	Met %	Se content mg/kg	GSH-Px EU	GSH mmol/g pro	TBARS-6 h mg/kg	TBARS-3 d mg/kg
0.00	0.32	$0.093^a \pm 0.009$	$1.71^{bc} \pm 0.61$	$40.47^{ab} \pm 0.83$	$1.87^d \pm 0.35$	$3.83^{de} \pm 0.35$
	0.40	$0.088^a \pm 0.005$	$1.97^{abc} \pm 0.19$	$39.45^{ab} \pm 1.06$	$2.53^{bc} \pm 0.31$	$4.24^{abcd} \pm 0.50$
	0.54	$0.085^{ab} \pm 0.009$	$2.35^a \pm 0.10$	$42.26^a \pm 3.92$	$3.11^a \pm 0.25$	$4.66^a \pm 0.33$
0.30	0.32	$0.083^{ab} \pm 0.006$	$2.00^{ab} \pm 0.29$	$39.95^{ab} \pm 1.09$	$2.82^{ab} \pm 0.32$	$4.33^{abc} \pm 0.28$
	0.40	$0.087^a \pm 0.001$	$1.79^{bc} \pm 0.26$	$38.35^b \pm 1.06$	$2.67^b \pm 0.26$	$4.14^{bcd} \pm 0.19$
	0.54	$0.087^a \pm 0.007$	$1.92^{abc} \pm 0.26$	$41.60^{ab} \pm 1.09$	$2.53^{bc} \pm 0.55$	$4.57^{ab} \pm 0.36$
0.60	0.32	$0.075^b \pm 0.010$	$1.67^{bc} \pm 0.31$	$38.41^b \pm 2.98$	$2.21^{cd} \pm 0.29$	$4.06^{cd} \pm 0.23$
	0.40	$0.085^{ab} \pm 0.005$	$1.89^{abc} \pm 0.30$	$41.24^{ab} \pm 2.09$	$2.49^{bc} \pm 0.12$	$3.94^{cd} \pm 0.24$
	0.54	$0.092^a \pm 0.006$	$1.50^c \pm 0.04$	$41.21^{ab} \pm 2.48$	$2.03^d \pm 0.22$	$3.45^e \pm 0.33$
0.00		0.089 ± 0.008	$2.01^a \pm 0.43$	40.73 ± 2.48	$2.50^a \pm 0.60$	$4.24^a \pm 0.51$
0.30		0.086 ± 0.006	$1.91^{ab} \pm 0.26$	39.96 ± 1.69	$2.67^a \pm 0.38$	$4.35^a \pm 0.32$
0.60		0.084 ± 0.010	$1.68^b \pm 0.28$	40.29 ± 2.68	$2.24^b \pm 0.28$	$3.82^b \pm 0.37$
	0.32	0.084 ± 0.011	1.80 ± 0.42	$39.61^b \pm 1.94$	$2.30^b \pm 0.50$	4.07 ± 0.34
	0.40	0.086 ± 0.004	1.88 ± 0.24	$39.68^b \pm 1.83$	$2.56^a \pm 0.24$	4.11 ± 0.34
	0.54	0.088 ± 0.008	1.92 ± 0.39	$41.69^a \pm 2.53$	$2.56^a \pm 0.57$	4.23 ± 0.65
P-value						
Se		0.218	0.04	0.68	0.003	0.0002
Met		0.295	0.58	0.04	0.048	0.4062
Se * Met		0.009	0.04	0.26	<0.0001	0.0002

[a,b] Means within a column lacking a common superscript differ ($P < 0.05$).

Table 1. The effect of dietary Se and Met supplementation of breeders on Se and GSH content, GSH-Px activity, and stability of lipid oxidation of progeny thigh.

2.2 GSH-Px activity
GSH-Px activity of progeny thigh was decreased ($P < 0.05$) in supplementation of 0.60 mg Se/kg diet of breeders compared to the control treatment; however, the main effect of Met supplementation was not significant(Table 1). Se and Met had a significant interaction ($P < 0.05$) with regard to GSH-Px activity (Table 1). Dietary supplementation of breeders with higher Se significantly decreased GSH-Px activity($P < 0.05$), but this effect was only significant when Met supplementation was 0.54% (Table 1). For the treatments not supplemented with Se, GSH-Px activity was increased by the supplementation of 0.54% Met compared to 0.32% ($P < 0.05$). The treatment that was not supplemented with Se and contained 0.54% of Met had the highest GSH-Px activity in progeny thigh, and the lowest GSH-Px activity was found in the groups that had Se and Met, both with the highest or the lowest levels.

2.3 GSH content
GSH content of progeny thigh was not influenced by dietary supplementation with Se (P > 0.05). Conversely, it was higher in subjects with the highest supplementation of Met(0.54%) (P < 0.05), and there was no significant difference between groups supplemented with 0.32% and 0.40% Met. Se and Met had no significant interaction(P > 0.05) on the concentration of GSH.

2.4 TBARS content
The TBARS content, expressed as milligram MDA equivalents per kilogram meat, was increased (P < 0.05) with chilled storage time (Table 1). Dietary supplementation of breeders

with 0.60 mg Se/kg diet reduced (P < 0.05) the TBARS content of progeny thigh at both 6 h and 3d (Table 1), but its effect was significant only when dietary supplementation with Met was 0.54%. With the exception of a significant reduction (P < 0.05) in TBARS content at 6 h, dietary supplementation with 0.30 mg Se/kg diet, with respect to that which was not supplemented, did not result in significant variations in the se substances at 3 d when breeders were supplemented with 0.54% of Met. This leads to the supposition that 0.30 mg Se/kg was not sufficient to guarantee adequate protection against oxidative phenomena when the lipid oxidation was greater after 3d under the meat storage conditions employed in this study. However, when the breeders received the diets with 0.32% of Met, 0.30 mg Se/kg dietary supplementation revealed the highest TBARS content (P < 0.05), and there was no evident difference (P > 0.05) between the control and the treatment supplemented with 0.6 mg Se/kg diet both at 6 h and 3 d. Irrespective of the effect of Se supplementation, addition of 0.54% and 0.40% Met increased TBARS content of progeny thigh (P < 0.05) compared to 0.32% at 6 h, and did not result in significant difference in the se reactive substances at 3d (P > 0.05). When the breeders were not supplemented with Se, TBARS content of progeny thigh was increased (P < 0.05) with the supplementation of Met at 6 h and 3 d. However, when the breeders received diets with 0.6 mg Se/kg, 0.54% Met supplementation decreased the TBARS content (P < 0.05) compared to 0.40% at 6 h and the other 2 levels at 3d, and there was no significant difference between treatments supplemented with 0.32% and 0.40% of Met (P > 0.05).

In summary, the results can be concluded as:

1. When breeders were received the higher levels of Met or Se, GSH-Px activity in their progeny's thigh muscle was decreased, while the Se concentration and the oxidative stability of muscular lipids were increased. When breeder hens were given a Met-deficient diet, supplementing with Se decreased the Se deposition in progeny thigh. With regard to lipid oxidation, 0.3 mg/kg maternal Se supplementation decreased the oxidative stability of muscle lipid and 0.6 mg/kg Se supplementation showed no difference from the control group. When breeders were fed a Se-deficient diet, the GSH-Px activity was significantly increased and the oxidative stability of progeny muscles was decreased with the supplementation of Met.

2. A significant interactive effect between maternal Se and Met on relative quantity of primary volatile oxidative compounds produced at the beginning of warmed-over flavour (WOF) was found (P<0.05), but the main effect of either Se or Met was not significant (P>0.05). Both the higher levels of Se and Met could result in the lower content of total aldehyde, hexanal, 1-pentanol, but the higher concentration of 2,3-octanedione and 2-pentyl-furan in progeny thigh muscle (P<0.05). There was no significant effects of maternal Se and Met supplementation on the content of volatile oxidative compounds produced at the later stage of WOF (P<0.05). The relative quantity of volatile oxidative compounds as a result of WOF development was significantly influenced by the chilled storage time (P<0.01). The contents of total aldehyde, total acids, hexanal, pentanal and 1-pentanol were found to negatively covary and decrease with increasing days of storage, while the relative quantities of total hydrocarbon, total ketone, octanal, nonanal, 1-octanol, 2-octen-1-ol, 1-octen-3-ol, 2,3- octanedione and 2-pentyl-furan increased with the storage time.

5.4 g of Met/kg treatment exhibited the highest concentrations of free fatty acids at 6 h (P<0.05); the contents of myristic acid, palmitoleic acid, linolenic acid and docosahexaenoic

acid at 6 h significantly increased as a result of maternal Se supplementation (P<0.05); the interactive effect of Se and Met on the contents of free fatty acids at 6 h was not significant (P>0.05). Also, there were no significant effects of dietary Se and Met supplementation of breeder hens on the concentrations of free fatty acids at 3 d. Free fatty acids and phospholipid fatty acids were all found to decrease with the prolong of storage time (P<0.01). The oxidative rates of phospholipid fatty acids were significantly higher than those of free fatty acids (P<0.01). The most decreased fatty acids from 6 h to 3 d both in free fatty acids and in phospholipid fatty acids were linolenic acid, palmitoleic acid and myristic acid.

Se is an essential component of GSH-Px, which plays an important role in the anti-oxidation system of tissue. Irrespective of Met supplementation levels, GSH-Px activity of progeny thigh was decreased with the supplementation of Se, in agreement with the results reported by Waschulewski and Sunde (1988a), who founded that when weaning rats received 0.5 mg Se/kg, the percentage of muscle Se present as GSH-Px was decreased at all of Met levels. Additionally, supplementation of the breeder's diet with Se increased the protective ability of progeny thigh against lipid oxidation, which was shown by lower TBARS values.

Met participates in methyl group metabolism and the synthesis of other sulfur amino acids, notably cysteine. Cysteineis required for the synthesis of GSH and taurine, which are essential compounds for host defense against oxidative stress (M`etayer and others 2007). GSH concentration of progeny thigh was significantly increased with the supplementation of Met. Lipid oxidation was not influenced by the supplementation of Met, except that TBARS values after 6 h were elevated with the supplementation of Met.
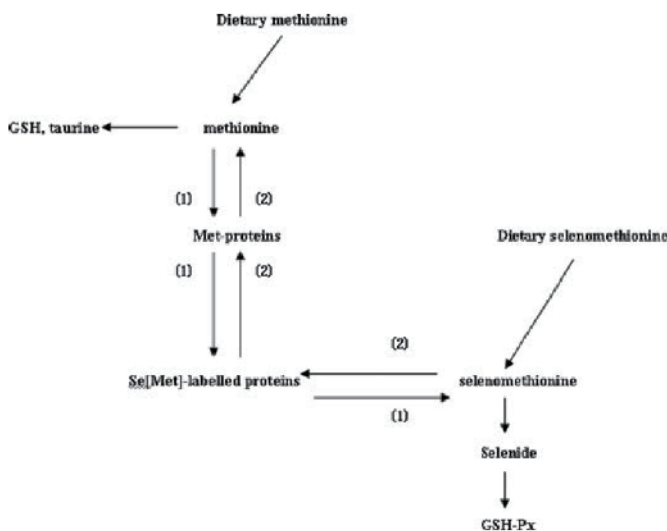
Fig. 1. Proposed schemeof the metabolism of Se (Se[Met]) and Met. Pathway 1, free Met, arising from dietary Met or from protein turnover, is incorporated into proteins for synthesis GSH-Px. Pathway 2, free Se, arisling from dietary Se [Met] or from protein turnover, is incorporated into proteins in place of Met as mediated by tRNA[Met] (McConell and Hofman 1972), so there is more Met for catabolizing GSH, taurine, and other sulfur-contalning antloxidants.

The interaction of dietary Met and Se can be explained because of the common metabolic pathways of these amino acids. The Se used in this experiment was mainly present in the

form of Se[Met]. Se[Met] can be an excellent analogue for Met in biochemical reactions because of the similar covalent radio of Se and S. As McConnell and Cho(1965) mentioned, Se[Met] is transported by the same intestinal transport system as Met, it is readily esterified to tRNA Met (Hoffman and others 1970), and it can substitute for Met during protein synthesis in eukaryotes (McConnell and Hoffman 1972). Thus Se[Met] can be metabolized by the same enzymes that incorporate Met into protein. It is also likely that Se[Met] follows the same catabolic pathways as Met until 1 of the C-Se bonds is broken (Sunde1984). These common pathways thus can be combined into a diagram of Se[Met] and Met metabolism (Figure 1) that could be used to illustrate and summarize the 2 fates of dietary Se[Met] and Met. When breeders received the Se-deficient diet, dietary supplementation with Met would release Se[Met] from body proteins, which could be converted to selenide by either of the 2 Met catabolic pathways as described by Steeleand Benevenga (1978) and Esakiand others(1982), thus Se from Se[Met] would be available for co-translational incorporation into GSH-Px and other seleno proteins as proposed by Sunde and Evenson (1987). The same effect of Met on the utilization of the deposited tissue Se for GSH-Px synthesis was reported by Waschulewski and Sunde (1988b). The ability of progeny thigh for preventing lipid oxidation was decreased concomitant with increased GSH-Px activity and decreased Se concentration. It was suggested that Met used for catabolizing to GSH, taurine, and other sulfur-containing antioxidants could be reduced (pathway1). When breeders were given sufficient Se (0.6 mg Se/kg) then decomposition of Met to GSH, taurine, and other sulfur-containing antioxidants was increased substantially as dietary Met was raised from deficient to adequate levels, and the protective ability of progeny thigh against lipid oxidation was significantly increased. It was supposed that the catabolism of Met increases the utilization of Se from dietary Se[Met] for protein synthesis, and thus increase the Se concentration of muscle (pathway2), which was contrary to the result of Waschulewski and Sunde (1988a) who found that when weanling rats were given 0.5 mg Se as Se[Met] per kilogram, muscle Se concentration was significantly decreased with the supplementation of Met. The difference would be related to the supplemented Met levels, the type of experiment animals, and the transport of nutriment from hen to embryo andthen to progeny. Similarly, when breeder stook adequate (0.54%) Met, dietary supplementation of breeders with Se substantially decreased the utilization of dietary Se[Met] for GSH-Px synthesis, and it led to preferential incorporation of Se[Met] into tissues in a form other than as GSH-Px. This resulted in elevated muscle Se levels and lower GSH-Px activity in Se-adequate treatment relative to the Se-deficient treatment. Also, dietary Se[Met] supplementation substantially increased the catabolism of Met for the synthesis of GSH, taurine, and other sulfur-containing anti oxidants, therefore increased the protective ability of muscle tissue against lipid oxidation (pathway 2). When breeders received a Met-deficient diet, supplementation of 0.3 mg Se[Met]/kg tended to increase the utilization of Se for GSH-Px synthesis and accelerate the catabolism of Se-labeled muscle proteins, which gave rise to more free Met for synthesis of muscle proteins and thereby decreased muscle anti oxidative ability (pathway 1). This was contrary to the result of Sunde and others(1981) and Waschulewski and Sunde (1988b) who reported that Se availability for GSH-Px synthesis from dietary Se[Met] was reduced when supplemented at less than 0.5 mg Se/kg in rats fed on a Met-deficient diet. However, increments of dietary Se[Met] to 0.6 mg/kg lessened this tendency; there was more Se for incorporation into muscle proteins instead of GSH-Px and more Met for synthesis of GSH, taurine, and other sulfur-containing anti oxidants. As a result, decreased GSH-Px activity and increased ability of protection against lipid oxidation were revealed (pathway 2).

Meat quality and antioxidant capacity of their male offspring

Meat quality (like color and drip loss) affected the acceptability at the time of consumer purchase to an extent. Meat discoloration was believed to be related to the effectiveness of the oxidation processes (Faustman and Cassens, 1990). Changes associated with oxidation include unpleasant tastes and odors, discoloration, protein solubility, and even potential formation of toxic compounds (Baron and Andersen, 2002). Lipid oxidation reduced the shelf life of meat and decreased nutritive and sensory quality of meat. Protein oxidation reduced meat product quality (Decker et al., 1993). Therefore, the meat industry has great interest in improving meat quality and optimizing meat color and water-holding capacity, limiting meat discoloration and loss of fluids (drip loss) because it implies a financial loss.

Four hundred fifty 52-wk-old Lang-shan breeding hens (dual-purpose type, an indigenous poultry breed of China) were randomly divided into 9 treatments with 5 replicates each treatment. They were fed corn-soybean diets with 0, 0.30, and 0.60 mg of Se/kg from Se yeast and 3.2, 4.0, and 5.4 g of dl-Met/kg, respectively. After incubation, 250 chickens each treatment were randomly divided into 5 replicates and fed the same diet. At 21 d old, 10 male chicks in each treatment were slaughtered. Color, water-holding capacity, and oxidative stability were examined to elucidate the effects of Se and Met supplementation of the maternal diets on of their male offspring meat at the early stage.

3. Experimental parameters measured

3.1 Se content

The influence of Se yeast, Met, and their interactions on the Se content in 21-d-old male offspring breast meat was remarkable ($P < 0.01$; Table 2). The Se content significantly increased with the increase of the maternal Se yeast ($P < 0.01$). However, the Se content in 5.4 g of Met/kg treatments were significantly less than those of 3.2 and 4.0 g of Met/kg treatments ($P < 0.01$).

Se (mg/kg)	Met (g/kg)	Se (μg/100 mg)	MDA (mg/kg)	Protein carbonyls (nm/mg)
0	3.2	$0.42 + 0.04^{e,D}$	$0.43 + 0.02^{a,A}$	$5.53 - 0.29^{a,A}$
	4.0	$0.49 \pm 0.06^{c,C}$	$0.30 \pm 0.03^{d,CD}$	$4.31 = 0.34^{be,BC}$
	5.4	$0.40 \pm 0.05^{c,D}$	$0.29 \pm 0.03^{d,D}$	$2.69 - 0.28^{c,D}$
0.3	3.2	$0.55 \pm 0.03^{b,B}$	$0.37 \pm 0.03^{b,B}$	$4.54 \pm 0.48^{b,B}$
	4.0	$0.60 \pm 0.04^{b,B}$	$0.29 \pm 0.07^{d,D}$	$3.88 - 0.38^{cd,BC}$
	5.4	$0.55 \pm 0.01^{b,B}$	$0.27 + 0.05^{d,D}$	$2.87 - 0.35^{c,D}$
0.6	3.2	$0.71 + 0.02^{Ax}$	$0.29 + 0.04^{d,D}$	$1.45 + 0.78^{be,B}$
	4.0	$0.69 + 0.04^{a,A}$	$0.33 + 0.06^{c,DC}$	$4.04 - 0.61^{bcc,DC}$
	5.4	$0.59 \pm 0.04^{b,B}$	$0.29 \pm 0.04^{d,D}$	$3.62 = 0.50^{c,C}$
0		$0.44 \pm 0.06^{c,C}$	$0.34 \pm 0.07^{a,A}$	$4.18 = 1.23^{a}$
0.3		$0.57 \pm 0.04^{b,B}$	$0.31 \pm 0.05^{b,B}$	$3.76 - 0.80^{b}$
0.6		$0.66 + 0.06^{a,A}$	$0.30 + 0.04^{b,B}$	$4.03 - 0.69^{ab}$
	3.2	$0.56 \pm 0.13^{b,A}$	$0.36 \pm 0.06^{a,A}$	$4.84 - 0.72^{a,A}$
	4.0	$0.59 + 0.10^{a,A}$	$0.31 + 0.04^{b,B}$	$4.08 - 0.47^{b,B}$
	5.4	$0.51 \pm 0.09^{c,C}$	$0.28 \pm 0.03^{c,C}$	$3.06 = 0.55^{c,C}$
P-value				
Se		0.0001	0.0001	0.0338
Met		0.0001	0.0001	0.0001
Se × Met		0.0033	0.0001	0.0002

[a-e] Means in a column with different superscripts are significantly different ($P < 0.05$).

[A-D] Means in a column with different superscripts are significantly different ($P < 0.01$).

Table 2. Effects of the Se yeast and Met supplementations of the maternal diets on Se, malondialdehyde (MDA), and protein carbonyl content of their male offspring breast meat (n = 10)

3.2 Protein and lipid oxidation

There were significant effects of interactions between maternal Se yeast and Met on carbonyl content (P < 0.01; Table 2). The carbonyl content significantly decreased with increase of Met supplementation (P < 0.01) and the carbonyl content of 0 mg of Se/kg treatments were higher than those of 0.3 mg Se/kg treatments (P < 0.01). Moreover, 0.30 and 0.60 mg of Se/kg treatments significantly decreased MDA content compared with those of 0 mg of Se/kg treatments (P < 0.01). The 4.0 and 5.4 g of Met/kg treatments significantly decreased MDA content compared with those of 3.2 g of Met/kg treatments (P < 0.01).

Se (mg/kg)	Met (g/kg)	L* value	a* value	b* value	Drip loss (%)
0	3.2	57.00 ± 3.89^{bcf,BC}	2.18 ± 0.18^{bc,B}	7.87 ± 0.85^{a,A}	2.16 ± 0.20^{a,A}
	4.0	61.45 ± 2.36^{a,A}	1.91 ± 0.32^{c,B}	7.33 ± 0.50^{ab,AB}	1.91 ± 0.23^{b,AB}
	5.4	54.10 ± 2.62^{d,C}	2.98 ± 0.33^{a,A}	6.24 ± 0.25^{c,C}	1.57 ± 0.30^{de,CD}
0.3	3.2	59.78 ± 1.94^{ab,AB}	2.30 ± 0.30^{a,B}	6.82 ± 0.57^{bc,BC}	1.83 ± 0.16^{bc,BC}
	4.0	57.13 ± 3.96^{bcf,BC}	2.25 ± 0.30^{bc,B}	6.62 ± 0.45^{bc,BC}	1.63 ± 0.25^{cd,BCD}
	5.4	55.89 ± 1.75^{cd,BC}	2.85 ± 0.38^{a,A}	6.23 ± 0.65^{c,C}	1.20 ± 0.15^{f,K}
0.6	3.2	57.05 ± 2.43^{bcf,BC}	2.25 ± 0.36^{bc,B}	6.69 ± 0.42^{bc,BC}	1.72 ± 0.18^{bcd,BC}
	4.0	55.93 ± 1.34^{cd,BC}	2.88 ± 0.38^{a,A}	6.74 ± 0.13^{bc,BC}	1.37 ± 0.19^{ef,DE}
	5.4	58.45 ± 3.01^{bc,AB}	3.10 ± 0.39^{a,A}	7.86 ± 0.77^{a,A}	1.33 ± 0.26^{f,DE}
0		57.52 ± 4.23	2.36 ± 0.54^{b,D}	7.15 ± 0.88^{a,A}	1.88 ± 0.34^{a,A}
0.3		57.60 ± 3.10	2.47 ± 0.42^{b,B}	6.36 ± 0.59^{b,B}	1.55 ± 0.32^{b,B}
0.6		57.14 ± 2.49	2.74 ± 0.51^{a,A}	7.10 ± 0.96^{a,A}	1.47 ± 0.27^{b,B}
	3.2	57.94 ± 3.05^{a}	2.24 ± 0.28^{b,B}	7.13 ± 0.81	1.90 ± 0.26^{a,A}
	4.0	58.17 ± 3.59^{a}	2.35 ± 0.52^{b,B}	6.90 ± 0.78	1.63 ± 0.31^{b,B}
	5.4	56.15 ± 3.02^{b}	2.97 ± 0.37^{a,A}	6.78 ± 0.97	1.37 ± 0.28^{c,C}
P-value					
Se		0.8247	0.0005	0.0050	0.0338
Met		0.0238	0.0001	0.1896	0.0001
Se × Met		0.0001	0.0012	0.0001	0.0002

$^{a-f}$Means in a column with different superscripts are significantly different ($P < 0.05$).

$^{A-E}$Means in a column with different superscripts are significantly different ($P < 0.01$).

Table 3. Effects of the Se yeast and Met supplementation of the maternal diets on color and drip loss of their male offspring breast meat (n = 10)

3.3 Meat color

The maternal Met significantly influenced L* value (0.01 < P < 0.05) and a* value (P < 0.01; Table 3). Supplementation of Met at 5.4 g/kg significantly increased a* value compared with 3.2 and 4.0 g of Met/kg (P < 0.01). The 0.60 mg of Se/kg treatment significantly increased a* value compared with 0 and 0.30 mg of Se/kg treatments (P < 0.01) and 0 mg of Se/kg treatment significantly increased b* value compared with 0.3 and 0.6 mg of Se/kg treatments (P < 0.01). Moreover, the interactions of the maternal Met and Se yeast significantly influenced L*, a*, and b* values (P < 0.01).

3.4 Drip loss

The Se yeast and Met supplementation significantly decreased drip loss (P < 0.01) (Table 3). Supplementation of Se at 0.30 and 0.60 mg/kg decreased drip loss compared with 0 mg of Se/kg treatment.

In summary, the results can be concluded as:

1. Supplementation of Met at 5.4g/kg significantly increased International Commission on Illumination a* value compared with 3.2 and 4.0 g of Met/kg (P<0.01). Supplementation of Se at 0.6 mg/kg significantly increased a* value compared 0 and 0.3 mg of Se/kg (P<0.01) and 0 mg of Se/kg significantly increased b* value compared with 0.30 and 0.60 mg of Se/kg (P<0.01).

2. Selenium supplemented at 0.30 and 0.60 mg/kg decreased drip loss compared with 0 mg of **Se**/kg and 4.0 and 5.4 g of **Met**/kg decreased drip loss compared with 3.2 g of **Met**/kg, respectively.

3. The carbonyl content of the myofibrillar protein significantly decreased with the increase of **Met** supplementation (P<0.01) and the carbonyl content of the 0 mg of **Se**/kg treatment was higher than the 0.3 mg of **Se**/kg treatment (P<0.01). Selenium supplementation at 0.30 and 0.60 mg/kg significantly decreased MDA content compared with that of   0 mg of **Se**/kg (P<0.01) and 4.0 and 5.4 g of **Met**/kg supplementation significantly decreased MDA content compared with that of 3.2 g of Met/kg(P<0.01). However, the higher levels of **Met** and **Se** (5.4 g/kg **Met** and 0.60 mg/kg **Se**) significantly increased reactive sulfhydryl content in the muscle.

4. The intermediate levels of **Met** and **Se** (4.0 g/kg Met and 0.30 mg/kg **Se**) significantly increased heat-induced gel hardness and water-holding capacity of the gel and the higher levels of **Met** and **Se** (5.4 g/kg **Met** and 0.60 mg/kg **Se**) decreased gel hardness and water-holding capacity of the gel to an extent.

Whether the Se was in an inorganic form or part of an organic molecule in the maternal diets would influence the Se content transferred to the developing embryo and the 2-wk-old chickens after hatch (Paton et al., 2002; Pappas et al., 2005). Maternal Se yeast could significantly increase the Se content of serum, liver, and muscle tissues in newborn chickens and quails (Surai, 2000). Pappas et al. (2005) reported that the high levels of maternal Se could significantly increase Se content of serum, liver, and muscle tissues from 2- to 4-wk-old chickens. In this study, all of the progeny were fed the diet with the same Se concentration throughout the experiment. Therefore, the differences in the Se content of the breast meat were due solely to the different Se content of the maternal diets.

Selenomethionine, the Se-containing analog of Met, is thought to be the common form of Se in foodstuffs of plant origin. Beilstein and Whanger (1986) reported that Selenomethionine supplementation could increase tissue Se levels substantially. This study also showed that 4.0 g of Met/kg supplementation of the maternal diet could increase Se content of the chicken meat. It might be due to the fact that tissue Se was present as selenomethionine when Se and Met were provided at high levels in the diets.

The oxidative stability of meat depends upon the balance between anti- and prooxidants. One approach to enhancing the oxidative stability of meat is to add antioxidants either into the diet of the animal or directly during processing. Selenium is an essential component of the antioxidant enzyme GSH-Px (Surai and Dvorska, 2002a, b) and Met plays a particularly important role in providing Cys for GSH synthesis (Beatty and Reed, 1980). Supplementing broiler diets with 0.25 mg/kg of Se substantially increased GSH-Px activity in breast (2.1-fold) and leg (4.1-fold) muscle and as a result decreased lipid peroxidation was detected (2.5-fold in breast muscle and 3.3-fold in leg muscles) after 4 d of storage at 4°C compared with the control group (Devore et al., 1983). Ryu et al. (1995) reported that the dietary Se from 1 to 8 mg/kg revealed only minor improvements in the oxidative stability of 42-d-old chicken meat and 8 mg/kg of Se supplementation in combination with 100 IU of α-tocopherol was more effective in reducing lipid oxidation during refrigerated storage. Several authors have measured protein oxidation in meat and meat products and have related this parameter to lipid oxidation (Mercier et al., 1998, 2004; Ventanas et al., 2006). In this study, the Se yeast and Met supplementation significantly decreased protein carbonyl and MDA content. Based on the fact presented above that the hen diets could affect Se

intake in the chicks and should modulate antioxidant enzyme GSH-Px activities, it is possible to suggest that GSH-Px contributes to the overall antioxidant defense of muscle, decreasing tissue susceptibility accomplished by organic Se supplementation of the hen diets.

The oxidative state of muscle pigments plays an important role in meat color. Redness is related to myoglobin content and its chemical state in meat (Mancini and Hunt, 2005). Ryu et al. (1995) reported that the dietary Se and α-tocopherol levels did not affect 42-d old chicken meat color. However, this present study indicated that the Se yeast and Met supplementation of the maternal diets could increase meat color stability to an extent. It might be due to the fact that that Se yeast and Met supplementation of the maternal diets could decrease protein oxidation to an extent.

Meat oxidation could decrease hydrolysis sensitivity, weaken protein degradation, and reduce water reservation among myofibrils, which increase juice loss of meat (Elisabeth and Steven, 2005). Drip is a dilute solution of the sarcoplasmic proteins. Factors that affect the state of myofibrillar proteins, like protein and lipid oxidation, will also affect drip loss. Carbonyl group formation is the main chemical modification of amino acids during oxidation. Lipid oxidation also could increase cell membrane permeability and induce juice loss (Cheah et al., 1995).

Postmortem changes include a decrease of the antioxidant defense system and an increase in the degree of lipid and protein oxidation. Glutathione peroxidase activity would be elevated if it was maintained postmortem. Therefore, we might expect a stabilizing effect of dietary Se supplementation during meat storage. Indeed, supplementing broiler diets with 0.25 mg/kg of Se substantially increased GSH-Px activity in breast (2.1-fold) and leg (4.1-fold) muscle and as a result decreased lipid peroxidation was detected (2.5-fold in breast muscle and 3.3-fold in leg muscles) after 4 d of storage at 4°C compared with the control group (Devore et al., 1983). It seems likely that a stabilizing effect of Se is associated with maintaining muscle membrane integrity. Edens (1996) reported that drip loss was decreased when organic Se was fed to broilers. Using a mode system based on red blood cell membrane stability, Edens (2001) confirmed a membrane-stabilizing effect of organic Se. This study indicated that the Se yeast and Met supplementation of the hen diets could decrease drip loss. It might be due to the fact that Se content in the meat could elevate and maintain the GSH-Px activity in meat and muscle membrane integrity.

Physical qualities and antioxidant capacity of the Breeding Eggs

The antioxidant system of chicken embryo is based on natural antioxidants (e.g., vitamin E, carotenoids, GSH; Surai et al., 1996, 2001a, b; Surai, 1999) and antioxidant enzymes [e.g., GSH-Px and catalase as well as antioxidant enzyme cofactors (Se, Zn, Mn, and Fe; Surai et al., 1999). Of these, vitamin E, carotenoids, and metals, including Se, are obtained from the maternal diet. Increasing antioxidant supplementation of the maternal diet can substantially increase their concentrations in developing chick tissues and significantly decrease their susceptibility to lipid peroxidation (Surai and Speake, 1998; Surai et al., 1999). Selenium is an integral part of GSH-Px and GSH-Px plays an important role in antioxidant defense in poultry (Surai and Dvorska, 2002a,b). The Se concentration in eggs depends on both Se content and form of dietary Se used in the maternal diet. Payne et al. (2005) reported that a Se-enriched yeast diet was more effective for increasing the egg Se content than a sodium selenite diet. Wakebe (1998) found that adding 0.3 mg of Se/kg from Se-Met to layers increased GSH-Px activity in both the yolk and the white of eggs.

Effect of Maternal Selenium and Methionine on Poultry
Products (Egg and Meat) Qualities and Oxidative Stability
279

Methionine, an essential dietary amino acid, is used to synthesize proteins and other amino acids. Moreover, Met plays a particularly important role in providing Cys for GSH synthesis (Beatty and Reed, 1980). These common S-containing amino acids and GSH are antioxidants (Mosharov et al., 2000).

Four hundred fifty 52-wk-old Langshan layer hens (dual-purpose type, an indigenous poultry breed of China) were randomly divided into 9 treatments with 5 replicates in each treatment. Birds were fed corn-soybean diets (0.13 mg of Se/kg) supplemented with 0, 0.30, and 0.60 mg/kg of Se from Se yeast and 3.2, 4.0, and 5.4 g of dl-Met/kg, respectively. Se Concentration, GSH-Px Activity, and GSH Concentration, Lipid Oxidation, Protein Carbonyl were examined to evaluate the effect of Se yeast and Met supplementation of the maternal diet on antioxidant activity of the breeding eggs.

4. Experiment parameters measured

4.1 Se concentration

As can be seen from Table 4, the inclusion of Se yeast in the diet significantly increased the Se concentration in the yolk (P < 0.01). Supplementation of Met at 4.0 and 5.4 g /kg in the diets significantly decreased the Se concentration in the egg yolk compared with 3.2 g of Met/kg (P < 0.01). Moreover, a combination of the 0.6 mg of Se/kg and 3.2 g of Met/kg treatments had the maximal Se concentration and adding 3.2 g of Met/kg alone had the minimal Se concentration.

Item	Met (g/kg)	Se (µg/g)	GSH-Px (U/g)	GSH (mg/g)	MDA (µg/g)
Se (mg/kg)					
0	3.2	$0.48 \pm 0.03^{g,E}$	$29.40 \pm 2.85^{b,B}$	$1.13 \pm 0.14^{de,BC}$	$6.34 \pm 0.79^{a,A}$
	4.0	$0.63 \pm 0.03^{ef,D}$	$17.63 \pm 3.47^{d,DC}$	$1.29 \pm 0.04^{ab,A}$	$3.71 \pm 0.65^{ed,DE}$
	5.4	$0.62 \pm 0.03^{f,D}$	$19.82 \pm 2.09^{d,C}$	$1.18 \pm 0.07^{bdce,ABC}$	$4.15 \pm 0.43^{e,CD}$
0.3	3.2	$0.84 \pm 0.03^{b,B}$	$36.42 \pm 3.34^{a,A}$	$1.23 \pm 0.08^{abcd,ABC}$	$5.07 \pm 0.59^{b,B}$
	4.0	$0.66 \pm 0.03^{d,D}$	$13.82 \pm 1.92^{e,DE}$	$1.30 \pm 0.09^{a,A}$	$4.18 \pm 0.56^{e,DC}$
	5.4	$0.66 \pm 0.02^{de,D}$	$25.60 \pm 3.31^{e,B}$	$1.11 \pm 0.14^{e,C}$	$3.14 \pm 1.13^{d,E}$
0.6	3.2	$0.95 \pm 0.04^{a,A}$	$17.88 \pm 3.84^{d,DC}$	$1.27 \pm 0.14^{abc,AB}$	$5.12 \pm 0.51^{b,B}$
	4.0	$0.85 \pm 0.03^{b,B}$	$26.91 \pm 3.96^{bc,B}$	$1.16 \pm 0.12^{cde,ABC}$	$4.87 \pm 0.35^{b,BC}$
	5.4	$0.80 \pm 0.03^{c,C}$	$12.68 \pm 1.42^{n,E}$	$1.12 \pm 0.04^{e,BC}$	$3.91 \pm 0.28^{ed,DE}$
0		$0.57 \pm 0.07^{c,C}$	$22.28 \pm 5.89^{b,B}$	1.20 ± 0.11	$4.74 \pm 1.32^{a,A}$
0.3		$0.72 \pm 0.09^{b,B}$	$25.28 \pm 9.84^{a,A}$	1.21 ± 0.13	$4.13 \pm 1.11^{b,B}$
0.6		$0.87 \pm 0.07^{a,A}$	$19.15 \pm 6.78^{e,C}$	1.18 ± 0.12	$4.63 \pm 0.65^{a,A}$
	3.2	$0.80 \pm 0.21^{a,A}$	$27.90 \pm 8.44^{a,A}$	$1.21 \pm 0.13^{a,AB}$	$5.51 \pm 0.86^{a,A}$
	4.0	$0.71 \pm 0.11^{b,B}$	$19.45 \pm 6.41^{b,B}$	$1.25 \pm 0.11^{a,A}$	$4.25 \pm 0.70^{b,B}$
	5.4	$0.69 \pm 0.08^{b,B}$	$19.37 \pm 5.86^{b,B}$	$1.14 \pm 0.09^{b,B}$	$3.74 \pm 0.81^{e,C}$
P-value					
Se		0.0001	0.0001	0.5464	0.0034
Met		0.0001	0.0001	0.0012	0.0001
Se × Met		0.0001	0.0001	0.0025	0.0001

[a] [g] Means in a column with different superscripts are significantly different ($P < 0.05$).
[A] [E] Means in a column with different superscripts are significantly different ($P < 0.01$).

Table 4. Effects of Se yeast and Met supplementation of the maternal diets on contents of Se, glutathione (GSH), and malondialdehyde (MDA) and glutathione peroxidase (GSH-Px) activity in the yolk of eggs

4.2 GSH concentration

The Met supplementation of the diets significantly influenced the GSH concentration in both the yolk and the albumen of eggs (P < 0.01; Table 4 and 5). The 5.4 g of Met/kg treatment decreased the GSH concentration in the egg yolk compared with 3.2 and 5.4 g of Met/kg, whereas the 5.4 g of Met/kg treatment significantly increased the GSH concentration in the

egg albumen compared with 3.2 and 4.0 g of Met/kg. Moreover, there was significant effect of the interactions between Se and Met on the GSH concentration in the egg yolk. However, increasing Se supplementation in the maternal diet did not significantly influence the GSH concentration in both the yolk and the albumen of eggs.

Se (mg/kg)	Met (g/kg)	GSH-Px (U/g)	GSH (mg/g)	Carbonyl group (nm/mg)
0	3.2	$292.00 \pm 20.51^{a,A}$	$2.56 \pm 0.12^{c,C}$	$5.01 \pm 0.39^{a,A}$
	4.0	$168.91 \pm 12.01^{de,CD}$	$2.72 \pm 0.16^{bc,BC}$	$4.64 \pm 0.48^{abcd,AB}$
	5.4	$131.78 \pm 16.61^{e,D}$	$2.97 \pm 0.30^{ab,AB}$	$4.37 \pm 0.50^{bcd,AB}$
0.3	3.2	$295.66 \pm 31.70^{a,A}$	$2.75 \pm 0.23^{bc,BC}$	$4.87 \pm 0.49^{abc,AB}$
	4.0	$178.57 \pm 21.46^{d,CD}$	$2.72 \pm 0.18^{bc,BC}$	$4.26 \pm 0.45^{cd,B}$
	5.4	$228.23 \pm 23.73^{c,B}$	$3.20 \pm 0.21^{a,A}$	$4.53 \pm 0.43^{abcd,AB}$
0.6	3.2	$252.29 \pm 17.97^{b,B}$	$2.77 \pm 0.27^{bc,BC}$	$4.74 \pm 0.52^{abc,AB}$
	4.0	$296.93 \pm 14.58^{a,A}$	$2.91 \pm 0.38^{ab,AB}$	$4.16 \pm 0.32^{d,B}$
	5.4	$187.72 \pm 13.05^{d,C}$	$2.96 \pm 0.15^{ab,AB}$	$4.39 \pm 0.63^{bcd,AB}$
0		$204.23 \pm 65.77^{c,B}$	2.75 ± 0.27	4.67 ± 0.52
0.3		$234.15 \pm 54.95^{b,A}$	2.89 ± 0.30	4.55 ± 0.51
0.6		$245.66 \pm 48.06^{a,A}$	2.88 ± 0.28	4.43 ± 0.54
	3.2	$279.98 \pm 30.57^{a,A}$	$2.69 \pm 0.23^{b,B}$	$4.87 \pm 0.46^{a,A}$
	4.0	$214.81 \pm 61.51^{b,B}$	$2.78 \pm 0.27^{b,B}$	$4.35 \pm 0.46^{b,B}$
	5.4	$189.26 \pm 39.40^{c,C}$	$3.03 \pm 0.25^{a,A}$	$4.43 \pm 0.51^{b,B}$
P-value				
Se		0.0001	0.0870	0.2159
Met		0.0001	0.0001	0.0006
Se × Met		0.0001	0.1262	0.5271

$^{a-e}$Means in a column with different superscripts are significantly different $(P < 0.05)$.
$^{A-D}$Means in a column with different superscripts are significantly different $(P < 0.01)$.

Table 5. Effects of Se yeast and Met supplementation of the maternal diets on the contents of glutathione (GSH) and carbonyl group and glutathione peroxidase (GSH-Px) activity in the albumen of eggs

4.3 GSH-Px activity

As can be seen from Tables 4 and 5, the inclusion of Se yeast and Met in the diets significantly increased or decreased the GSH-Px activity in eggs ($P < 0.01$). The inclusion of Se yeast in the maternal diets increased GSH-Px activity in both the yolk and the albumen of eggs. However, adding 0.6 mg of Se/kg decreased the GSH-Px activity in the egg yolk compared with 0 and 0.3 mg of Se/kg. The GSH-Px activity in the albumen of eggs significantly decreased with increasing Met supplementation, whereas the albumen GSH-Px activity of the 3.2 g of Met/kg treatment was significantly higher than those of the 4.0 and 5.4 g of Met/kg treatments. In the egg yolk, the inclusion of the 0.3 mg of Se/kg and 3.2 g of Met/kg treatments had the maximal GSH-Px activity, whereas a combination of the 0.6 mg of Se/kg and 5.4 g of Met/kg treatments had the minimal GSH-Px activity. In the egg albumen, a combination of the 0.3 mg of Se/kg and 3.2 g of Met/kg treatments had the maximal GSH-Px activity and adding 5.4 g of Met/kg alone had the minimal GSH-Px activity.

4.4 Lipid oxidation

The influence of Se yeast, Met, and their interactions on the MDA content in the egg yolk was remarkable ($P < 0.01$; Table 2). The MDA content in the egg yolk significantly decreased with Met supplementation ($P < 0.01$). However, adding 0.3 mg of Se/kg to the diets significantly decreased the MDA content in the egg yolk compared with the 0 and 0.6 mg of Se/kg treatments. In the egg yolk, a combination of 0.3 mg of Se/kg and 5.4 g of Met/kg had the minimal MDA content and adding 3.2 g of Met/kg alone to the diet had the maximal MDA content.

Effect of Maternal Selenium and Methionine on Poultry
Products (Egg and Meat) Qualities and Oxidative Stability
281

4.5 Protein oxidation

Table 5 showed that Met supplemented at 4.0 and 5.4 g/kg (irrespective of Se supplementation) decreased carbonyl content in the egg albumen compared with the 3.2 g of Met/kg treatment. Adding 3.2 g of Met/kg alone to the diet created the maximal carbonyl content in the egg albumen, whereas a combination of the 0.6 mg of Se/kg and 4.0 g of Met/kg treatments created the minimal carbonyl content.

In summary, the results can be concluded as:

1. Supplementing 0.3 mg/kg **Se** increased egg albumen weight, but decreased egg yolk weight. However, supplementing 5.4 g of DL-**Met** increased egg albumen weigh, decreased egg yolk weight. There was no significant interaction between **Se** and **Met** on egg albumen weight, egg yolk weight, egg shape index and haugh unit ($P > 0.05$), while they significantly influenced egg yolk weight, egg hell weight and egg albumen height ($P < 0.05$).

2. Increasing Se yeast supplementation significantly increased **Se** concentration in the egg yolk ($P < 0.01$) and the **Se** concentration of the 3.2 g of **Met**/kg treatment was higher than those of the 4.0 and 5.4 g of **Met**/kg treatments. Adding 0.3 mg of **Se**/kg to the diet significantly increased **GSH-Px** activity in the egg yolk compared with 0 and 0.6 mg of **Se**/kg ($P < 0.01$) and increasing **Se** yeast supplementation significantly increased the **GSH-Px** activity in the egg albumen ($P < 0.01$). Increasing **Met** supplementation significantly decreased the **GSH-Px** activity in the egg albumen and yolk ($P < 0.01$). Methionine supplemented at 3.2 and 4.0 g/kg significantly increased glutathione concentration in the egg yolk compared with 5.4 g of **Met**/kg ($P < 0.01$) and increasing **Met** supplementation increased the glutathione concentration in the egg albumen. Increasing **Met** supplementation significantly decreased **MDA** concentration in the egg yolk ($P < 0.01$) and **Se** supplemented at 0 and 0.6 mg/kg increased the malondialdehyde concentrations in the egg yolk compared with 0.3 mg of **Se**/kg ($P < 0.01$). Methionine supplemented at 4.0 and 5.4 g/kg significantly decreased carbonyl concentration compared with 3.2 g of Met/kg.

Selenium concentration in the egg yolk depends on both the Se content and form in the maternal diet. Several authors (Cantor, 1997; Paton et al., 2000; Surai, 2000) reported that organic Se (e.g., Se-Met) is more efficiently deposited in the egg yolk. Davis and Fear (1996) found that both sodium selenite and Se-Met fed at 2 mg/kg increased the Se concentration in the egg yolk compared with a diet that was not supplemented. Paton et al. (2002) found that increasing Se levels (irrespective of source) produced a significant linear increase in egg Se concentration and the linear responses were different for the different Se sources in all egg components. The Se used in this experiment is the Se-enriched yeast that is produced by growing the yeast *Saccharomyces cerevisiae* in a high-Se medium (AAFCO, 2003). Beilstein and Whanger (1986) and kelly and Power (1995) reported that the majority of the Se in Se yeast is Se-Met, a Se analog of Met. Selenomethionine can be an excellent analog for Met in biochemical reactions because of the similar covalent radii of Se and S. The result of this current study showed that increasing Se supplementation significantly increased Se deposition in the egg yolk. However, adding 4.0 and 5.4 g of Met/kg to the maternal diet significantly decreased the Se concentration in the egg yolk compared with 3.2 g of Met/kg. It is possible that the chemical similarity between Se-Met and Met allows the body to use them interchangeably in protein synthesis (Surai, 2002), which makes it possible to build Se reserves in the other parts of eggs and decreases Se concentration in the egg yolk during storage.

Met supplementation of the diet significantly influenced the GSH concentration in both the yolk and the albumen of eggs. It might be due to the fact that Met provided plenty of Cys during metabolism for GSH synthesis when the hens were fed an excess of Met (Beatty and Reed, 1980). However, increasing dietary Se yeast supplementation did not significantly influence the GSH concentration. A possible reason is that when an excess of Se yeast is supplemented in the diet in the form of Se-Met, this amino acid will be nonspecifically incorporated into various proteins (Surai, 2002), which decreases GSH synthesis.

Selenium is an essential component of a variety of selenoproteins, the best known of which is GSH-Px. The GSH-Px family of enzymes is a crucial player in the antioxidant system, neutralizing potential threats to the integrity of cellular macromolecules by eliminating hydrogen peroxide and detoxifying lipid hydroperoxides (Brigelius-Flohé, 1999). The Se derived from hen is deposited in eggs and is distributed among the developing tissues during embryogenesis (Gaal et al., 1995; Surai, 2000; Paton et al., 2002). Consequently, the Se supplementation of the maternal diet has an effect on the GSH-Px activity in the egg yolk. Wakebe (1998) found that adding 0.3 mg of Se/kg to the diet increased GSH-Px activity in both the yolk and the albumen of eggs. Surai (2000) reported that the GSH-Px activity in the liver of newly hatched chicks significantly decreased when the hens were fed a low level of Se diet. This current study also showed that the inclusion of Se yeast in the maternal diet increased GSH-Px activity in both the yolk and the albumen of eggs. However, adding 0.6 mg of Se/kg to the diet decreased the GSH-Px activity in the egg yolk compared with the 0 and 0.3 mg of Se/kg treatments. The mechanism of this observation is not known. A possible reason is that high dietary Se-Met is preferentially incorporated into proteins rather than used for GSH-Px when Met is limiting in the maternal diet (Waschulewski and Sunde, 1988).

Methionine participates in methyl group metabolism and the synthesis of other S amino acids, notably Cys. Cysteine is required for the synthesis of GSH and taurine, which are important compounds for host defense against oxidative stress (Métayer et al., 2008). This current study showed that increasing Met supplementation in the maternal diet decreased the GSH-Px activity in both the yolk and the albumen of the eggs. It is possible that adding an excess of Met to the diets leads to preferential incorporation of Se-Met into the body (e.g., eggs) in a form other than as GSH-Px (Zhao et al., 2009). Thus, the utilization of Se-Met supplementation for GSH-Px synthesis substantially decreases when the hens are fed an excess of Met. This study also indicated that a combination of the 0.3 mg of Se/kg and 3.2 g of Met/kg treatments had the maximal GSH-Px activity in the egg yolk and the second largest GSH-Px activity (slightly lower than that of the 0.6 mg of Se/kg and 4.0 g of Met/kg treatments) in the eggs, which probably means that inclusion of 0.3 mg of Se/kg and 3.2 g of Met/kg in the diet provides enough Se to the egg for the requirement for maximum Se-GSH-Px activity. It is possible that an appropriate amount of Se in the diet achieves the GSH-Px activity through Se-GSH-Px gene expression and cytosolic mRNA stabilization (Chris-tensen and Burgener, 1992) or regulates the level of GSH-Px mRNA in a posttranscriptional step (Toyoda et al., 1990).

Egg quality decreases during storage. This process is associated with biochemical changes, including lipid and protein oxidation. Lipid oxidation causes loss of nutrition. One such product is MDA, which has long been considered as an index of oxidative rancidity (Cortinas et al., 2005). Increasing Se concentration in combination with other antioxidants (vitamin E and carotenoids) could be an effective means to prevent the damaging effect of

free radicals produced in eggs (Surai, 2002). This study demonstrated that adding 0.3 mg of Se/kg to the diet significantly decreased the MDA content in the egg yolk compared with 0 and 0.6 mg of Se/kg treatments. This can be explained as a result of increased GSH concentration and GSH-Px activity and changes in lipid composition (Noble and Cocchi, 1990).

However, the MDA contents of adding 0.6 mg of Se/kg to the diet were higher than that of the 0.3 mg of Se/kg treatment. The mechanism of this observation is not known. This study also showed that Met supplementation of the diet significantly decreased the MDA content in the egg yolk. This might be due to the fact that Met provides plenty of Cys for GSH synthesis when the hens are fed an excess of Met (Beatty and Reed, 1980), which inhibits lipid oxidation and decreases MDA content in eggs.

Eggs can be divided into 3 components: albumen, yolk, and shell. Albumen, which contains approximately 67% of the protein content of the egg (Romanoff and Romanoff, 1949), provides more proteins for assimilation into tissue during embryonic development (Finkler et al., 1998). Egg storage before incubation can be associated with protein oxidation within the egg albumen. Carbonyl contents can be considered as a marker for protein oxidation because amino acid residues of proteins, such as Lys, Met, and Cys, can be oxidized to carbonyl derivatives by oxidative stresses (Butterfield et al., 1998). Therefore, protein oxidation affects the physicochemical and functional properties of egg white. This current study indicated that adding Se and Met to the hens' diets could decrease carbonyl content in the egg albumen. Adding 3.2 g of Met/kg alone to the hens' diets created the maximal carbonyl content in the egg albumen, probably due to the fact that the eggs from hens fed a low-Se diet had low GSH content in the egg albumen. However, the inclusion of 0.6 mg of Se/kg and 4.0 g of Met/kg in the hens' diets created the minimal carbonyl content in the egg albumen. This can be explained as a result of the higher GSH content and highest GSH-Px activity (Table 3), thus slowing the rate of protein oxidation (Pappas et al., 2005).

To sum up, a conclusion was drawn that **Se** yeast and **Met** supplementation of the maternal diets could improve egg quality, meat quality, meat protein functionalities and the oxidative stability of meat proteins and lipid to varying degrees.

5. References

AAFCO. 2003. Page 285 in Feed Ingredient Definitions. 57. Mineral Products. Tentative T57:163 Official Publication. Association of American Feed Control Officials Inc., Olympia, WA.

Angelbeck, J.H., Du Bru, E.F. 1983. The effect of neonatal testosterone on specific male and female patterns of phosphorylated cytosolic proteins in the rat preoptichypothalamus, cortex and amygdala. Brain Res 264(2):277–83.

Baker, D.H. 2006. Comparative species utilization and toxicity of sulfur amino acids. J Nutr 136(6):1670S–5S.

Baron, C. P., and J. H. Andersen. 2002. Myoglobin-induced lipid oxidation. A review. J. Agric. Food Chem. 50:3887–3897.

Beatty, P. W., and D. J. Reed. 1980. Involvement of the cystathionine pathway in the biosynthesis of glutathione by isolated rat hepatocytes. Arch. Biochem. Biophys. 204:80–87.

Beilstein, M. A., and P. D. Whanger. 1986. Deposition of dietary organic and inorganic selenium in rat erythrocyte proteins. J. Nutr. 116:1701–1710.

Beilstein, M. A., and P. D. Whanger. 1986. Deposition of dietary organic and inorganic selenium in rat erythrocyte proteins. J. Nutr. 116:1701–1710.

Brigelius-Flohé, R. 1999. Tissue-specific functions of individual glutathione peroxidases. Free Radic. Biol. Med. 27:951–965.

Butterfield, D. A., T. Koppal, B. Howard, R. Subramaniam, N. Hall, K. Hensley, S. Yatin, K. Allen, M. Aksenov, M. Aksenova, and J. Carney. 1998. Structural and functional changes in proteins induced by free radical-mediated oxidative stress and protective action of the antioxidants N-tert-butyl-α-phe-nylnitrone and vitamin E. Ann. N. Y. Acad. Sci. 854:448–462.

Cantor, A. H. 1997. The role of selenium in poultry nutrition. Pages 155–164 in Biotechnology in the Feed Industry. T. P. Lyons and K. A. Jacques, ed. Proc. Alltech 13th Annu. Symp. Nottingham University Press, UK.

Cheah, K. S., A. M. Chreah, and D. J. Krausgrill. 1995. Effect of dietary supplementary vitamin E on pig meat quality. Meat Sci.39:255–265.

Christensen, M. J., and K. W. Burgener. 1992. Dietary selenium stabilises glutathione peroxidase mRNA in rat liver. J. Nutr. 122:1620–1626.

Cortinas, L., A. Barroeta, C. Villaverde, J. Galobart, F. Guardiola, and M. D. Baucells. 2005. Influence of the dietary polyunsaturation level on chicken meat quality: Lipid oxidation. Poult. Sci. 84:48–55.

Daun C, Akesson B. 2004. Comparison of glutathione peroxidase activity, and of total and soluble selenium content in two muscles from chicken, turkey, duck, ostrich and lamb. Food Chem 85:295–303.

Davis, R. H., and J. Fear. 1996. Incorporation of selenium into egg proteins from dietary selenite. Br. Poult. Sci. 37:197–211.

Decker, E. A., Y. L. Xiong, J. T. Calvert, A. D. Crum, and S. P. Blanchart. 1993. Chemical, physical and functional properties of oxidized turkey white muscle myofibrillar proteins. J. Agric.

Food Chem. 41:186–189.

Desai M, Crowther, N.J., Ozanne, S.E., Lucas A, Hales, C.N. 1995. Adult glucose and lipid metabolism may be programmed during fetal life. Biochem Soc Trans 23(2):331–5.

Devore, V. R., G. R. Colnago, L. S. Jensen, and B. E. Greene. 1983. Thiobarbituric acid values and glutathione peroxidase activity in meat from chickens fed a selenium-supplemented diet. J. Food Sci. 48:300–301.

Edens, F. W. 1996. Organic selenium: From feathers to muscle integrity to drip loss. Five years onward: No more selenite. Pages 165–185 in Biotechnology in the Feed industry. Proceedings of Alltech's 12th Annual Symposium. T. P. Lyons and K. A.Jacques, ed. Nottingham University Press, Nottingham, UK.

Edens, F. W. 2001. Involvement of Sel-Plex in physiological stability and performance of broiler chickens. Pages 349–376 in Biotechnology in the Feed Industry. Proceedings of Alltech's 17th Annual Symposium. T. P. Lyons and T. A. Jacques, ed. Nottingham University Press, Nottingham, UK.

Elisabeth, H. L., and M. L. Steven. 2005. Mechanisms of waterholding capacity of meat: The role of postmortem biochemical and structural changes. Meat Sci. 71:194–204.

Esaki N, Nakamura T, Tanaka H, Soda K. 1982. Selenocysteine lyase, a novel enzyme that specifically acts on selenocysteine. Mammalian distribution and purification and properties of pig liver enzyme. J Biol Chem 257:4386–91.

Faustman, C., and R. G. Cassens. 1990. The biochemical basis for discoloration in fresh meat: A review. J. Muscle Foods 1:217–243.

Finkler, M. S., J. B. Van Orman, and P. R. Sotherland. 1998. Experimental manipulation of egg quality in chickens: Influence of albumen and yolk on the size and body composition of near-term embryos in a precocial bird. J. Comp. Physiol. B 168:17–24.

Gaal, T., M. Mezes, R. C. Noble, J. Dixon, and B. K. Speake. 1995. Development of antioxidant capacity in tissues of the chick embryo. Comp. Biochem. Physiol. B 112:711–716.

Hoffman, J.L., McConnell, K.P., Carpenter, D.R. 1970. Aminoacylation of Escherichia coli methionine tRNA by selenomethionine. Biochim Biophys Acta 199:531–4.

Kelly, M. P., and R. F. Power. 1995. Fractionation and identification of the major selenium containing compounds in selenized yeast. J. Dairy Sci. 78(Suppl. 1):237. (Abstr.)

Lucas A, Morley R, Cole, T.J., Gore, S.M., Lucas, P.J., Crowle P, Pearse R, Boon, A.J., Powell R. 1990. Early diet in preterm babies and developmental status at 18 months. Lancet 335(8704):1477–81.

Lucas A, Baker, B.A., Desai M, Hales, C.N. 1996. Nutrition in pregnant or lactating rats programs lipid metabolism in the offspring. Br J Nutr 76(4):605–12.

Mancini, R. A., and M. C. Hunt. 2005. Current research in meat color. Meat Sci. 71:100–121.

McConnell, K.P., Cho, G.J. 1965. Transmucosal movement of selenium. Am J Physiol 208:1191–5.

McConnell, K.P., Hoffman, J.L. 1972. Methionine-selenomethionine parallels in rat liver polypeptide chain synthesis. FEBS Lett 24:60–2.

Mercier Y, Gattellier P, Viau M, Remingnon H, Renerre M. 1998. Effect of dietary fat and vitamin E on lipid and protein oxidation in turkey meat during storage. Meat Sci 48:301–17.

Mercier, Y., P. Gatellier, and M. Renerre. 2004. Lipid and protein oxidation in vitro, and antioxidant potential in meat from Charolais cows finished on pasture or mixed diet. Meat Sci.66:467–473.

Mercier, Y., P. Gatellier, M. Viau, H. Remignon, and M. Renerre.1998. Effect of dietary fat and vitamin E on color stability and on lipid and protein oxidation in turkey meat during storage. Meat Sci. 48:301–318.

Métayer, S., I. Seiliez, A. Collin, S. Duchêne, Y. Mercier, P. A. Geraert, and S. Tesseraud. 2008. Mechanisms through which sulfur amino acids control protein metabolism and oxidative status. J. Nutr. Biochem. 19:207–215.

Mosharov, E., M. R. Cranford, and R. Banerjee. 2000. The quantitatively important relationship between homocysteine metabolism and glutathione synthesis by the transsulfuration pathway and its regulation by redox changes. Biochemistry 39:13005–13012.

Noble, R. C., and M. Cocchi. 1990. Lipid metabolism in the neonatal chicken. Prog. Lipid Res. 29:107–140.

Oliver, C. N., B. W. Alin, E. J. Moerman, S. Goldstein, and E. R. Stadtman. 1987. Age-related changes in oxidized proteins. J. Biol. Chem. 262:5488–5491.

Pappas, A. C., T. Acamovic, N. H. C. Sparks, P. F. Surai, and R. M. McDevitt. 2005. Effects of supplementing broiler breeder diets with organic selenium and polyunsaturated fatty acids on egg quality during storage. Poult. Sci. 84:865–874.

Pappas, A. C., F. Karadas, P. F. Surai, and B. K. Speake. 2005. The selenium intake of the female chicken influences the selenium status of her progeny. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 142:465–474.

Paton, N. D., A. H. Cantor, A. J. Pescatore, M. J. Ford, and C. A. Smith. 2000. Effect of dietary selenium source and level of inclusion on selenium content of incubated eggs. Poult. Sci. 79(Suppl.1):40. (Abstr.)

Paton, N. D., A. H. Cantor, A. J. Pescatore, M. J. Ford, and C. A. Smith. 2002. The effect of dietary selenium source and level on the uptake of selenium by developing chick embryos. Poult. Sci. 81:1548–1554.

Payne, R. L., T. K. Lavergne, and L. L. Southern. 2005. Effect of inorganic versus organic selenium on hen production and selenium concentration. Poult. Sci. 84:232–237.

Racanicci, A.M.C, Danielsen B, Menten, J.F.M, Reginato-d'Arce, M.A.B., Skibsted, L.H. 2004. Antioxidant effect of dittany (Origanum dictamnus) in pre-cooked meat balls during chill-storage in comparation to rosemary (Rosmarinus officinalis). Eur Food Res Technol 218:521–4.

Romanoff, A. L., and A. J. Romanoff. 1949. The Avian Egg. John Wiley and Sons, New York, NY.

Ryu, Y. C., M. S. Rhee, K. M. Lee, and B. C. Kim. 1995. Effects of different levels of dietary supplemental selenium on performance, lipid oxidation, and colour stability of broiler chicks. Poult. Sci.84:809–815.

Snoeck A, Remacle C, Reusens B, Hoet, J.J. 1990. Effect of a low-protein diet during pregnancy on the fetal rat endocrine pancreas. Biol Neonate 57(2):107–18.

Steele, R.D., Benevenga, N.J. 1978. Identification of 3-methylthiopropionic acid as an intermediate in mammalian methionine metabolism. J Biol Chem 253:7844–50.

Sunde, R.A. 1984. The biochemistry of selenoproteins. J AmOil Chem Soc 61:1891–900.

Sunde, R.A., Evenson, J.K. 1987. Serine incorporation into the selenocysteine moiety of glutathione peroxidase. J Biol Chem 262:933–7.

Effect of Maternal Selenium and Methionine on Poultry
Products (Egg and Meat) Qualities and Oxidative Stability
287

Sunde, R.A., Gutzke, G.E., Hoekstra,W.G. 1981. Effect of dietarymethionine on the biopotency of selenite and selenomethionine in the rat. J Nutr 111:76–86.

Surai, P. F. 2000. Effect of selenium and vitamin E content of the maternal diet on the antioxidant system of the yolk and the developing chick. Br. Poult. Sci. 41:235–243.

Surai, P. F., and N. C. Sparks. 2000. Tissue-specific fatty acid and α-tocopherol profiles in male chickens depending on dietary tuna oil and vitamin E provision. Poult. Sci. 79:1132–1142.

Surai, P. F., and J. E. Dvorska. 2002a. Effect of selenium and vitamin E on lipid peroxidation in thigh muscle tissue of broiler breeder hens during storage. Arch. Geflugelkd. 66:120.

Surai, P. F., and J. E. Dvorska. 2002b.Effect of selenium and vitamin E content of the breeder's diet on lipid peroxidation in breast muscles during storage. Proceedings of Australian Poultry Science Symposium, Sydney. 14:187–192.

Surai, P. F. 1999. Tissue-specific changes in the activities of antioxidant enzymes during the development of the chicken embryo. Br. Poult. Sci. 40:397–405.

Surai, P. F. 2002. Selenium in poultry nutrition: 2.Reproduction, egg and meat quality and practical applications. World's Poult. Sci. J. 58:431–450.

Surai, P. F., R. C. Noble, and B. K. Speake. 1996. Tissue specific differences in antioxidant distribution and susceptibility to lipid peroxidation during development of the chick embryo. Biochem. Biophys. Acta 1304:1–10.

Surai, P. F., and B. K. Speake. 1998. Distribution of carotenoids from the yolk to the tissues of the chick embryo. J. Nutr. Biochem.9:645–651.

Surai, P. F., B. K. Speake, and N. C. Spark. 2001a. Carotenoids in avian nutrition and embryonic development: 1. Absorption, availability and levels in plasma and egg yolk. Poult. Sci. 38:1–27.

Surai, P. F., B. K. Speake, and N. C. Spark. 2001b. Carotenoids in avian nutrition and embryonic development: 2. Antioxidant properties and discrimination in embryonic tissues. Poult. Sci.38:117–145.

Toyoda, H., S. Himeno, and N. Imura. 1990. Regulation of glutathione peroxidase mRNA level by dietary selenium manipulation. Biochim. Biophys. Acta 1049:213–215.

Ventanas, S., M. Estevez, J. F. Tejeda, and J. Ruiz. 2006. Protein and lipid oxidation in longissimus dorsi and dry-cured loin from Iberian pigs as affected by crossbreeding and diet. Meat Sci. 72:647–655.

Wakebe, M. 1998. Organic selenium and egg freshness. Patent 10-23864. Feed for meat chickens and feed for laying hens. Japanese Patent Office. Application Heisei 8-179629.

Waschulewski, I.H., Sunde, R.A. 1988a. Effect of dietary methionine on the tissue selenium and glutathione peroxidase (EC 1.11.1.9) activity in rats given selenomethionine. Br J Nutr 60:57–68.

Waschulewski, I.H., Sunde, R.A. 1988b. Effect of dietary methionine on utilization of tissue selenium from dietary selenomethionine for glutathione peroxidase in the rat. J Nutr 118:367–74.

Wilson, J.X., Lui, E.M.K., Del RF. 1992. Developmental profiles of antioxidants and trace elements in chick embryo.Mech Ageing Dev 65:51–64.

Zhao, L. Y., S. Q. Xu, R. Q. Zhao, Z. Q. Peng, and X. J. Pan. 2009. Effects of selenium and methionine supplementation of breeder hen diets on selenium concentration and oxidative stability of lipids in the thigh muscles of progeny. J. Food Sci. 74:569–574.

Soy and Soy-Based Foods: Role in Health and Nutrition

Ana Villares, Ana García-Lafuente, Irene Palacios,
Miguel Lozano, Carlos Moro and Eva Guillamón
*Centro para la Calidad de los Alimentos, Instituto Nacional de Investigación y
Tecnología Agraria y Alimentaria (INIA),
Campus Universitario Duques de Soria, Soria
Spain*

1. Introduction

The soybean seeds (*Glycine max*) originate from north-eastern Asian regions, especially China and Korea and, since its introduction to American colonies in 1765 (Hymowitz and Harlan 1983), its consumption has been worldwide spread. Soybeans are an economically important crop, which has been traditionally used for animal feed as well as for human food. In this field, soy has been a valuable resource for humankind by providing excellent proteins and other nutrients and, in many countries, soybeans and soy-based foods are considered as a staple food thanks to its low cost and high availability (Khetarpaul et al. 2004). Presently, in the market, there are several soy-based products and ingredients such as soy sauce, tofu, soy protein, soy-based beverages and fermented foods, like miso, tempeh, natto, etc. Besides the excellent nutritional properties such as the high protein content and the presence of polyunsaturated fatty acids, as well as the absence of cholesterol and lactose; soy has demonstrated to provide a preventive effect in the occurrence and development of several common diseases.

In the developed countries, the incidence and prevalence of illnesses like diabetes, osteoporosis, cancer, or cardiovascular diseases, among others, are becoming highly important and some of them constitute the leading causes of mortality. Generally, oxidative damage, inflammatory responses or lipid accumulation as well as other more complex biochemical processes are involved in the disease development mechanisms. Scientific studies have revealed that most of these illnesses are strongly related to genetic and/or environmental factors. In this field, the diet has shown to play an important role in preventing the appearance or even reducing the associated symptoms. The lower incidence of these diseases in eastern societies, where the soy consumption is considerably high, remarks the importance of diet and highlights the beneficial properties of soy. Many of the soy benefices have been attributed to the occurrence of several kinds of phytochemicals, such as isoflavones, soyasaponins, phytates, or protease inhibitors. These bioactive compounds seem to provide a preventive effect by means of reducing or even inhibiting the mechanisms of the disease development. Hence, some of their most important properties, for instance, the antioxidant, the antiinflammatory or the estrogenic activities, may be involved in the disease prevention.

In this chapter, the most important aspects of soy composition will be discussed in terms of nutritional profile and the occurrence of bioactive compounds. The influence of soy consumption on the disease prevention and the role that the phytochemicals play in the mechanisms of health promotion will be reviewed.

2. Nutritional profile of soy

Nowadays, there is a clear increase in the amounts of commercial soy-based foods so that the soy consumption has risen greatly. Some products are becoming increasingly popular and they are largely consumed by people in general, such as soy sauces and soy-based beverages. On the other hand, soy protein products are largely used as ingredients in meat products, breads, soups, and beverages, among others. New soy foods are continuously being developed and actually include cheese, salami, drinks and vegetarian meat substitutes. Traditionally, soy has been consumed either as fermented or as nonfermented foods (Wang and Murphy 1996; Umphress et al. 2005). The most common fermented soy-based foods include tempeh, miso, natto, sufu, soy sauce, and douchi, whereas the nonfermented foods comprise fresh soybeans, soybean sprouts, soymilk, tofu, and protein enriched foods such as soy protein flours and/or grits, textured soy protein and soy protein concentrates or isolates. Nutrient composition can be influenced not only by the genetics, the cultivar, or the growth conditions, but also by the processing and refinement; therefore, significant variations can be detected in the soybeans and soy-based foods nutritional profile (Grieshop and Fahey 2001; Grieshop et al. 2003).

Whole soy protein content usually ranges between 30 and 48% (Cai et al. 1997; Esteves et al. 2010). Glycinin toghether with α-, β-, and γ-conglycinins are soybean globulins and they represent the major components of the storage proteins of soy seeds. The seeds also contain bioactive proteins, including α-amylase, cytochrome *c*, lectin, lipoxygenase, urease and protease inhibitors (Wolf 1970). Soy is rich in essential amino acids, like lysine, isoleucine and leucine; and also in other dispensable ones, such as glutamic and aspartic acids. Nevertheless, sulphur-amino acids (cysteine and especially methionine), tryptophan and valine are limiting amino acids in soy (Aletor 2010; Anuonye et al. 2010).

Lipid content is considerably high in soy (17-25%) (Cai et al. 1997) although it is not usually a disadvantage because soy is generally used to produce edible oil and fat. Soy provides polyunsaturated fatty acids, being linoleic acid the most abundant, occurring between 42 and 58%. Significant amounts of oleic (20-25%), palmitic (8-11%), linolenic (1-8%) and stearic (3-6%) acids are also found whereas fatty acids with higher carbon number (eicosanoic, eicosenoic, docosanoic and tetracosanoic acids) are only detected in trace amounts (0.1-0.5%) (Vieira et al. 1999; Yuan and Chang 2007).

Carbohydrates constitute approximately the 30-35% of the whole soy seed, especially polysaccharide and dietary fibre. Starch, hemicelluloses, cellulose, stachylose, sucrose, raffinose, arabinose and glucose are included in its carbohydrate fraction. Dietary fibre, as non-starch polysaccharides, is mainly found in the hulls and the content range between 9 and 16% of whole soy seed (Esteves et al. 2010). The insoluble fraction is the predominant, comprising approximately 74-78%. It is mainly composed of arabinose, galactose, glucose, xylose and uronic acids. Soluble fibre ranges from 22 to 26% and the principal monomers are arabinose, galactose and uronic acids (Redondo-Cuenca et al. 2006).

Among the nutritionally important major minerals (table 1), whole soy shows high contents of potassium, phosphorous, calcium, magnesium and sodium (Vieira et al. 1999; Aletor 2010).

	K	P	Ca	Mg	Na
Soy content (mg/100 g DW)	1600-1900	450-650	170-320	215-270	10-21

Table 1. Major minerals present in soy (DW: dried weight).

Furthermore, other trace minerals are also found (table 2), for instance, iron, zinc, manganese, cooper and selenium, nickel and chromium (Anuonye et al. 2010).

	Fe	Zn	Mn	Cu	Se	Ni	Cr
Soy content (mg/100 g DW)	13-19	3-5	2-3	~2	~2	~1	~1

Table 2. Minor minerals detected in soy (DW: dried weight).

Soy contains water soluble vitamins (table 3), such as ascorbic acid (vitamin C) whose content varies from 0.03 to 35 mg/100 g dried weight, riboflavin (B_2) from 0.7 to 0.9 mg/100 g dried weight and B_6, which is the most abundant (2-5.5 mg/100 g dried weight) (Anuonye et al. 2010). Vitamin E, a lipid soluble vitamin is also detected in soy; thus, four kinds of tocopherol compounds are found in soy: α-tocopherol, whose content varies from 0.4 to 8 mg/100 g dried weight; γ-tocopherol, from 4 to 80 mg/100 g dried weight; δ-tocopherol, from 1 to 50 mg/100 g dried weight; and a trace amount of β-tocopherol (Kasim et al. 2010; Li et al. 2010).

	Vit. C	Vit. B_2	Vit. B_6	α-Toc.	γ-Toc.	δ-Toc.	β-Toc.
Soy content (mg/100 g DW)	0.03-35	0.7-0.9	2-5.5	0.4-8	4-80	1-50	traces

Table 3. Vitamins found in soy (DW: dried weight).

3. Bioactive compounds occurring in soy

Soy is a source not only of proteins, vitamins and minerals, but also of many bioactive compounds, such as isoflavones, protease inhibitors, saponins, and phytates. Substantial changes of phytochemical content in soybean seeds are found depending on the source because, as well as the nutritional profile, the functional composition is strongly affected by genetic and environmental factors, together with the processing conditions. The great importance of these compounds is based on their biochemical activity which results in health promotion and disease prevention. The activity of phytochemicals in the human body may be strongly influenced by absorption and bioavailability. Thus, relative disposition appears to be determined by the chemical nature, in terms of solubility and susceptibility to degradation by gut microorganisms.

3.1 Isoflavones

Soy has attracted much attention due to its healthy benefits associated with the presence of isoflavones. They comprise a group of naturally occurring flavonoids, which are heterocyclic phenols, possessing three aromatic rings with several hydroxyl groups. Soy contains three main types of isoflavones occurring in different chemical forms, which are the aglycones (daidzein, genistein and glycitein), the β-glucosides (daidzin, genistin and glycitin), the 6″-O-acetyl-β-glucosides and 6″-O-malonyl-β-glucosides. Furthermore, the 4′-

methyl ethers of daidzein and genistein, formononetin and biochanin A, are also detected in soy (figure 1).

R₁	R₂	R₃	

R_1	R_2	R_3	
H	H	OH	Daidzein
OH	H	OH	Genistein
H	OCH₃	OH	Glycitein
H	H	OCH₃	Formononetin
OH	H	OCH₃	Biochanin-A

R_1	R_2	R_3	
H	H	H	Daidzin
OH	H	H	Genistin
H	OCH₃	H	Glycitin
H	H	COCH₃	6''-O-Acetyldaidzin
OH	H	COCH₃	6''-O-Acetylgenistin
H	OCH₃	COCH₃	6''-O-Acetylglycitin
H	H	COCH₂COOH	6''-O-Malonyldaidzin
OH	H	COCH₂COOH	6''-O-Malonylgenistin
H	OCH₃	COCH₂COOH	6''-O-Malonylglycitin
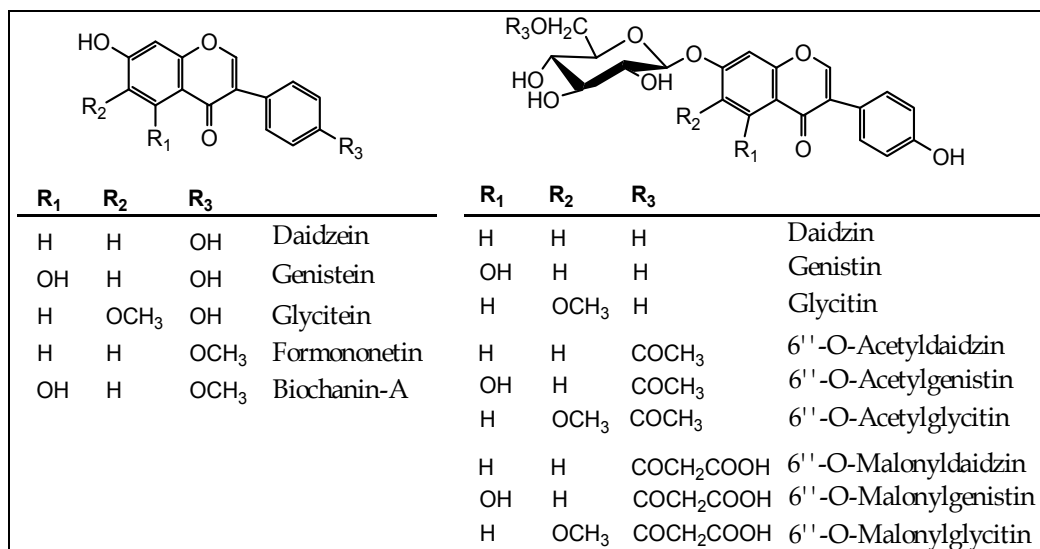
Fig. 1. Chemical structure of the main isoflavones occurring in soy.

Total isoflavone content in whole soy usually ranges from 50 to 450 mg/100 g dried weight (Kim et al. 2005). Generally, the β-glucosides account for the 30-35%, the 6″-O-malonyl-β-glucosides are the most abundant (50-65%), the aglycone content is less important (4-12%) and the 6″-O-acetyl-β-glucosides are usually scarce (0-5%) (Franke et al. 1999; Genovese et al. 2006). These percentages may change significantly when soy is processed since the 6″-O-malonyl-β-glucosides are instable under heat so that they are converted into the corresponding aglycones (Villares et al. 2011). Daizein, genistein and their derivatives are commonly the most abundant isoflavones in soy whereas glycitein is sometimes only detected as traces. About 80-90% of total seed isoflavones are located in the cotyledons (Tsukamoto et al. 1995).

Isoflavones have attracted a great deal of attention due to their antioxidant (Kao and Chen 2006; Chung et al. 2008), antiinflammatory (Park et al. 2007; García-Lafuente et al. 2009), and antiallergic (Chang et al. 2000) properties. These compounds have shown to reduce the risk of cardiovascular disease (Jackman et al. 2007), and promote the inhibition of cancer cell growth (Sarkar and Li 2003; Kao et al. 2007; Davis et al. 2008). Furthermore, soy intake plays an important role in the prevention of several ailments including osteoporosis, and menopausal symptoms (Dijsselbloem et al. 2004; Phrakonkham et al. 2007; Coxam 2008; Ma et al. 2008) since the isoflavones act as antiestrogens (Okamoto et al. 2006; Zhang et al. 2007) and tyrosine protein kinase inhibitors (Papazisis et al. 2006).

3.2 Lectins and trypsin inhibitors

Lectin (hemagglutinin or agglutinin) is a highly specific carbohydrate-binding protein, with the highest affinity for *N*-acetyl-D-galactosamine, and an important role in biological recognition. Soybeans seeds contain between 300 and 600 mg/100 g of lectins (Gu et al. 2010), which is approximately 0.2 – 1% of the soy protein (Rizzi et al. 2003; Anta et al. 2010).

Trypsin inhibitors comprise several protein and peptides including the Bowman-Birk inhibitors, the Kunitz inhibitors and lunasin. Soybean Bowman-Birk inhibitor (BBI) is a polypeptide of 71 amino acids belonging to the serine-protease inhibitor family. BBI tighyly interacts with trypsin or chymotrypsin and strongly inhibits their enzymatic activities (Odani and Ikenaka 1972). The Kunitz soybean inhibitor has been widely studied and consists of a single polypeptide chain crosslinked by two disulfide bridges, which inhibits trypsin and, in a lesser extent, chymotrypsin. Lunasin is a 43 amino acid peptide originally isolated from soy (De Mejia et al. 2004). Trypsin inhibitors usually range from 3000 to 6000 mg/100 g of soy seed (Gu et al. 2010), which comprise between 30 and 125 mg per gram of protein (Esteves et al. 2010).

Lectins and protease inhibitors have been usually considered as antinutrients because they may reduce the nutritional value of soybean (Machado et al. 2008; Ma and Wang 2010). Nevertheless, their intake have shown preventing properties against several diseases, for instance cancer (de Lumen 2005). Soy proteins seem to reduce the total lipids and cholesterol levels in the liver of rats (Potter 1995; Nagaoka et al. 1999). The Bowman-Birk soybean inhibitor has demonstrated to be involved in antiinflammatory processes preventing the development of cancer and coronary diseases (Dia et al. 2008). Furthermore, other peptides from soy have shown antihypertensive properties in spontaneously hypertensive rats (Chen et al. 2004; Gouda et al. 2006).

3.3 Saponins

Saponins are triterpenes or steroid aglycones with one or more sugar chains. The chemical structure comprises a hydrophobic nucleus (sapogenin) to which hydrophilic sugar chains are bound (Guclu-Ustundag and Mazza 2007). Saponins from soy, usually referred as soyasaponins, are classified in A, B and E groups depending on the chemical structure of the aglycones (Shiraiwa et al. 1991). Group A saponins have a hydroxyl group at the C-21 position whereas group B saponins have a hydrogen atom at the same position. Group E saponins are considered the oxidation products from group B saponins and differ from groups A and B by having a carbonyl group at C-22. In figure 2 the chemical structure of main soyasaponins is depicted.

Soy is the primary dietary source of saponins, which are generally in the hypocotyl, and the three groups of soyasaponins occur. The total content in soy may vary from 140 to 975 mg/100 g dried weight (Tsukamoto et al. 1995) and the group B usually accounts for up to 70% of total soyasaponins (Paucar-Menacho et al. 2010), being the subgroups I, αg, βg and γg the most abundant. In the contrary, the saponins II, III, IV, V and γa are found in low amounts (Hubert et al. 2005). The group A soy saponins comprises several species depending on the sugar composition and the position to be attached the sugar moiety, generally the C-3 although sometimes the C-22 position of the aglycones. Among them, subgroup Aa is the most abundant (Paucar-Menacho et al. 2010).

The group A soyasaponins are associated with the undesirable bitter and astringent taste of soy; nevertheless, the group B and E saponins and their labile precursor 2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one – conjugated saponin (DDMP) have shown several health benefits. Thanks to their amphiphilic nature, saponins have been added to pharmaceutical preparations the enhance absorption of bioactive compounds or drugs. In addition, they have been employed as immunological adjuvants in vaccine formulations due to their immune enhancing properties (Kensil et al. 2004). For instance, saponins appear to inhibit the infectivity of the AIDS virus (Nakashima et al. 1989; Vlietinck et al. 1998) and the

Group A soyasaponins		
R₁	**Sugar**	
xyl(2,3,4-triacetyl)-ara	glcU-gal-glc	Soyasaponin Aa
glc(2,3,4,6-tetraacetyl)-ara	glcU-gal-glc	Soyasaponin Ab
glc(2,3,4,6-tetraacetyl)-ara	glcU-gal-rha	Soyasaponin Ac
glc(2,3,4,6-tetraacetyl)-ara	glcU-ara-glc	Soyasaponin Ad
xyl(2,3,4-triacetyl)-ara	glcU-gal	Soyasaponin Ae
glc(2,3,4,6-tetraacetyl)-ara	glcU-gal	Soyasaponin Af
xyl(2,3,4-triacetyl)-ara	glcU-ara	Soyasaponin Ag
glc(2,3,4,6-tetraacetyl)-ara	glcU-ara	Soyasaponin Ah

Group A

Group B soyasaponins		
R₁	**Sugar**	
OH	glcU-gal-rha	Soyasaponin βg
O-DDMP	glcU-ara-rha	Soyasaponin βa
O-DDMP	glcU-gal	Soyasaponin γg
O-DDMP	glcU-ara	Soyasaponin γa
O-DDMP	glcU-gal-glc	Soyasaponin αg
O-DDMP	glcU-ara-glc	Soyasaponin αa
OH	glcU-gal-rha	Soyasaponin I
OH	glcU-ara-rha	Soyasaponin II
OH	glcU-gal	Soyasaponin III
OH	glcU-ara	Soyasaponin IV
OH	glcU-gal-glc	Soyasaponin V

Groups B and E

Group E soyasaponins		
R₁	**Sugar**	
=O	glcU-gal-rha	Soyasaponin I
=O	glcU-ara-rha	Soyasaponin II
=O	glcU-gal	Soyasaponin III
=O	glcU-ara	Soyasaponin IV
=O	glcU-gal-glc	Soyasaponin V

Fig. 2. Chemical structure of the saponins occurring in soy.

herpes simplex virus type 1 (Hayashi et al. 1997). Soyasaponins are reported to be hepatoprotective (Kinjo et al. 2003) and have also shown a preventive effect on the growth of human colon cancer cells (Tsai et al. 2010). The chemical structure significantly influences the saponins bioactivity; thus, individual soyasaponin I exhibits lipid peroxidation inhibitory effects *in vitro*, antiinflammatory properties in mice with TNBS-induced colitis (Lee et al. 2010), and potentially reduces metastatic ability of cancer cells (Chang et al. 2006). Moreover, soyasaponins I and III are reported to induce apoptosis in cells involved in hepatocarcinoma (Zhang and Popovich 2010).

3.4 Phytates

Phytic acid (*myo*-inositol-1,2,3,4,5,6-hexakis dihydrogen phosphate, figure 3) is the main storage source of phosphorus and minerals in many plant tissues. Besides phytate (the salt of phytic acid), other inositol phosphates, such as inositol pentaphosphates and inositol tetraphosphates, exist although to a much lower extent (Schlemmer et al. 2009). Phytic acid is found in many seeds and soy content varies from 1 to 3% (Esteves et al. 2010).Phytates have been traditionally regarded as antinutrients because they form complexes with proteins and minerals resulting in enzyme inhibition and mineral deficiencies.
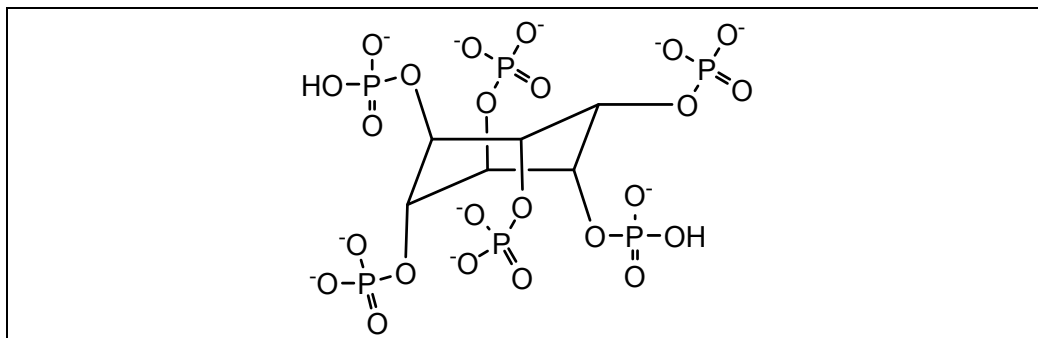
Fig. 3. Chemical structure of *myo*-inositol-1,2,3,4,5,6-hexakis dihydrogen phosphate.

Nevertheless, phytates intake has shown beneficial properties, for instance, blood glucose levels, serum total cholesterol and LDL cholesterol are significantly reduced after their intake (Lee et al. 2006; Lee et al. 2007). Furthermore, their consumption demonstrated to increase bone mineral density, thus, preventing osteopororis (Lopez-Gonzalez et al. 2008). Phytates also inhibit the crystallisation of calcium salts (oxalates and phosphates) avoiding the formation of kidney stones (Grases et al. 2000). The chelating effect of phytates avoids the excess iron accumulation and, therefore, provides a protective effect in Parkinson's disease (Xu et al. 2008) and antioxidant properties by inhibiting the Fenton oxidative reaction and the iron mediated lipid peroxidation (Graf and Eaton 1990; Rimbach and Pallauf 1998). On the other hand, phytate play an important role in cancer prevention and control of experimental tumor growth, progression and metastasis. Phytate appear to reduce cell proliferation and also induces differentiation of malignant cells. Hence, phytate has shown to decrease cancer biomarkers of colon, liver, lung, prostate, breast, skin and soft tissue carcinogenesis in rats and/or mice (Jenab and Thompson 1998; Jenab and Thompson 2000; Shamsuddin 2002; Vucenik and Shamsuddin 2004). Furthermore, the parent compound inositol appears to enhance the anticancer effect of conventional chemotherapy in addition to control cancer metastasis and improve quality of life (Vucenik and Shamsuddin 2006). Thus, the combination of phytate plus inositol holds great promise in cancer treatments (Vucenik and Shamsuddin 2003; Singh and Agarwal 2005).

4. Disease prevention

In table 4, the most important effects of the bioactive compounds occurring in soy linked to the main mechanisms related to common diseases prevention are shown.

In addition to the excellent nutritional properties of soy, the occurrence of natural phytochemicals showing a number of health protective effects has triggered the soy consumption. Presently, soybeans and soy-based foods are consumed worldwide and they can be considered as good candidates to be included in a healthy diet. Soy intake has been related to the prevention of several diseases, for instance, cancer, Alzheimer, osteoporosis, or coronary heart diseases.

Most of the health benefits of soy have been associated with the occurrence of isoflavones; nevertheless, as commented in the previous section, other phytochemicals also play an important role in the disease prevention. The studies have been performed not only by evaluating the soy intake but also the effect of supplementation with the isolated bioactive compounds. Generally, the observed effects are dose-dependent so as to show a preventive or the opposite activity deppending on the concentration.

Disease	Compound	Main effects
Cardiovascular disease	Isoflavones	Reduction of total lipids and triglycerides Inhibition of oxidative stress Decrease in bood pressure Inhibition of NO and TNF-α
	Phytate	Reduction of total homocysteine, transferrin saturation, and ferritin
	Saponins	Hypocholesterolemic properties Reduction of elevated blood sugar Inhibition of lipid peroxidation
	Soy proteins	Total cholesterol and lipids reduction Antihypertensive properties
Cancer	Isoflavones	Reduction of oxidative damage Inhibition of cancer cell proliferation Activity in estrogen and progesterone receptors Inhibiton of protein tyrosine kinases Cessation of DNA, RNA, and protein synthesis of the carcinogenic cells Induction of apoptosis
	Trysin inhibitors	Suppression of cancer cell invasion Reduction of tumor generation and cell proliferation Inhibition of tumor promotion by avoiding the digestion of proteins Reduction of carcinogens adsorption
	Saponins	Suppression of the inflammatory response Induction of apoptosis Antiestrogenic activity Inhibition of tumor cell metastasis Antimutagenic activity effect Bile acid binding action Normalization of carcinogen-induced cell proliferation
Osteoporosis	Isoflavones	(Effects have not reached a consensus)
	Phytate	Reduction of bone mineral density loss
Alzheimer	Isoflavones	Inhibition of amyloid-β-peptide fibril formation Suppression of the inflammatory response Reduction of oxidative damage

Table 4. Main mechanisms of action observed for the bioactive compounds present in soy related to disease prevention.

4.1 Cardiovascular disease

Cardiovascular diseases (CVD) remain the largest cause of death in developing countries. Coronary heart disease (CHD) and cerebrovascular disease, which includes stroke and transient ischemic attacks, are the main CVD incidents. Coronary heart disease is significantly more frequent than stroke, and increases considerably after menopause as a

consequence of higher levels of low density lipoproteins (LDL) and total cholesterol (Rosano et al. 2007). Cardiovascular diseases have a multifactorial aetiology and the pathogenesis of arterial forms of CVD is associated with atherosclerosis, an inflammatory process that develops at specific locations within the arterial tree. Potential risk biomarkers have been identified, such as lipid and lipoprotein metabolism (LDL and HDL cholesterol and triacylglycerol amounts), the haemostatic function, the oxidative damage, the homocysteine metabolism, and blood pressure (Mensink et al. 2003). The relationship between the biomarker and the risk has not clearly reached a consensus; hence, only LDL and HDL cholesterol, triacylglycerol, homocysteine and blood pressure are well-validated and generally accepted biomarkers.

Asiatic populations, whose diet is particularly rich in soy, show low rates of CVD, whereas groups moved to western societies lose this protection (Nagata 2000; Zhang et al. 2003). This fact highlights the cardiovascular disease protection by the occurring phytochemicals in soy, specially isoflavones and other flavonoid derivatives. Epidemiological studies clearly evidence that isoflavones and soy bioactive proteins are involved in CVD prevention (Cano et al. 2010; Jenkins et al. 2010); thus, they have demonstrated to reduce total cholesterol, triglycerides and LDL. A possible mechanism could involve the modulation of LDL receptor levels in human liver (Carroll and Kurowska 1995). Furthermore, dietary supplementation with soy isoflavones favorably alters insulin resistance and controls glycemic levels (Jayagopal et al. 2002). Recent studies have demonstrated that daily ingestion of purified soy isoflavones or soy-based foods significantly decreases blood pressure in adults (Matsui et al. 2010; Taku et al. 2010). On the other hand, individual isoflavones appear to play an important role in other biochemical processes related to CVD, for instance, genistein and daidzein seem to inhibit the nitric oxide (NO) and tumour necrosis factor alpha (TNF-alpha) production, showing genistein a greater inhibitory effect (Gottstein et al. 2003). Regarding to hemostasis, there is still uncertainty about their role in the hemostatic equilibrium; to date, these phytochemicals are involved in platelet aggregation mechanisms, including the blockage of the calcium channels (Dobrydneva et al. 2002) or the thromboxane A2 receptor (Muñoz et al. 2009), the reduction in the density of thromboxane A2 receptors (Garrido et al. 2006), or the interference with different platelet singnalling pathways triggered by thrombin (Navarro-Nuñez et al. 2009). On the other hand, phytates have been also evaluated in terms of protecting against CVD risks in postmenopausal women. Combined with soy protein, they have shown a potential atherosclerotic prevention because of the reduction of total homocysteine, transferrin saturation, and ferritin (Hanson et al. 2006). Furthermore, other biochemicals found in soy are also involved in CVD prevention, for instance, soyasaponins show hypocholesterolemic properties because they can form insoluble complexes with cholesterol so as to inhibit the intestinal absorption, and saponins could interfere with the enterohepatic circulation of bile acids by forming mixed micelles, which are blocked to be reabsorbed by the terminal ileum (Oakenfull and Sidhu 1990). Soyasaponins also reduce blood sugar when it is elevated and lipid peroxidation levels. In addition, soy proteins have shown an overall total cholesterol reduction in postmenopausal women (Mackey et al. 2000) as well as other peptides from soy have shown antihypertensive properties (Chen et al. 2004; Gouda et al. 2006).

The similar activities observed for the different bioactive compounds occurring in soy may indicate that the disease prevention could be achieved by the same or very similar mechanism. In a soy-based diet, the intake of different types of phytochemicals may favour

the synergistical interaction so as to induce preventive effects in cardiovascular disease that could not be observed with isolated pure compounds.

4.2 Cancer

Cancer is a leading cause of death worldwide. Prostate, breast, lung and colon cancers are the most common, being breast in women and prostate cancer in men the most prevalent. Despite genetic factors, diet may prevent against cancer appearance and development. In this field, soy-based foods are known to be inversely associated with the cancer risk. The strongest protective effect of the soy intake was reported for breast, prostate, and colon cancers (Adlercreutz et al. 1995; Wang and Kurzer 1997). The occurring phytochemicals, including isoflavones, phytates, saponins, and protease inhibitors, are believed to contribute to the biological effects observed after soy consumption (Messina et al. 1994).

The cancer protective properties of isoflavones may be attributed to their antioxidant (Ibrahim et al. 2008), and antiestrogenic (Cederroth and Nef 2009) actions, or their antimutagenic and antiproliferative activities by means of different mechanisms, such as the inhibition of protein tyrosine kinases or via cessation of DNA, RNA, and/or protein synthesis of the carcinogenic cells (Hirano et al. 1994; Kurzer and Xu 1997). Furthermore, isoflavones from soy show the ability of inhibiting the transformation, the differentiation (Constantinou and Huberman 1995), and the angiogenesis (Fotsis et al. 1995) of the carcinogenic cells, together with the induction of apoptosis (Kyle et al. 1997). Meta-analysis of epidemiological studies shows that soy isoflavones consumption contributes to reduce the risk of breast cancer incidence; hence, the protective effect of soy was only observed among studies conducted in Asian but not in Western populations (Dong and Qin 2011). The preventive effect is significantly important in post-menopausal women, according to the estrogenic activity of isoflavones, which is commonly associated to the anticarcinogenic mechanism of protection (Kang et al. 2010). Similarly, soy isoflavones have shown a preventive effect in prostate cancer (Severson et al. 1989; Kucuk 2010). In addition, a meta-analysis performed for Japanese and Korean populations demonstrated that consumption of non-fermented rather than fermented soy-based foods may reduce gastric cancer risk (Kim et al. 2011). On the other hand, soy protein fractions also play an important role in cancer prevention. Protease inhibitors may act as anticarcinogenic agents by means of different mechanisms, including the suppression of reactive oxygen species (superoxide anion free radicals) formation by stimulated neutrophils; inhibition of tumor promotion; growing cancer cells deprivation of essential amino acids by avoiding the digestion of proteins; and the adsorption of carcinogens during passage through the digestive tract, by acting as an insoluble fibre (Friedman and Brandon 2001). In this field, a Kunitz type trypsin inhibitor isolated from Korean large black soybeans appeared to exert antiproliferative activity toward CNE-2 and HNE-2 nasopharyngeal cancer cells, MCF-7 breast cancer cells, and Hep G2 hepatoma cells (Fang et al. 2010). The Kunitz type protease inhibitor, bikunin, has shown to suppress cancer cell invasion *in vitro* and metastasis *in vivo* (Kobayashi et al. 2004). Finally, lunasin seems to reduce tumor generation and cell proliferation in breast tumor sections (Hsieh et al. 2010). Other bioactive compounds occurring in soy, such as saponins, have shown anticarcinogenic properties in colon, liver or breast cancer cell lines. The proposed mechanisms of action include direct cytotoxicity, induction of apoptosis, antiestrogenic activity, inhibition of tumor cell metastasis, antimutagenic activity effect, bile acid binding action, and normalization of carcinogen-induced cell proliferation (Rao and Sung 1995). Group B saponins seem to reduce colon cancer cell proliferation possibly by

suppressing the inflammatory responses (Kim et al. 2004; Ellington et al. 2005). Thus, soyasaponin-supplemented diets could reduce the incidence of aberrant crypt foci, which suggest that they may play an important role in the incidence of colon cancer (Koratkar and Rao 1997).

4.3 Osteoporosis

Estrogen deficiency is involved in many of the proposed mechanisms taking place during the development of several menopausal-associated diseases, such as osteoporosis, breast cancer, and cardiovascular diseases. Primary type 1 or postmenopausal osteoporosis is a reduction of the bone mineral density (BMD) resulting in a significant risk factor for fracture, generally in vertebrae, forearm and hip. Soy has been evaluated as a protective agent against BMD loss due to the widely recognised estrogenic activity of isoflavones. Isoflavones can be considered as possible selective estrogen receptor modulators, which may bind to estrogen receptors and selectively stimulate or inhibit estrogen-like action (Setchell 2001). The preventive estrogenic activity could act by means of different mechanisms, for instance, the direct modulation of osteoblast and osteoclast activity; the regulation of the resorptive effects of parathyroid hormone; the inhibition of tyrosine-kinase activity with subsequent changes in the activity of alkaline phosphatase; or the inhibition of interleukin-1 release, a potent bone resorption agent (Bitto et al. 2010; Pilsakova et al. 2010).

Studies with animals have proved that soy isoflavones may prevent bone density loss. Protection effects may be significantly modified by the addition of prebiotic fructooligosaccharides, which increase the isoflavone bioavailability. Therefore, a genistin-rich diet combined with the intake of fructooligosaccharides is capable of preventing loss of bone mineral density by increasing whole-body, right femur, and fourth lumbar bone mineral density in rats (Hooshmand et al. 2010). Similarly, the addition vitamins D and K and calcium enrichment may enhance the isoflavone preventive effects in bone mass (Jeon et al. 2009). Nevertheless, published studies with humans are inconsistent and do not clearly support soy protective effect against bone loss. Some of the meta-analysis including data from more than 1000 menopausal women revealed that daily ingestion of soy isoflavones may increase lumbar spine BMD whereas no significant effects are shown in femoral neck, hip, and trochanter BMD (Taku et al. 2010). In contrast, epidemiological studies did not found a clear relationship between soy intake and prevention of bone loss and consequently fractures in perimenopausal and postmenopausal Western women (Lagari and Levis 2010; Ricci et al. 2010). One of the potential inconsistencies among the epidemiological studies could be the differences in the isoflavone metabolism. In this field, equol has shown stronger estrogenic activity than the parent compound daidzein (Ishimi 2009). Other authors propose that the phytate intake associated to a soy-rich diet could provide protection against osteoporosis since phytates may reduce BMD loss (Lopez-Gonzalez et al. 2008).

4.4 Alzheimer's disease

The neurodegenerative pathology Alzheimer's disease is the most common form of dementia among people above 65 years old (Hendrie 1998), being more common in women regarding to men (Vina and Lloret 2010).

Neuronal dysfunction, eventually leading to dementia, is caused by accumulation of filamentous proteins, such as the amyloid-β peptide, which provokes reactive oxygen species (ROS) generation, increase in Ca^{2+} levels and other cytotoxic stimuli. Estrogenic compounds protect cells against mitochondrial toxicity of amyloid-β peptide toxicity; hence, soy isoflavones have demonstrated to prevent Alzheimer's disease by inhibiting the

amyloid-β peptide fibril formation *in vitro*. Among the isoflavones tested, the aglycones and equol (a metabolite from daidzein) caused the greater inhibition (up to 30%) (Henry-Vitrac et al. 2010). On the other hand, soy isoflavones prevent the occurrence of Alzheimer's disease because of the antiinflammatory and antioxidant properties (Hsieh et al. 2009).

4.5 Other beneficial effects

In addition to the described diseases prevention, numerous other benefits are reported to be associated with soy-containing diets. For instance, as commented before, soy seems to provide protective effects against obesity by lowering the LDL and total cholesterol levels; and diabetes by reducing the glucose concentration. Furthermore, soybean inhibitors may protect against induced pancreatitis (Jurkowska et al. 1992), gastrointestinal mucosal injury (Funk and Baker 1991), and kidney diseases (Kinjo et al. 1998). Soyasaponins show significant inhibitory effects on the prevalence of herpes labialis and inhibit the replication of human cytomegalovirus and influenza virus (Hayashi et al. 1997).

5. Conclusion

Soy and soy-based foods have demonstrated to be good candidates to be included in healthy diets. Soy seeds provide high amounts of protein, dietary fibre, polyunsaturated fatty acids, vitamins and minerals whereas cholesterol and lactose levels are considerably low. Soy has attracted much interest not only because of its excellent nutritional properties, but also due to the occurrence of a number of bioactive compounds, such as isoflavones, saponins, protease inhibitors or phytates, among others. Many of the healthy benefits attributed to soy intake are due to the high content of these phytochemicals. Disease prevention comprises a wide range of mechanisms of action and some of these bioactive compounds have shown to be involved not only in a unique reaction but in some of them. Among the most important properties, the bioactive compounds exert antioxidant, antiinflammatory, or estrogenic activities, which have been demonstrated to be implicated in the health promotion and disease prevention. Concluding, according to the epidemiological studies, soy may provide a protective effect against common diseases, for instance, cancer, cardiovascular disease, osteoporosis or even Alzehimer's disease.

6. Acknowledgment

7. References

Adlercreutz, C. H. T., B. R. Goldin, S. L. Gorbach, K. A. V. Hockerstedt, S. Watanabe, E. K. Hamalainen, M. H. Markkanen, T. H. Makela, K. T. Wahala, T. A. Hase and T. Fotsis (1995). Soybean phytoestrogen intake and cancer risk. *Journal of Nutrition*, Vol. 125, No. 7, pp. 1960-1960.

Aletor, O. (2010). Soyabean meal versus soyabean protein isolate: A comparative study of the nutritive and functional attributes. *Journal of Food Agriculture & Environment*, Vol. 8, No. 2, pp. 34-38.

Anta, L., M. L. Marina and M. C. Garcia (2010). Simultaneous and rapid determination of the anticarcinogenic proteins Bowman-Birk inhibitor and lectin in soybean crops by perfusion RP-HPLC. *Journal of Chromatography A*, Vol. 1217, No. 45, pp. 7138-7143.

Anuonye, J. C., J. O. Onuh, E. Egwim and S. O. Adeyemo (2010). Nutrient and Antinutrient Composition of Extruded Acha/Soybean Blends. *Journal of Food Processing and Preservation*, Vol. 34, No., pp. 680-691.

Bitto, A., F. Polito, F. Squadrito, H. Marini, R. D'Anna, N. Irrera, L. Minutoli, R. Granese and D. Altavilla (2010). Genistein Aglycone: A Dual Mode of Action Anti-Osteoporotic Soy Isoflavone Rebalancing Bone Turnover Towards Bone Formation. *Current Medicinal Chemistry*, Vol. 17, No. 27, pp. 3007-3018.

Cai, T. D., K. C. Chang, M. C. Shih, H. J. Hou and M. Ji (1997). Comparison of bench and production scale methods for making soymild and tofu from 13 soybean varieties. *Food Research International*, Vol. 30, No., pp. 659-668.

Cano, A., M. A. Garcia-Perez and J. J. Tarin (2010). Isoflavones and cardiovascular disease. *Maturitas*, Vol. 67, No. 3, pp. 219-226.

Carroll, K. K. and E. M. Kurowska (1995). Soy consumption and cholesterol reduction - Review of animal and human studies. *Journal of Nutrition*, Vol. 125, No. 3, pp. S594-S597.

Cederroth, C. R. and S. Nef (2009). Soy, phytoestrogens and metabolism: A review. *Molecular and Cellular Endocrinology*, Vol. 304, No. 1-2, pp. 30-42.

Constantinou, A. and E. Huberman (1995). Genistein as an inducer of tumor-cell diferentiation - Possible mechanisms of action. *Proceedings of the Society for Experimental Biology and Medicine*, Vol. 208, No. 1, pp. 109-115.

Coxam, V. (2008). Phyto-oestrogens and bone health. *Proceedings of the Nutrition Society*, Vol. 67, No., pp. 184-195.

Chang, C. Y., L. J. Huang, J. P. Wang, C. M. Teng, S. C. Chen and S. C. Kuo (2000). Synthesis and anti-platelet, anti-inflammatory and anti-allergic activities of methoxyisoflavanquinone and related compounds. *Chemical & Pharmaceutical Bulletin*, Vol. 48, No., pp. 964-973.

Chang, W. W., C. Y. Yu, T. W. Lin, P. H. Wang and Y. C. Tsai (2006). Soyasaponin I decreases the expression of alpha 2,3-linked sialic acid on the cell surface and suppresses the metastatic potential of B16F10 melanoma cells. *Biochemical and Biophysical Research Communications*, Vol. 341, No. 2, pp. 614-619.

Chen, J. R., S. C. Yang, K. Suetsuna and J. C. J. Chao (2004). Soybean protein-derived hydrolysate affects blood pressure in spontaneously hypertensive rats. *Journal of Food Biochemistry*, Vol. 28, No. 1, pp. 61-73.

Chung, M. J., N.-J. Sung, C.-S. Park, D.-K. Kweon, A. Mantovani, T.-W. Moon, S.-J. Lee and K.-H. Park (2008). Antioxidative and hypocholesterolemic activities of water-soluble puerarin glycosides in HepG2 cells and in C57 BL/6J mice. *European Journal of Pharmacology*, Vol. 578, No., pp. 159-170.

Davis, D. D., E. S. Díaz-Cruz, S. Landini, Y.-W. Kim and R. W. Brueggemeier (2008). Evaluation of synthetic isoflavones on cell proliferation, estrogen receptor binding affinity, and apoptosis in human breast cancer cells. *Journal of Steroid Biochemistry and Molecular Biology*, Vol. 108, No., pp. 23-31.

De Lumen, B. O. (2005). Lunasin: A cancer-preventive soy peptide. *Nutrition Reviews*, Vol. 63, No. 1, pp. 16-21.

De Mejia, E. G., M. Vasconez, B. O. De Lumen and R. Nelson (2004). Lunasin concentration in different soybean genotypes, commercial soy protein, and isoflavone products. *Journal of Agricultural and Food Chemistry*, Vol. 52, No. 19, pp. 5882-5887.

Dia, V. P., M. A. Berhow and E. G. De Mejiat (2008). Bowman-Birk Inhibitor and Genistein among Soy Compounds That Synergistically Inhibit Nitric Oxide and Prostaglandin E-2 Pathways in Lipopolysaccharide-Induced Macrophages. *Journal of Agricultural and Food Chemistry*, Vol. 56, No. 24, pp. 11707-11717.

Dijsselbloem, N., W. V. Berghe, A. D. Naeyer and G. Haegeman (2004). Soy isoflavone phyto-pharmaceuticals in interleukin-6 affections Multi-purpose nutraceuticals at the crossroad of hormone replacement, anti-cancer and anti-inflammatory therapy. *Biochemical Pharmacology*, Vol. 68, No., pp. 1171-1185.

Dobrydneva, Y., R. L. Williams, G. Z. Morris and P. F. Blackmore (2002). Dietary phytoestrogens and their synthetic structural analogues as calcium channel blockers in human platelets. *Journal of Cardiovascular Pharmacology*, Vol. 40, No. 3, pp. 399-410.

Dong, J. Y. and L. Q. Qin (2011). Soy isoflavones consumption and risk of breast cancer incidence or recurrence: a meta-analysis of prospective studies. *Breast Cancer Research and Treatment*, Vol. 125, No. 2, pp. 315-323.

Ellington, A. A., M. Berhow and K. W. Singletary (2005). Induction of macroautophagy in human colon cancer cells by soybean B-group triterpenoid saponins. *Carcinogenesis*, Vol. 26, No. 1, pp. 159-167.

Esteves, E. A., H. S. D. Martino, F. C. E. Oliveira, J. Bressan and N. M. B. Costa (2010). Chemical composition of a soybean cultivar lacking lipoxygenases (LOX2 and LOX3). *Food Chemistry*, Vol. 122, No. 1, pp. 238-242.

Fang, E. F., J. H. Wong and T. B. Ng (2010). Thermostable Kunitz trypsin inhibitor with cytokine inducing, antitumor and HIV-1 reverse transcriptase inhibitory activities from Korean large black soybeans. *Journal of Bioscience and Bioengineering*, Vol. 109, No. 3, pp. 211-217.

Fotsis, T., M. Pepper, H. Adlercreutz, T. Hase, R. Montesano and L. Schweigerer (1995). Genistein, a dietary ingested isoflavonoid, inhibits cell-proliferation and in-vitro angiogenesis. *Journal of Nutrition*, Vol. 125, No. 3, pp. S790-S797.

Franke, A. A., J. H. Hankin, M. C. Yu, G. Maskarinec, S.-H. Low and L. J. Custer (1999). Isoflavone Levels in Soy Foods Consumed by Multiethnic Populations in Singapore and Hawaii. *Journal of Agricultural and Food Chemistry*, Vol. 47, No., pp. 977-986.

Friedman, M. and D. L. Brandon (2001). Nutritional and health benefits of soy proteins. *Journal of Agricultural and Food Chemistry*, Vol. 49, No. 3, pp. 1069-1086.

Funk, M. A. and D. H. Baker (1991). Effect of soy products on methotrexane toxicity in rats. *Journal of Nutrition*, Vol. 121, No. 10, pp. 1684-1692.

García-Lafuente, A., E. Guillamón, A. Villares, M. A. Rostagno and J. A. Martínez (2009). Flavonoids as anti-inflammatory agents: implications in cancer and cardiovascular disease. *Inflammation Research*, Vol. 58, No. 9, pp. 537-552.

Garrido, A., M. P. De la Maza, S. Hirsch and L. Valladares (2006). Soy isoflavones affect platelet thromboxane A(2) receptor density but not plasma lipids in menopausal women. *Maturitas*, Vol. 54, No. 3, pp. 270-276.

Genovese, M. I., J. Davila and F. M. Lajolo (2006). Isoflavones in Processed Soybean Products from Ecuador. *Brazilian Archives of Biology and Technology*, Vol. 49, No., pp. 853-859.

Gottstein, N., B. A. Ewins, C. Eccleston, G. P. Hubbard, I. C. Kavanagh, A. M. Minihane, P. D. Weinberg and G. Rimbach (2003). Effect of genistein and daidzein on platelet aggregation and monocyte and endothelial function. *British Journal of Nutrition*, Vol. 89, No. 5, pp. 607-615.

Gouda, K. G. M., L. R. Gowda, A. G. A. Rao and V. Prakash (2006). Angiotensin I-converting enzyme inhibitory peptide derived from glycinin, the 11S globulin of soybean (Glycine max). *Journal of Agricultural and Food Chemistry*, Vol. 54, No. 13, pp. 4568-4573.

Graf, E. and J. W. Eaton (1990). Antioxidant Function of Phytic Acid. *Free Radical Biology and Medicine*, Vol. 8, No. 1, pp. 61-69.

Grases, F., J. G. March, R. M. Prieto, B. M. Simonet, A. Costa-Bauza, A. Garcia-Raja and A. Conte (2000). Urinary phytate in calcium oxalate stone formers and healthy people - Dietary effects on phytate excretion. *Scandinavian Journal of Urology and Nephrology*, Vol. 34, No. 3, pp. 162-164.

Grieshop, C. M. and G. C. Fahey (2001). Comparison of quality characteristics of soybeans from Brazil, China, and the United States. *Journal of Agricultural and Food Chemistry*, Vol. 49, No. 5, pp. 2669-2673.

Grieshop, C. M., C. T. Kadzere, G. M. Clapper, E. A. Flickinger, L. L. Bauer, R. L. Frazier and G. C. Fahey (2003). Chemical and nutritional characteristics of United States soybeans and soybean meals. *Journal of Agricultural and Food Chemistry*, Vol. 51, No. 26, pp. 7684-7691.

Gu, C. M., H. B. Pan, Z. W. Sun and G. X. Qin (2010). Effect of Soybean Variety on Anti-Nutritional Factors Content, and Growth Performance and Nutrients Metabolism in Rat. *International Journal of Molecular Sciences*, Vol. 11, No. 3, pp. 1048-1056.

Guclu-Ustundag, O. and G. Mazza (2007). Saponins: Properties, applications and processing. *Critical Reviews in Food Science and Nutrition*, Vol. 47, No. 3, pp. 231-258.

Hanson, L. N., H. M. Engelman, D. L. Alekel, K. L. Schalinske, M. L. Kohut and M. B. Reddy (2006). Effects of soy isoflavones and phytate on homocysteine, C-reactive protein, and iron status in postmenopausal women. *American Journal of Clinical Nutrition*, Vol. 84, No. 4, pp. 774-780.

Hayashi, K., H. Hayashi, N. Hiraoka and Y. Ikeshiro (1997). Inhibitory activity of soyasaponin II on virus replication in vitro. *Planta Medica*, Vol. 63, No. 2, pp. 102-105.

Hendrie, H. C. (1998). Epidemiology of dementia and Alzheimer's disease. *American Journal of Geriatric Psychiatry*, Vol. 6, No. 2, pp. S3-S18.

Henry-Vitrac, C., H. Berbille, J. M. Merillon and X. Vitrac (2010). Soy isoflavones as potential inhibitors of Alzheimer beta-amyloid fibril aggregation in vitro. *Food Research International*, Vol. 43, No. 8, pp. 2176-2178.

Hirano, T., M. Gotoh and K. Oka (1994). Natural flavonoids and lignans are potent cytostatic agents against human HL-60 cells. *Life Sciences*, Vol. 55, No. 13, pp. 1061-1069.

Hooshmand, S., S. Juma and B. H. Arjmandi (2010). Combination of Genistin and Fructooligosaccharides Prevents Bone Loss in Ovarian Hormone Deficiency. *Journal of Medicinal Food*, Vol. 13, No. 2, pp. 320-325.

Hsieh, C. C., B. Hernandez-Ledesma, H. J. Jeong, J. H. Park and B. O. de Lumen (2010). Complementary Roles in Cancer Prevention: Protease Inhibitor Makes the Cancer Preventive Peptide Lunasin Bioavailable. *Plos One*, Vol. 5, No. 1, pp.

Hsieh, H. M., W. M. Wu and M. L. Hu (2009). Soy isoflavones attenuate oxidative stress and improve parameters related to aging and Alzheimer's disease in C57BL/6J mice treated with D-galactose. *Food and Chemical Toxicology*, Vol. 47, No. 3, pp. 625-632.

Hubert, J., M. Berger and J. Dayde (2005). Use of a simplified HPLC-UV analysis for soyasaponin B determination: Study of saponin and isoflavone variability in soybean cultivars and soy-based health food products. *Journal of Agricultural and Food Chemistry*, Vol. 53, No. 10, pp. 3923-3930.

Hymowitz, T. and J. R. Harlan (1983). Introduction of soybean to North-America by Bowen, Samuel in 1765. *Economic Botany*, Vol. 37, No. 4, pp. 371-379.

Ibrahim, W. H., H. M. Habib, C. K. Chow and G. G. Bruckner (2008). Isoflavone-Rich Soy Isolate Reduces Lipid Peroxidation in Mouse Liver. *International Journal for Vitamin and Nutrition Research*, Vol. 78, No. 4-5, pp. 217-222.

Ishimi, Y. (2009). Soybean Isoflavones in Bone Health. Food Factors for Health Promotion. 61: 104-116.

Jackman, K. A., O. L. Woodman and C. G. Sobey (2007). Isoflavones, equol and cardiovascular disease: Pharmacological and therapeutic insights. *Current Medicinal Chemistry*, Vol. 14, No., pp. 2824-2830.

Jayagopal, V., P. Albertazzi, E. S. Kilpatrick, E. M. Howarth, P. E. Jennings, D. A. Hepburn and S. L. Atkin (2002). Beneficial effects of soy phytoestrogen intake in postmenopausal women with type 2 diabetes. *Diabetes Care*, Vol. 25, No. 10, pp. 1709-1714.

Jenab, M. and L. U. Thompson (1998). The influence of phytic acid in wheat bran on early biomarkers of colon carcinogenesis. *Carcinogenesis*, Vol. 19, No. 6, pp. 1087-1092.

Jenab, M. and L. U. Thompson (2000). Phytic acid in wheat bran affects colon morphology, cell differentiation and apoptosis. *Carcinogenesis*, Vol. 21, No. 8, pp. 1547-1552.

Jenkins, D. J. A., A. Mirrahimi, K. Srichaikul, C. E. Berryman, L. Wang, A. Carleton, S. Abdulnour, J. L. Sievenpiper, C. W. C. Kendall and P. M. Kris-Etherton (2010). Soy Protein Reduces Serum Cholesterol by Both Intrinsic and Food Displacement Mechanisms. *Journal of Nutrition*, Vol. 140, No. 12, pp. 2302S-2311S.

Jeon, B. J., J. Ahn and H. S. Kwak (2009). Effect of Isoflavone-Enriched Milk on Bone Mass in Ovariectomized Rats. *Journal of Medicinal Food*, Vol. 12, No. 6, pp. 1260-1267.

Jurkowska, G., G. Grondin, S. Masse and J. Morisset (1992). Soybeab trypsin-inhibitor and cerulein accelerate recovery of cerulein-induced pancreatitis in rats. *Gastroenterology*, Vol. 102, No. 2, pp. 550-562.

Kang, X. M., Q. Y. Zhang, S. H. Wang, X. Huang and S. Jin (2010). Effect of soy isoflavones on breast cancer recurrence and death for patients receiving adjuvant endocrine therapy. *Canadian Medical Association Journal*, Vol. 182, No. 17, pp. 1857-1862.

Kao, T. H. and B. H. Chen (2006). Functional Components in Soybean Cake and Their Effects on Antioxidant Activity. *Journal of Agricultural and Food Chemistry*, Vol. 54, No., pp. 7544-7555.

Kao, T. H., R.-F. S. Huang and B. H. Chen (2007). Antiproliferation of Hepatoma Cell and Progression of Cell Cycle as Affected by Isoflavone Extracts from Soybean Cake. *International Journal of Molecular Sciences*, Vol. 8, No., pp. 1095-1110.

Kasim, N. S., S. Gunawan, M. Yuliana and Y. H. Ju (2010). A Simple Two-Step Method for Simultaneous Isolation of Tocopherols and Free Phytosterols from Soybean Oil Deodorizer Distillate with High Purity and Recovery. *Separation Science and Technology*, Vol. 45, No. 16, pp. 2437-2446.

Kensil, C. R., A. X. Y. Mo and A. Truneh (2004). Current vaccine adjuvants: An overview of a diverse class. *Frontiers in Bioscience*, Vol. 9, No., pp. 2972-2988.

Khetarpaul, N., R. B. Grewal, R. Goyal and R. Garg (2004). Development of partially defatted soy flour and *dhal*. *Food Chemistry*, Vol. 87, No., pp. 355-359.

Kim, H. Y., R. Yu, J. S. Kim, Y. K. Kim and M. K. Sung (2004). Antiproliferative crude soy saponin extract modulates the expression of I kappa B alpha, protein kinase C, and cyclooxygenase-2 in human colon cancer cells. *Cancer Letters*, Vol. 210, No. 1, pp. 1-6.

Kim, J., M. Kang, J. S. Lee, M. Inoue, S. Sasazuki and S. Tsugane (2011). Fermented and non-fermented soy food consumption and gastric cancer in Japanese and Korean populations: A meta-analysis of observational studies. *Cancer Science*, Vol. 102, No. 1, pp. 231-244.

Kim, J. J., S. H. Kim, S. J. Hahn and I. M. Chung (2005). Changing soybean isoflavone composition and concentrations under two different storage conditions over three years. *Food Research International*, Vol. 38, No., pp. 435-444.

Kinjo, J., T. Hirakawa, R. Tsuchihashi, T. Nagao, M. Okawa, T. Nohara and H. Okabe (2003). Hepatoprotective constituents in plants 14. Effects of soyasapogenol B, sophoradiol, and their glucuronides on the cytotoxicity of tert-butyl hydroperoxide to HepG2 cells. *Biological & Pharmaceutical Bulletin*, Vol. 26, No. 9, pp. 1357-1360.

Kinjo, J., M. Imagire, M. Udayama, T. Arao and T. Nohara (1998). Studies on the hepatoprotective drugs part 3 - Studies on the constituents of the leguminous plants part 56 - Structure-hepatoprotective relationships study of soyasaponins I-IV having soyasapogenol B as aglycone. *Planta Medica*, Vol. 64, No. 3, pp. 233-236.

Kobayashi, H., M. Suzuki, N. Kanayama and T. Terao (2004). A soybean Kunitz trypsin inhibitor suppresses ovarian cancer cell invasion by blocking urokinase upregulation. *Clinical & Experimental Metastasis*, Vol. 21, No. 2, pp. 159-166.

Koratkar, R. and A. V. Rao (1997). Effect of soya bean saponins on azoxymethane-induced preneoplastic lesions in the colon of mice. *Nutrition and Cancer-an International Journal*, Vol. 27, No. 2, pp. 206-209.

Kucuk, O. (2010). Soy Isoflavones in Prostate Cancer Treatment. *Drug Metabolism Reviews*, Vol. 42, No., pp. 24-24.

Kurzer, M. S. and X. Xu (1997). Dietary phytoestrogens. *Annual Review of Nutrition*, Vol. 17, No., pp. 353-381.

Kyle, E., L. Neckers, C. Takimoto, G. Curt and R. Bergan (1997). Genistein-induced apoptosis of prostate cancer cells is preceded by a specific decrease in focal adhesion kinase activity. *Molecular Pharmacology*, Vol. 51, No. 2, pp. 193-200.

Lagari, V. S. and S. Levis (2010). Phytoestrogens and bone health. *Current Opinion in Endocrinology Diabetes and Obesity*, Vol. 17, No. 6, pp. 546-553.

Lee, I. A., Y. J. Park, H. K. Yeo, M. J. Han and D. H. Kim (2010). Soyasaponin I Attenuates TNBS-Induced Colitis in Mice by Inhibiting NF-kappa B Pathway. *Journal of Agricultural and Food Chemistry*, Vol. 58, No. 20, pp. 10929-10934.

Lee, S. H., H. J. Park, H. K. Chun, S. Y. Cho, S. M. Cho and H. S. Lillehoj (2006). Dietary phytic acid lowers the blood glucose level in diabetic KK mice. *Nutrition Research*, Vol. 26, No. 9, pp. 474-479.

Lee, S. H., H. J. Park, H. K. Chun, S. Y. Cho, H. J. Jung, S. M. Cho, D. Y. Kim, M. S. Kang and H. S. Lillehoj (2007). Dietary phytic acid improves serum and hepatic lipid levels in aged ICR mice fed a high-cholesterol diet. *Nutrition Research*, Vol. 27, No. 8, pp. 505-510.

Li, H. Y., H. C. Liu, Y. P. Han, X. X. Wu, W. L. Teng, G. F. Liu and W. B. Li (2010). Identification of QTL underlying vitamin E contents in soybean seed among multiple environments. *Theoretical and Applied Genetics*, Vol. 120, No. 7, pp. 1405-1413.

Lopez-Gonzalez, A. A., F. Grases, P. Roca, B. Mari, M. T. Vicente-Herrero and A. Costa-Bauza (2008). Phytate (myo-Inositol Hexaphosphate) and Risk Factors for Osteoporosis. *Journal of Medicinal Food*, Vol. 11, No. 4, pp. 747-752.

Ma, D. F., L. Q. Qin, P. Y. Wang and R. Katoh (2008). Soy isoflavone intake increases bone mineral density in the spine of menopausal women: Meta-analysis of randomized controlled trials. *Clinical Nutrition*, Vol. 27, No. 1, pp. 57-64.

Ma, Y. T. and T. Wang (2010). Deactivation of Soybean Agglutinin by Enzymatic and Other Physical Treatments. *Journal of Agricultural and Food Chemistry*, Vol. 58, No. 21, pp. 11413-11419.

Mackey, R., A. Ekangaki and J. A. Eden (2000). The effects of soy protein in women and men with elevated plasma lipids. *Biofactors*, Vol. 12, No. 1-4, pp. 251-257.

Machado, F. P. P., J. H. Queiroz, M. G. A. Oliveira, N. D. Piovesan, M. C. G. Peluzio, N. M. B. Costa and M. A. Moreira (2008). Effects of heating on protein quality of soybean flour devoid of Kunitz inhibitor and lectin. *Food Chemistry*, Vol. 107, No. 2, pp. 649-655.

Matsui, T., X. L. Zhu, K. Shiraishi, T. Ueki, Y. Noda and K. Matsumoto (2010). Antihypertensive Effect of Salt-Free Soy Sauce, a New Fermented Seasoning, in Spontaneously Hypertensive Rats. *Journal of Food Science*, Vol. 75, No. 4, pp. H129-H134.

Mensink, R. P., A. Aro, E. Den Hond, J. B. German, B. A. Griffin, H. U. ter Meer, M. Mutanen, D. Pannemans and W. Stahl (2003). PASSCLAIM - Diet-related cardiovascular disease. *European Journal of Nutrition*, Vol. 42, No., pp. 6-27.

Messina, M. J., V. Persky, K. D. R. Setchell and S. Barnes (1994). Soy intake and cancer risk - A review of the in-vitro and in-vivo data. *Nutrition and Cancer-An International Journal*, Vol. 21, No. 2, pp. 113-131.

Muñoz, Y., A. Garrido and L. Valladares (2009). Equol is more active than soy isoflavone itself to compete for binding to thromboxane A(2) receptor in human platelets. *Thrombosis Research*, Vol. 123, No. 5, pp. 740-744.

Nagaoka, S., K. Miwa, M. Eto, Y. Kuzuya, G. Hori and K. Yamamoto (1999). Soy protein peptic hydrolysate with bound phospholipids decreases micellar solubility and cholesterol absorption in rats and Caco-2 cells. *Journal of Nutrition*, Vol. 129, No. 9, pp. 1725-1730.

Nagata, C. (2000). Ecological study of the association between soy product intake and mortality from cancer and heart disease in Japan. *International Journal of Epidemiology*, Vol. 29, No. 5, pp. 832-836.

Nakashima, H., K. Okubo, Y. Honda, T. Tamura, S. Matsuda and N. Yamamoto (1989). Inhibitory effect of glycosides like saponin from soybean on the infectivity of HIV in vitro. *Aids*, Vol. 3, No. 10, pp. 655-658.

Navarro-Nuñez, L., J. Rivera, J. A. Guerrero, C. Martinez, V. Vicente and M. L. Lozano (2009). Differential effects of quercetin, apigenin and genistein on signalling pathways of protease-activated receptors PAR(1) and PAR(4) in platelets. *British Journal of Pharmacology*, Vol. 158, No. 6, pp. 1548-1556.

Oakenfull, D. and G. S. Sidhu (1990). Could saponins be a useful treatment for hypercholesterolemia. *European Journal of Clinical Nutrition*, Vol. 44, No. 1, pp. 79-88.

Odani, S. and T. Ikenaka (1972). Studies on soybean trysin-inhibitors 4. Complete amino acid sequence and anti-proteinase sites of Bowman-Birk soybean proteinase inhibitor. *Journal of Biochemistry*, Vol. 71, No. 5, pp. 839-&.

Okamoto, Y., A. Suzuki, K. Ueda, C. Ito, M. Itoigawa, H. Furukawa, T. Nishihara and N. Kojima (2006). Anti-Estrogenic Activity of Prenylated Isoflavones from *Millettia pachycarpa*: Implications for Pharmacophores and Unique Mechanisms. *Journal of Health Science*, Vol. 52, No., pp. 186-191.

Papazisis, K. T., T. G. Kalemi, D. Zambouli, G. D. Geromichalos, A. F. Lambropoulos, A. Kotsis, L. L. Boutis and A. H. Kortsaris (2006). Synergistic effects of protein tyrosine kinase inhibitor genistein with camptothecins against three cell lines in vitro. *Cancer Letters*, Vol. 233, No., pp. 255-264.

Park, J.-S., M.-S. Woo, D.-H. Kim, J.-W. Hyun, W.-K. Kim, J.-C. Lee and H.-S. Kim (2007). Anti-Inflammatory Mechanisms of Isoflavone Metabolites in Lipopolysaccharide-Stimulated Microglial Cells. *Journal of Pharmacology and Experimental Therapeutics*, Vol. 320, No., pp. 1237-1245.

Paucar-Menacho, L. M., J. Amaya-Farfan, M. A. Berhow, J. M. G. Mandarino, E. G. de Mejia and Y. K. Chang (2010). A high-protein soybean cultivar contains lower isoflavones and saponins but higher minerals and bioactive peptides than a low-protein cultivar. *Food Chemistry*, Vol. 120, No. 1, pp. 15-21.

Phrakonkham, P., J. Chevalier, C. Desmetz, M.-F. Pinnert, R. Bergès, E. Jover, M.-J. Davicco, C. Bennetau-Pelissero, V. Coxam, Y. Artur and M.-C. Canivenc-Lavier (2007). Isoflavonoid-based bone-sparing treatments exert a low activity on reproductive

organs and on hepatic metabolism of estradiol in ovariectomized rats. *Toxicology and Applied Pharmacology*, Vol. 224, No., pp. 105-115.

Pilsakova, L., I. Riecansky and F. Jagla (2010). The Physiological Actions of Isoflavone Phytoestrogens. *Physiological Research*, Vol. 59, No. 5, pp. 651-664.

Potter, S. M. (1995). Overview of Proposed Mechanisms for the Hypocholesterolemic Effect of Soy. *Journal of Nutrition*, Vol. 125, No. 3, pp. S606-S611.

Rao, A. V. and M. K. Sung (1995). Saponins as anticarcinogens. *Journal of Nutrition*, Vol. 125, No. 3, pp. S717-S724.

Redondo-Cuenca, A., M. J. Villanueva-Suarez, M. D. Rodriguez-Sevilla and I. Mateos-Aparicio (2006). Chemical composition and dietary fibre of yellow and green commercial soybeans (Glycine max). *Food Chemistry*, Vol. 101, No. 3, pp. 1216-1222.

Ricci, E., S. Cipriani, F. Chiaffarino, M. Malvezzi and F. Parazzini (2010). Soy Isoflavones and Bone Mineral Density in Perimenopausal and Postmenopausal Western Women: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Journal of Womens Health*, Vol. 19, No. 9, pp. 1609-1617.

Rimbach, G. and J. Pallauf (1998). Phytic acid inhibits free radical formation in vitro but does not affect liver oxidant or antioxidant status in growing rats. *Journal of Nutrition*, Vol. 128, No. 11, pp. 1950-1955.

Rizzi, C., L. Galeoto, G. Zoccatelli, S. Vincenzi, R. Chignola and A. D. B. Peruffo (2003). Active soybean lectin in foods: quantitative determination by ELISA using immobilised asialofetuin. *Food Research International*, Vol. 36, No. 8, pp. 815-821.

Rosano, G. M. C., C. Vitale, G. Marazzi and M. Volterrani (2007). Menopause and cardiovascular disease: the evidence. *Climacteric*, Vol. 10, No., pp. 19-24.

Sarkar, F. H. and Y. Li (2003). Soy Isoflavones and Cancer Prevention. *Cancer Investigation*, Vol. 21, No. 5, pp. 744-757.

Schlemmer, U., W. Frolich, R. M. Prieto and F. Grases (2009). Phytate in foods and significance for humans: Food sources, intake, processing, bioavailability, protective role and analysis. *Molecular Nutrition & Food Research*, Vol. 53, No., pp. S330-S375.

Setchell, K. D. R. (2001). Soy isoflavones - Benefits and risks from nature's selective estrogen receptor modulators (SERMs). *Journal of the American College of Nutrition*, Vol. 20, No. 5, pp. 354S-362S.

Severson, R. K., A. M. Y. Nomura, J. S. Grove and G. N. Stemmermann (1989). A prospective-study of demographics, diet, and prostate-cancer among men of japanese ancestry in Hawaii. *Cancer Research*, Vol. 49, No. 7, pp. 1857-1860.

Shamsuddin, A. M. (2002). Anti-cancer function of phytic acid. *International Journal of Food Science and Technology*, Vol. 37, No. 7, pp. 769-782.

Shiraiwa, M., K. Harada and K. Okubo (1991). Composition and content of saponins in soybean seed according to variety, cultivation year and maturity. *Agricultural and Biological Chemistry*, Vol. 55, No. 2, pp. 323-331.

Singh, R. P. and R. Agarwal (2005). Prostate cancer and inositol hexaphosphate: Efficacy and mechanisms. *Anticancer Research*, Vol. 25, No. 4, pp. 2891-2903.

Taku, K., N. Lin, D. L. Cai, J. W. Hu, X. H. Zhao, Y. M. Zhang, P. Y. Wang, M. K. Melby, L. Hooper, M. S. Kurzer, S. Mizuno, Y. Ishimi and S. Watanabe (2010). Effects of soy

isoflavone extract supplements on blood pressure in adult humans: systematic review and meta-analysis of randomized placebo-controlled trials. *Journal of Hypertension*, Vol. 28, No. 10, pp. 1971-1982.

Taku, K., M. K. Melby, J. Takebayashi, S. Mizuno, Y. Ishimi, T. Omori and S. Watanabe (2010). Effect of soy isoflavone extract supplements on bone mineral density in menopausal women: meta-analysis of randomized controlled trials. *Asia Pacific Journal of Clinical Nutrition*, Vol. 19, No. 1, pp. 33-42.

Tsai, C. Y., Y. H. Chen, Y. W. Chien, W. H. Huang and S. H. Lin (2010). Effect of soy saponin on the growth of human colon cancer cells. *World Journal of Gastroenterology*, Vol. 16, No. 27, pp. 3371-3376.

Tsukamoto, C., S. Shimada, K. Igita, S. Kudou, M. Kokubun, K. Okubo and K. Kitamura (1995). Factors Affecting Isoflavone Content in Soybean Seeds: Changes in Isoflavones, Saponins, and Composition of Fatty Acids at Different Temperatures during Seed Development. *Journal of Agricultural and Food Chemistry*, Vol. 43, No. 5, pp. 1184-1192.

Umphress, S. T., S. P. Murphy, A. A. Franke, L. J. Custer and C. L. Blitz (2005). Isoflavone content of foods with soy additives. *Journal of Food Composition and Analysis*, Vol. 18, No., pp. 533-550.

Vieira, C. R., L. C. Cabral and A. C. O. De Paula (1999). Proximate composition and amino acid, fatty acid and mineral contents of six soybean cultivars for human consumption. *Pesquisa Agropecuaria Brasileira*, Vol. 34, No. 7, pp. 1277-1283.

Villares, A., M. A. Rostagno, A. García - Lafuente, E. Guillamon and J. A. Martínez (2011). Content and Profile of Isoflavones in Soy-Based Foods as a Function of the Production Process. *Food and Bioprocess Technology*, Vol. 4, No. 1, pp. 27-38.

Vina, J. and A. Lloret (2010). Why Women Have More Alzheimer's Disease Than Men: Gender and Mitochondrial Toxicity of Amyloid-beta Peptide. *Journal of Alzheimers Disease*, Vol. 20, No., pp. S527-S533.

Vlietinck, A. J., T. De Bruyne, S. Apers and L. A. Pieters (1998). Plant-derived leading compounds for chemotherapy of human immunodeficiency virus (HIV) infection. *Planta Medica*, Vol. 64, No. 2, pp. 97-109.

Vucenik, I. and A. M. Shamsuddin (2003). Cancer inhibition by inositol hexaphosphate (IP6) and inositol: From laboratory to clinic. *Journal of Nutrition*, Vol. 133, No. 11, pp. 3778S-3784S.

Vucenik, I. and A. M. Shamsuddin (2004). IP-6 and Inositol against Breast Cancer: Modulation of PKC delta and p27(Kip1). *Anticancer Research*, Vol. 24, No. 5D, pp. 539.

Vucenik, I. and A. M. Shamsuddin (2006). Protection against cancer by dietary IP6 and inositol. *Nutrition and Cancer*, Vol. 55, No. 2, pp. 109-125.

Wang, C. F. and M. S. Kurzer (1997). Phytoestrogen concentration determines effects on DNA synthesis in human breast cancer cells. *Nutrition and Cancer-an International Journal*, Vol. 28, No. 3, pp. 236-247.

Wang, H.-J. and P. A. Murphy (1996). Mass Balance Study of Isoflavones during Soybean Processing. *Journal of Agricultural and Food Chemistry*, Vol. 44, No., pp. 2377-2383.

Wolf, W. J. (1970). Soybeans proteins - Their functional, chemical, and physical properties. *Journal of Agricultural and Food Chemistry*, Vol. 18, No. 6, pp. 969-&.

Xu, Q., A. G. Kanthasamy and M. U. B. Reddy (2008). Neuroprotective effect of the natural iron chelator, phytic acid in a cell culture model of Parkinson's disease. *Toxicology*, Vol. 245, No. 1-2, pp. 101-108.

Yuan, S. H. and S. K. C. Chang (2007). Selected odor compounds in soymilk as affected by chemical composition and lipoxygenases in five soybean materials. *Journal of Agricultural and Food Chemistry*, Vol. 55, No. 2, pp. 426-431.

Zhang, E. J., K. M. Ng and K. Q. Luo (2007). Extraction and purification of isoflavones from soybeans and characterization of their estrogenic activities. *Journal of Agricultural and Food Chemistry*, Vol. 55, No. 17, pp. 6940-6950.

Zhang, W. and D. G. Popovich (2010). Group B Oleanane Triterpenoid Extract Containing Soyasaponins I and III from Soy Flour Induces Apoptosis in Hep-G2 Cells. *Journal of Agricultural and Food Chemistry*, Vol. 58, No. 9, pp. 5315-5319.

Zhang, X. L., X. O. Shu, Y. T. Gao, G. Yang, Q. Li, H. L. Li, F. Jin and W. Zheng (2003). Soy food consumption is associated with lower risk of coronary heart disease in Chinese women. *Journal of Nutrition*, Vol. 133, No. 9, pp. 2874-2878.

"Okara" a New Preparation of Food Material with Antioxidant Activity and Dietary Fiber from Soybean

Kohei Suruga[1], Akihisa Kato[2], Kazunari Kadokura[1], Wataru Hiruma[1],
Yoshihiro Sekino[1], C. A. Tony Buffington[3] and Yasuhiho Komatsu[4,5]

[1]*Kibun Foods INC.*
[2]*D & C Veterinary Clinic*
[3]*The Ohio State University*
[4]*Kitazato Institute for Life Science Kitazato University*
[5]*Sun R & D Institute for Natural Medicines Co., INC.*
[1,2,4,5]*Japan*
[3]*USA*

1. Introduction

Okara (OC) is a byproduct of the production of soybean foods such as tofu and soy milk. It is a nutrient-rich product, containing about 25% protein, 20% fat and 33% dietary fiber on a dry basis. Approximately 700,000 tons of okara are produced in Japan each year, some of which has been utilized as a feed for domestic animals and as a fertilizer (O'Toole, 1999). Most of it, however, is discarded as industrial waste because is perishable and other uses for it have not been identified (Ohno & Shoda, 1993), which has created social and environmental problems. Although many papers have reported methods for fermentation (Matsuo, 1997; Jiang et al., 2005; Mizumoto et al., 2006), extraction (Quitain, 2006) and digestion (Kasai et al., 2004), development of effective methods for OC utilization remain an important and difficult challenge. So, as part of a study increase utilization of OC, we have developed a new food product by combining fermented OC with fruits, especially banana. We investigated two properties of this food, reactive oxygen scavenging activity, the effect of including it as part of diet therapy for obesity using dogs as a model.

2. Reactive oxygen scavenging activity of the new food material from OC

Several investigators and some individual enterprises have been studying how to use OC as a new type of food, or in another industrial fields. For example, Maeda Y. developed a method of extracting water-soluble polysaccharides from OC and used them as an emulsifer or viscoelastic reagent (Maeda, 1992). Matsuo M. investigated the composition and properties of OC fermented by *Aspergillus oryzae* (*A. oryzae*) and *Rhizopus oligosporus* (*R. oligosporus*) and reported that the fermented OC could be a useful high-fiber, low-energy food material (Matsuo, 1989a; 1989b). Tempeh, a soy-based food originating in Indonesia, also is fermented by *R. oligosporus*. Today, it is still the most popular soy-based protein food in Indonesia (Golbitz, 1995). The antioxidant activity of OC fermented by *A. oryzae* also has

been evaluated, and the 80% methanol extract of it showed high antioxidant activity (Matsuo, 1997).

Today, many foods are imported to Japan from all over the world. Approximately 1,700,000 tons of fruits imported each year (http://www.kanbou.maff.go.jp /www/jk/kajitu/ 15kajitu.pdf; The Ministry of Agriculture. Forestry and Fisheries of Japan, published results, 2003), including one million tons of banana imported from Philippine and some other countries. Banana is one of the most popular fruits in the world, and represents ca. 50% of all fruits imported into Japan. More that 10% of the imported volume of banana are wasted, however, because of inadequate quality for the retail market. We have a thought that this is quite large volume, which also creates social problems, also might be recycled as a food. Bananas are known to contain antioxidant compounds such as vitamins, flavonoids and phenolic compounds, and the antioxidant activity of banana is higher than that of other fruits. Using photon emission scavenging of reactive oxygen by XYZ system, Someya S. et al. determined that the antioxidant activity of banana was approximately 85-fold and 510-fold greater than that of grapefruit and lemon, respectively (Someya et al., 2003). In addition, these researchers found that the antioxidant activity of banana peel was ca. 1.2-fold that of banana pulp. Based on this information, we have tried to prepare a new food material with high reactive oxygen scavenging activity (ROSA) using fermented OC and banana.

Lately, many people have an interest in reactive oxygen and antioxidant because of the positive relationship between some diseases and reactive oxygen species. The ROSA of cereals and the synergistic effects of combinations of materials have been investigated by XYZ system. Akiyama Y. et al. found a synergistic effect on ROSA between rice and soybean, and between rice and green tea (Akiyama et al., 2002). In their report, they presumed that the thiamin in rice, catechins in green tea, and isoflavones in soybean were the major constituents contributing to the synergistic effects. However, a synergistic effect of fermented OC and banana on ROSA has not previously been reported.

Here, we present a new product created by combination of fermented of OC (OT) and banana, and investigate the synergistic effects of OT and banana on ROSA using photon emission scavenging reactive oxygen by XYZ system.

2.1 Materials and methods
2.1.1 OC fermentation

The method of fermentation of OC described by Aoki H. et al. was utilized with some modifications (Aoki et al., 2003). OC (Food Chemifa Co., Ltd.) was soaked in 0.2% sodium citrate (Wako Pure Chemical Industries) at room temperature for 60 min, and then steamed at 121°C for 20 min. The steamed OC was then cultured with *R. oligosporus* (Akita Konno Co., Ltd.) at 30°C for 24 h under aerobic conditions. After incubation, the fermented product, OT, was lyophilized.

2.1.2 Extraction of ethanol-soluble and water-soluble fraction

Extraction of ethanol-soluble and water-soluble fraction of the mixture of OT and banana (OTB) was based on methods described by Mizuno M. et al. (Mizuno et al., 1998), Nakamura T. et al. (Nakamura et al., 2004) and Matsuo M. (Matsuo, 2005). Lyophilized OTB powder (5 g) was extracted with 80% ethanol (50 mL) at 45°C for 24 h. After extraction with 80% ethanol, the remaining residue was extracted with ultra pure water (50 mL) at room temperature for 24 h. The 80% ethanol-soluble fraction, water-soluble fraction, and the residue after extraction with 80% ethanol and water were lyophilized to powder.

2.1.3 Measurement of reactive oxygen scavenging activity

ROSA was measured using the XYZ system as described by Okubo K. (Okubo, 2002). The lyophilized sample (30 mg), saturated KH_2CO_3 in 10% (Vol/Vol) acetaldehyde (1000 µL), and 0.6% H_2O_2 (1000 µL) were mixed in a 24-well micro titer plate and photon emission was monitored with a Bio-Emission Detector Model Bio-ED21 (E&T Corporation).

2.2 Results and discussion
2.2.1 Photon emission scavenging reactive oxygen of OTB by XYZ system

The XYZ system to measure scavenging activity for reactive oxygen was developed by Arai S. et al. in the process of research on soybean saponins (Arai et al., 2001). The characteristics of this system are (1) short measuring time, (2) simultaneous measurement of 10-20 samples at once, (3) simplified measurement technique, and (4) applicability to liquid and solid samples (Yoshiki et al., 2004). In the case of the ferric thiocyanate method or DPPH radical scavenging method, only liquid extraction samples can be measured. In contrast, the photon emission scavenging reactive oxygen by XYZ system permits analysis of materials of diverse form such as solid extraction residues as well as liquid extraction samples. Researchers in this field would like to have an experimental system able to measure the antioxidant activity of materials of diverse from such as solid type sample like extraction residues for bioresource science.

We next attempted to determine the ROSA of lyophilized OC, lyophilized OT, and lyophilized OTB. OT and banana (B) were mixed (OT:B ratio 1:1 by weight) and the mixture was lyophilized. The photon emission images, obtained using a charge-coupled device camera, are shown in Fig. 1. In this system, photon emission is observed when H_2O_2 reacts with antioxidants and mediators. The intensity of photon emission increases with the radical scavenging ability of the sample.

The photon emission intensity revealed that of OC had almost the same ROSA as that of OT, 1,093 cd/m^2 and 1,266 cd/m^2, respectively. The photon intensity activity of OTB, however, was 34,405 cd/m^2, ca. 30-fold stronger that of either OC or OT alone.
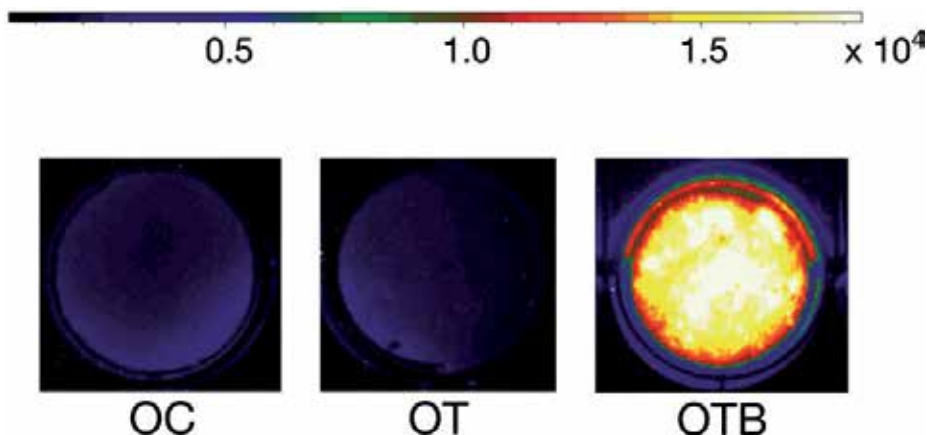
Detection image of photon emission from OC, OT and OTB using a charge-coupled device camera. OC, lyophilized okara; OT, lyophilized okara fermented by R. oligosporus; OTB, lyophilized OT-banana combination. The lyophilized sample (30 mg), saturated KH2CO3 in 10% (Vol/Vol) acetaldehyde (1000 µL), and 0.6% H2O2 (1000 µL) were mixed in a 24-well micro titer plate.

Fig. 1. Photon emission of OC, OT and OTB.

2.2.2 Effect of mixing ratio of OT and banana on photon emission scavenging reactive oxygen by XYZ system

To investigate the relationship between the ROSA and the mixing ratio of OT:B (by weight), mixtures of 4:1, 4:3, 1:1, 3:4, 1:4, and dry banana powder were prepared. All samples were lyophilized, and the ROSA was measured using the method of photon emission scavenging reactive oxygen by XYZ system (Fig. 2). All of the prepared OTB mixtures, exhibited a synergistic effect on ROSA. The photon emission intensity of OTB 4:3 was highest of all the OTB mixtures at 36,906 cd/m^2, some 3-fold and 30-fold more potent than that of dry banana powder (photon intensity: 11,531 cd/m^2) or OT (photon intensity: 1,266 cd/m^2), respectively.
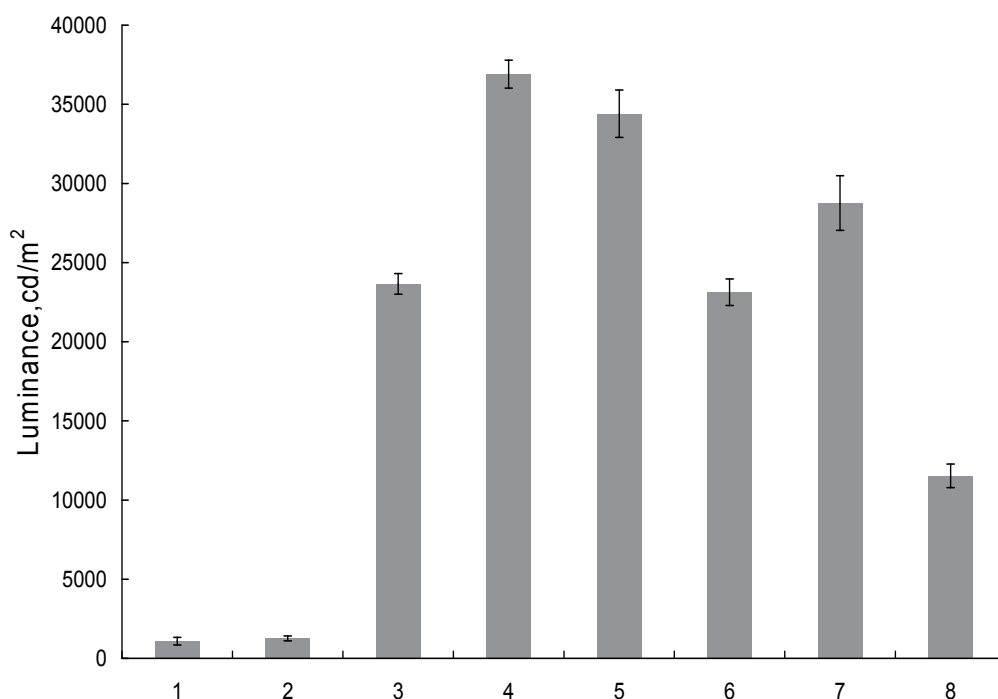
1, lyophilized OC; 2, lyophilized OT; 3, lyophilized OTB 4:1; 4, lyophilized OTB 4:3; 5, lyophilized OTB 1:1; 6, lyophilized OTB 3:4; 7, lyophilized OTB 1:4; 8, lyophilized banana. The lyophilized sample (30 mg), saturated KH$_2$CO$_3$ in 10% (Vol/Vol) acetaldehyde (1000 μL), and 0.6% H$_2$O$_2$ (1000 μL) were mixed in a 24-well micro titer plate. Data represent the mean ± SD (n=5).

Fig. 2. Effect of mixing ratio (by weight) of OT and banana on photon emission intensity.

The effect of various concentrations of OTB 4:3 (0 mg, 0.03 mg, 3 mg, 10 mg, and 30 mg) on ROSA is shown in Fig. 3. The ROSA of OTB 4:3 increased with increasing OTB 4:3 concentrations. Gulcin I. investigated the effect of concentration of L-carnitine on antioxidant activity, and reported that the antioxidant power of L-carnitine increased with increasing concentration (Gulcin, 2006). There is also a report that the antioxidant effect of 3-hydroxyanthranilic acid (HAA) from soybean increased with increasing the added amount of HAA (Esaki et al., 1996). Our results were similar to that of those reports, and confirmed the reproducibility of ROSA of OTB 4:3.
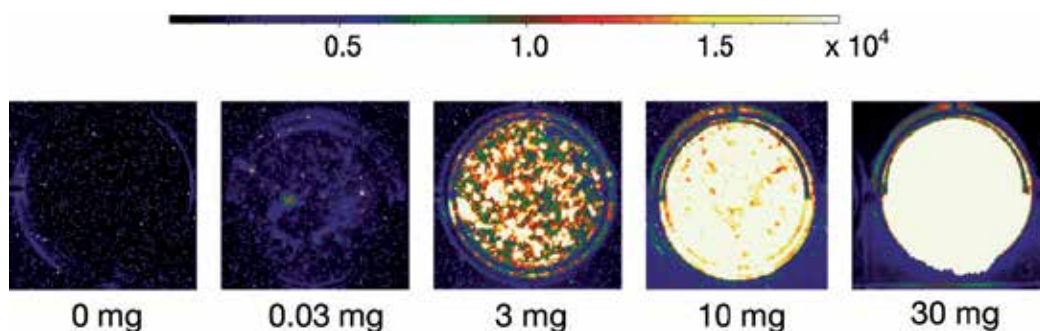
Imaging detection of photon emission from OTB 4:3 using a charge-coupled device camera. The lyophilized OTB 4:3 (0 mg, 0.03 mg, 3 mg, 10 mg, and 30 mg), saturated KH_2CO_3 in 10% (Vol/Vol) acetaldehyde (1000 µL), and 0.6% H_2O_2 (1000 µL) were mixed in a 24-well micro titer plate.

Fig. 3. Photon emission of various concentration of OTB 4:3.

2.2.3 Photon emission scavenging reactive oxygen of OTB extraction by XYZ system

The 80% ethanol-soluble fraction and water-soluble fraction of OTB were extracted to investigate the ROSA of extracted OTB by XYZ system. In this experiment, the 80% ethanol-soluble and water-soluble fractions were extracted from OTB 4:3 (Fig. 4), because the ROSA of OTB 4:3 was greater than that of other OTB mixture (see preceding section and Fig. 2). The ROSA of whole OTB 4:3 (30 mg), 80% ethanol-soluble fraction (30 mg), water-soluble fraction (30 mg), and extraction residue after extraction with 80% ethanol and water were measured using XYZ system, and the results were shown in Fig. 5. Contrary to the prediction, the ROSA of 80% ethanol-soluble fraction and water-soluble fraction were lower than that of other samples. On the other hand, ROSA of the extraction residue of OTB 4:3 (photon intensity: 42,062 cd/m^2) was higher than that of both the 80% ethanol-soluble fraction (photon intensity: 312 cd/m^2) and the water-soluble fraction (photon intensity: 4,528 cd/m^2). From these results, it was concluded that the effective ROSA of OTB 4:3 was attributable to components in the residue left after extraction with 80% ethanol and water. Iwai K. et al. studied the antioxidant activity of blueberry using the XYZ-dish method and reported that the activity of the residue left after extraction with distilled water was greater than that of the water solution (Iwai et al., 2001b). Someya S. et al. investigated the chemiluminescence properties of banana extracts and extraction residue after extraction with water by XYZ system and demonstrated that the photon emission intensity of extraction residue was 5.7-fold that of extracts (Someya et al., 2003). Our results agree with the data by Iwai K. et al. and Someya S. et al.. The residue left after extraction with 80% ethanol and water of OTB 4:3 has many bioactive components, and dietary fiber may be one of the most important substances there. OC and OT contained dietary fiber at a level of ca. 50% of dry weight (Matsuo, 1989b). Approximately 2 g of dietary fiber is contained in 100 g of banana (Forster et al., 2002). The dietary fiber in foods has protective effects against cardiovascular disease and atherosclerosis (Anderson et al., 1990; Anderson, 1995; Rimm et al., 1996; Van Horn, 1997). In addition to these functions, dietary fiber may show the photon emission scavenging reactive oxygen by XYZ system. Prosky L. et al. reported the method of extraction and determination of insoluble, soluble, and total dietary fiber in foods and food products (Prosky, 1988). In the method, foods and food products were treated by

thermostable α-amylase and amyloglucosidase for extraction of dietary fiber. The ethanol-soluble and water-soluble fraction of OTB 4:3 did not contain dietary fiber, because thermostable α-amylase and amyloglucosidase treatments were not done during OTB 4:3 extraction. The ROSA of the extraction residue of OTB 4:3 may have been higher than that of the 80% ethanol-soluble and water-soluble fractions because the dietary fiber may have remained in the residue after extraction with 80% ethanol and water. The antioxidant components in OTB and the mechanism of a synergistic effect of OT and banana on ROSA are still unclear, and we currently are examining these further.
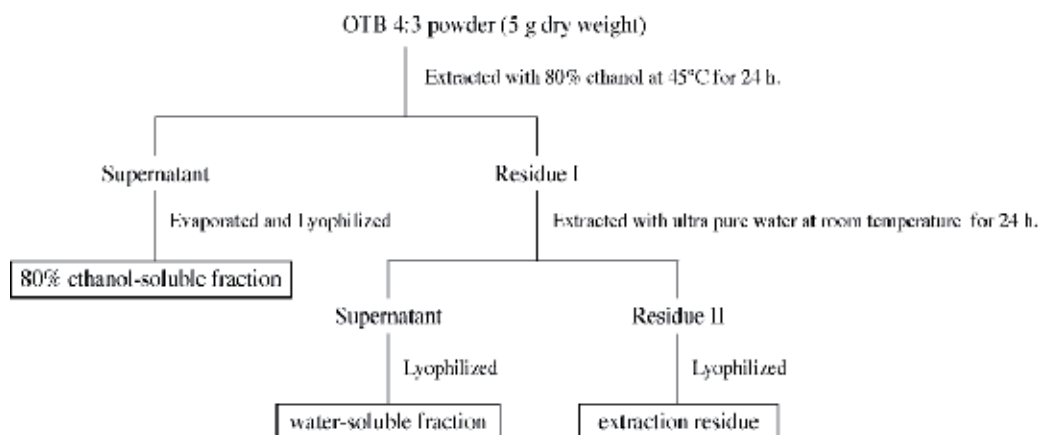
Fig. 4. Extraction procedure of ethanol-soluble and water-soluble fraction of OTB 4:3.
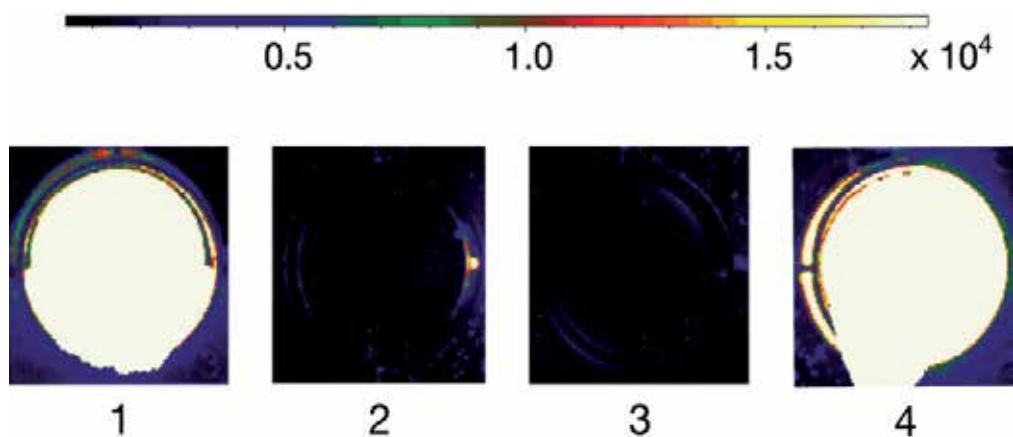
1, lyophilized OTB 4:3; 2, lyophilized 80% ethanol-soluble fraction from OTB 4:3; 3, lyophilized water-soluble fraction from OTB 4:3; 4, lyophilized extraction residue after extraction with 80% ethanol and water. Imaging detection of photon emission from OTB 4:3, 80% ethanol-soluble fraction, water-soluble fraction, and their residue using a charge-coupled device camera. The lyophilized sample (30 mg), saturated KH_2CO_3 in 10% (Vol/Vol) acetaldehyde (1000 µL), and 0.6% H_2O_2 (1000 µL) were mixed in a 24-well micro titer plate.

Fig. 5. Photon emission of OTB 4:3 (OTB 4:3) extractions.

"Okara" a New Preparation of Food Material with
Antioxidant Activity and Dietary Fiber from Soybean
317

2.2.4 Comparison of OTB with commercial organic green tea powder on photon emission scavenging reactive oxygen by XYZ system

Recently, many reports have appeared concerning about the antioxidant activity of various foods. One of the representative antioxidant foods is green tea, because ascorbic acid, α-tocopherol, and catechins are found at high concentrations in green tea. Iwai K. et al. investigated the antioxidant activity of commercially available tea and found that the antioxidant activity of tea infusions was stronger than that of canned tea (Iwai et al., 2001a). We compared the ROSA of OTB 4:3 powder with that of two kinds of commercially available organic green tea powder (Fig. 6), and found that the ROSA of OTB 4:3 was nearly equal to that of the tea samples (The photon intensity of lane 4 and lane 5 of Fig. 6 was 48,961 cd/m^2 and 49,914 cd/m^2, respectively). In addition, we had received data that showed the lipid peroxide concentration in the serum of mice that took the OTB 4:3 tended to decrease (Sugiyama H., School of Medicine, Akita University, personal communication). These data show that OTB 4:3 has high ROSA, and could be useful as a novel antioxidant food material and/or nutritional supplement.
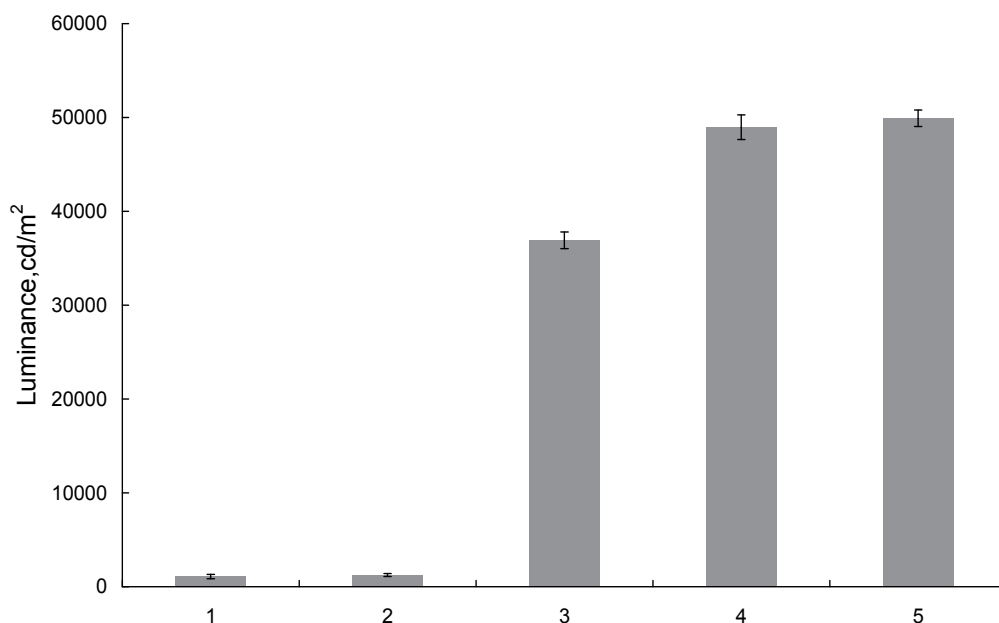
1, lyophilized OC; 2, lyophilized OT; 3, lyophilized OTB 4:3; 4 and 5, two kinds of commercial organic green tea powder. The lyophilized sample (30 mg), saturated KH_2CO_3 in 10% (Vol/Vol) acetaldehyde (1000 μL), and 0.6% H_2O_2 (1000 μL) were mixed in a 24-well micro titer plate. Data represent the mean ± SD (n=5).

Fig. 6. Photon intensity of OTB 4:3 and commercial organic green tea.

3. Diet effect of the new food material from OC on overweight and/or obesity dogs

An increased prevalence of obesity recently has been reported worldwide, both in developed and developing countries. Overweight and obesity have been associated with several chronic diseases and disabilities, including type 2 diabetes, cardiovascular diseases,

hypertension, certain types of cancer, and premature death (Chopra et al., 2002; Lew, 1985). Mokdad, A. H. et. al. reported that in Unites States, the number of patients with obesity and has been increasing in men and women of all ages, all races, all educational levels, and all smoking levels (Mokdad et al., 2003). Therefore, the health status and health problem related to obesity are becoming more and more important issues.

The prevalence of obesity is increasing in companion dogs as well as in human beings. Companion dogs are an indispensable part of people's lives, and many owners consider their dogs to be part of their family. Several investigators have reported a relationship between health and obesity in dogs. Stone, R. et al. reported the higher serum concentrations of triglycerides and cholesterol in obese dogs suggesting an association between metabolic derangements and obesity in dogs similar to those observed in human beings (Stone et al, 2009). Increasing rates of obesity in dogs also have been associated with increased rates of osteoarthritis, insulin resistance and certain neoplasias (Laflamme, 2006). Heuberger, R. and Wakshiag, J. estimated that owners who ate nutrient-rich, calorie-poor diets had normal weight dogs, whereas owners who fed more table scraps had overweight dogs (Heuberger & Wakshlag, 2011). In addition, non-obese dogs consumed significantly more crude dietary fiber in their diets (Heuberger & Wakshlag, 2011).

As mentioned, OC contains a rather large amount of dietary fiber. In general, increasing dietary fiber intake promotes body fat loss (Roberts et al., 2002; Slavin, 2005). Matsumoto K et al. investigated the effect of OC on the prevention of obesity using a mouse model (Matsumoto et al., 2007). However, to our knowledge there is no published literature describing the effects of consumption of OTB on obesity in dogs. To begin to address this question, we conducted a pilot clinical research study to assess the effect of addition of OTB to the usual diet on overweight and obesity dogs.

3.1 Materials and methods
3.1.1 Case selection
Candidate dogs for weight loss study were selected from out-patient dogs that were relatively healthy except for overweight and/or obesity. The veterinarian estimated the body fat of the dogs using the Body Condition Score (BCS, Fig. 7), and diagnosed overweight (BCS=4) and/or obese (BCS=5) dogs admitted for usual care. For weight loss to succeed, one must select suitable candidate patient dogs and owners, those who concerned about "their"obesity and ready to make changes.

3.1.2 Weight loss study program
The diet effect of OTB paste (moisture: ca.60%) was examined in consultation with the pet owners about the obese state of their dogs (Fig. 8). Owners substituted OTB paste (OTBp) for 30% to 50% of the dog's usual food to maintain the volume of the food equivalent to the original by weight as shown in Fig. 9. The effectiveness of this addition was evaluated by periodic measurement of body weight. In some cases, laboratory examinations also were performed.

3.1.3 Blood biochemical clinical examination
Blood tests were performed on two of the six cases according to conventional methods. Blood samples were collected at the starting date of the study, and again at the end of the test. Blood tests were performed using a PocH-100iv, Sysmex, Tokyo, Japan, and blood biochemical examinations were performed using a Spotchem EZ #sp-4430 Arkray, Tokyo, Japan.
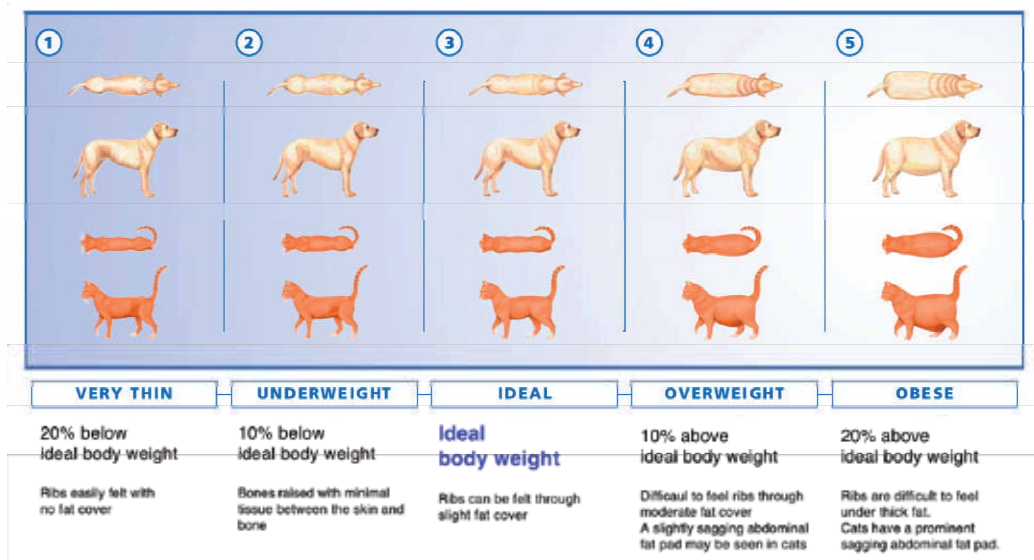
Fig. 7. Body Condition Score (BCS).

Fig. 8. The obesity dog (case No. 05709).

30 % to 50 % by weight of the daily ration of dog food was replaced with OTBp.

Fig. 9. The modified food used in weight loss study program.

3.2 Results and discussion

The nutrition facts of OTBp were shown in Table 1. As shown in the Table 1, OTBp contains only 137 kcal per 100 grams as fed.

Per 100 g edible portion

Nutrition Facts	
Energy	137 kcal
Water	63.8 g
Protein	3.8 g
Lipid	1.3 g
Ash	1.3 g
Carbohydrate	25.1 g
Fiber	4.7 g
Sodium	19.6 mg

Test Analysis by Japan Food Analysis Center

Table 1. Nutrition facts of OTB paste.

Prior to the weight loss study, we tested the response to OTBp of dogs that were selected at random from usual clinic patents. We found that 42 of 51 dogs, nearly 80%, ate the OTBp, suggesting good acceptance by dogs. Of the eight owners enrolled in the weight loss after oral informed consent, only two dropped out of the study because their dogs refused to eat the diet. The remaining six dogs completed the study. Average for the changes in body weight is shown in Fig. 10.

Patient No.	Canine Strain	sex	Age	BCS at start	Body weight (kg)[a]						BCS at end	Max Reduce %	Period days	Satis-faction[b]
					point 0	point 1	point 2	point 3	point 4	point 5				
05803	Miniature Dachshund	♀	7.5	4	7.00	6.95	6.75	6.60	6.55	6.55	3	6.4%	75	excellent
					100.0%	99.3%	96.4%	94.3%	93.6%	93.6%				
03767	Miniature Schnauzer	♂spay	5.5	5	9.60	9.80	9.65	9.50	9.55	9.65	5	1.0%	37	good
					100.0%	102.1%	100.5%	99.0%	99.5%	100.5%				
05634	Miniature Pinscher	♀	3.5	5	5.75	5.70	5.65	5.60	5.45	---	3	5.2%	42	excellent
					100.0%	99.1%	98.3%	97.4%	94.8%					
05709	Miniature Dachshund	♀spay	12.3	5	8.85	8.30	8.20	8.10	7.85	7.80	3	11.9%	53	excellent
					100.0%	93.8%	92.7%	91.5%	88.7%	88.1%				
06081	Papillon	♂	4.5	5	6.50	6.55	6.40	6.40	6.36	6.34	4	2.5%	50	excellent
					100.0%	100.8%	98.5%	98.5%	97.8%	97.5%				
05645	Miniature Dachshund	♀spay	6.5	4	6.70	6.75	6.75	6.60	6.65	---	4	1.5%	37	fair
					100.0%	100.7%	100.7%	98.5%	99.3%					
	mean		6.63	4.67	100.00%	99.30%	97.85%	96.52%	95.61%	94.94%	3.67	4.75%	49.00	
	±SD		3.10	0.52	0.00%	2.91%	3.00%	2.98%	4.14%	5.36%	0.82	4.08%	14.35	

[a] Body weight of the dogs were measured every seven or ten days.
[b] Owner's satisfaction was as follows; excellent, good, fair and poor.

Table 2. Dog characteristics and body weight changes.
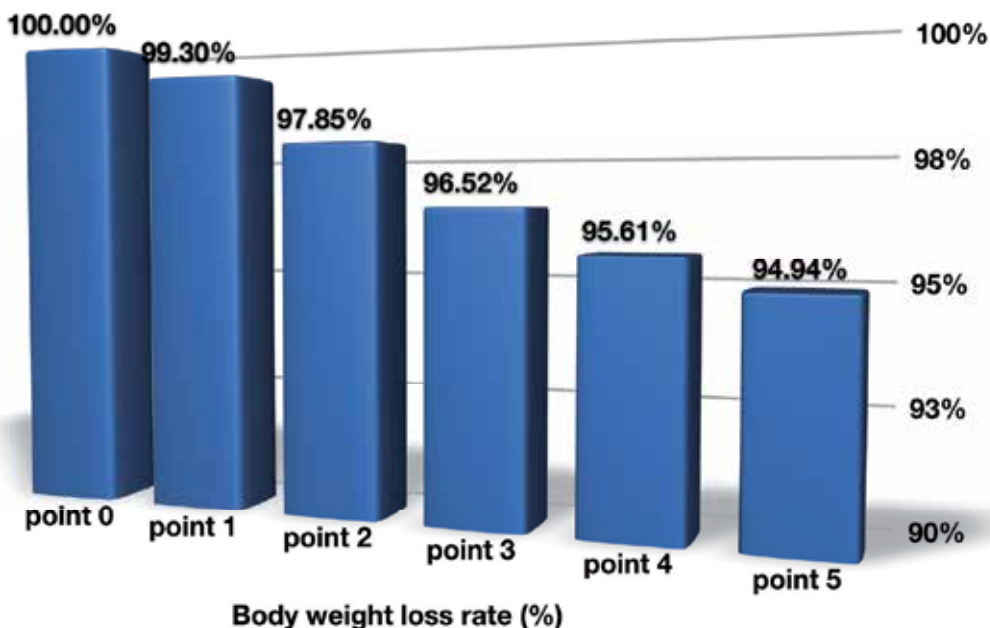
Fig. 10. Body weight loss rate (%) of dogs (n=6).

Lose of body weight was clearly seen in three cases, #05803, #05634 and #05709, during the study, and the dog's owners were satisfied with the program. In case of #06081, a body weight gain was observed between the beginning of the study and the seven day weight (point 1), but after that the body weight decreased and the owner was also satisfied with these results. On the other hand, in two cases (#03767 and #05645), the final body weight (point 5, at the end of the study) of the dogs was almost same as that of point 0, at the beginning of the study. In these cases, the body weight of the dogs increased up to point 2, decreased from point 2 to point 3, and increased again after point 3.

The mean percent loss of the body weight in the overwight and/or obesity dogs (n=6) is shown in Fig. 10. As can be seen in Fig. 10 and Table 2, feeding OTBp resulted in a average body weight loss of about 5%, and a body fat (BCS) loss of approximately 20% during the 35 days of the study. Although preliminary, these results suggest a beneficial effect of addition of OTBp to the diet on body fat loss in overweight and/or obese dogs.

Blood and serum biochemical evaluations were carried out in two cases, #03767 and #06081, and the results aree shown in Table 3. The results revealed that OTBp did not exhibit any adverse reactions or influences on health conditions of the dogs through out the period of study. In additiont, OTBp treatment lowered the alkaline phosphatase activity (ALP IU/L) of the dogs between the initial (day 0) and final measurements, suggesting that OTBp might have an improving effect on liver function. Nishi, S. et al. investigated the antiobesity effects of seaberry leaf polyphenol (SBLPP) juice using male mice (ddY) fed a high-fat diet. In the paper, they reported that the body weight of experimental mice fed a high-fat and SBLPP juice for eight weeks was significantly lower than that of control levels, and that the SBLPP juice also lowered the blood glutamic pyruvic transaminase (GPT) and ALP activities in the mice. (Nishi et al., 2007). Swaroop, A. et al. and Yang, Z. G. et al. reported that the SBLPP had potent antioxidant activity (Swaroop et al., 2005; Yang et al., 2007). Taken together, these results suggest that OTBp may improve liver function, possibly due to its potent antioxidant activity.

ITEM	#03767		#06081	
	DAY 0	DAY31	DAY 0	DAY50
WBC x10^2/µL	100.00	93.00	93.00	140.00
RBC x10^4/µL	845.00	889.00	916.00	938.00
HCT %	56.10	58.40	56.30	56.70
PLT x10^4/µL	46.80	48.40	36.20	22.40
Glu mg/dl	105.00	101.00	94.00	97.00
T-Cho mg/dl	> 400.0	> 400.0	> 400.0	> 400.0
BUN mg/dl	13.00	13.00	14.00	14.00
T-Bil	< 0.2	< 0.2	< 0.2	< 0.2
GOT IU/L	14.00	10.00	11.00	14.00
GPT IU/L	92.00	86.00	72.00	76.00
T-Pro g/dl	8.00	7.60	6.40	6.10
GGT IU/L	< 10.0	< 10.0	< 10.0	< 10.0
ALP IU/L	2115.00	1547.00	212.00	< 130.0

Table 3. The results of blood test and biochemical examination on case #03767 and #06081.

The results of this pilot study are consistent with those of other investigations of soybean components on obesity. For example, Jenkins, D. J. et al. reported that consumption of a low-energy diet containing soy protein had signnificant anti-obesity effects (Jenkins et al., 1989). Goodman-Gruen, D. and Kritz-Silverstein, D. investigated the beneficial effects of soy phytoestrogen, and isoflavones on excess body weight (Goodman-Gruen and Kritz-Silverstein, 2001). Moreover, in a study of genetically obese mice (yellow KK), Aoyama, T. et al. reported that soy-protein and its hydrolysate (active tetrapeptides) were effective for weight reduction (Aoyama et al., 2000). From these reports, we hypothesized that OTBp might contain effective components from the soybean residue, OC. OTBp also is dilute in energy, and contains a large amount of dietary fiber (Table 1). Some reports showed that the dietary fiber has an effect on reducing serum cholestrol concentrations by increasing fecal bile acid excretion (Andersson et al., 2002; Chau and Huang, 2005; van Benneleum, 2005). Ble-Castillo, J. L. et al. reported that addition of a banana supplement containing dietary fiber to the diet significantly lowered body weight and increased insulin sensitivity (Ble-Castillo et al, 2010). Slavin, J. L. et al. reported that increasing intake of dietary fiber resulted in decreased feelings of hunder, and played a role in control of energy balance (Slavin et al, 2005). Epidemiological research also has shown the effectiveness of dietary fiber intake on preventing obesity (Robert et al, 2002).

Many papers also have shown obesity to be associated with increased oxidative stress in human beings and mice, which could be associated with obesity-associated metabolic syndrome (Keaney et al., 2003; Vincent et al., 1999; Furukawa et al., 2004). Hogan, S. et al. demonstrated that grape pomace extract had significant antioxidant capacity, and exerted an anti-inflammatory activity in subjects with diet-induced obesity (Hogan et al, 2010). Shen, X. H. et al. reported that the antioxidant vitamin E could play an important role in the treatment of obesity-related diseases (Shen et al., 2010). Given the high antioxidant activity we found in OTB (see preceding section). The effects of OTBp on fat loss in overweight

and/or obesity dogs may have resulted from its combination of properties of low energy, presence of effective soybean compnents, amount of dietary fiber, and potent antioxidant activity.

Another important consideration for the success of diet therapy for weight loss is the attitude of to owner toward their pet, as shown in the recent report of the the relationship between feeding patterns and obesity in dogs by Heuberger, R. and Wakshlag, J. mentioned previously. Thus, we studied pets whose owners were conserned about their pet's weight and wanted to make a change. Under these circumstances, we found that addition of OTBp might be an effective treatment for dog owners as well as for their obese pets. The detailed mechanisms of the OTBp effect are still unclear because we have not yet conducted any laboratory experiments to clarify the diet effects of OTBp. We need basic experiments using obesity model mice.

4. Conclusion

In this paper, we have described our efforts to use OC to develop a new food material from OC by fermentation with *R. oligosporus* and combination with banana. We have investigated two activities, ROSA, and the effects of the new food material from OC on obese dogs. First, in the ROSA studies, a synergistic effect on ROSA between OT and banana was observed, and the activity of OTB was approximately 30-fold more potent than that of OC alone by XYZ system. We concluded that ROSA of OTB was attributable to components contained in the dietary fiber portion of the residue after extraction with 80% ethanol and water. This is the first report about the synergistic effect of fermented OC and banana on ROSA by XYZ system (Suruga et al., 2007). Secondary, OTBp showed a dietary effect on obesity in dogs. This clinical study was a part of application researches of the OTB. In the veterinary clinical study, OTBp reduced the body weight and BCS in the obese dogs in a time-dependent manner during the study. Moreover, the ALP activity of the two dogs was lowered than that of the control (0 day). This result indicated that OTBp could have improving effect on the liver function because of the potent antioxidant activity present in OTB. From these results, OTBp is thought to have an improving effect on liver function, and a diet effect on the body weight without any adverse reactions. OTB prepared from OC by fermentation and combination with banana could be developed as a useful, novel food material and/or nutritional supplement with both antioxidant and dietary effects. Further research and development is undergoing.

5. Acknowledgments

6. References

Akiyama, Y., Yoshiki, Y. & Okubo, K. (2002). Studies on reactive oxygen scavengers in cereals. *Journal of Photon Emission Scavenging Reactive Oxygen by XYZ system*, 1, 37-44 (in Japanese).

Anderson, JW., Deakins, DA., Floore, TL., Smith, BM. & Whitis, SE. (1990). Dietary fiber and coronary heart disease. *Crit. Rev. Food Sci. Nutr.*, 29, 95-147.

Anderson, JW. (1995). Dietary fiber, complex carbohydrate and coronary heart disease. *Can. J. Cardiol.*, 11, 55G-63G.

Andersson, M., Ellegard, L. & Andersson, H. (2002). Oat bran stimulates bile acid synthesis within 8 h as measured by 7 α -hydroxy-4-cholesten-3-one. *Am. J. Clin. Nutr.*, 76, 1111-1116.

Aoki, H., Uda, I., Tagami, K., Furuya, Y., Endo, Y. & Fujimoto, K. (2003). The production of a new *Tempeh*-like fermented soybean containing a high level of γ-aminobutyric acid by anaerobic incubation with *Rhizopus*. *Biosci. Biotech. Biochem.*, 67, 1018-1023.

Aoyama, T., Fukui, K., Nakamori, T., Mashimoto, Y., Endo, Y., Yamamoto, T., Takamatsu, K. & Sugano, M. (2000). Effect of soy and milk whey protein isolates and their hydrolysates on weight reduction in genetically obese mice. *Biosci. Biotech. Biochem.*, 64, 2594-2600.

Arai, S., Osawa, T., Ohigashi, H., Yoshikawa, M., Kaminogawa, S., Watanabe, M., Ogawa, T., Okubo, K., Watanabe, S., Nishino, S., Shinohara, K., Esashi, T. & Hirahara, T. (2001). A mainstay of functional food science in Japan-History, present status, and future outlook. *Biosci. Biotech. Biochem*, 65, 1-13.

Bendixen, H. (2004). Major increase in prevalence of overwight and obesity between 1987 and 2001 among Danish adults. *Obes. Res.*, 12, 1464-1472.

Ble-Castillo, JL., Asparicio-Trapala, MA. Francisco-Luria, MU., Cordova-Uscanga, R., Rodriguez-Hernandez, A., Mendez, JD. & Diaz-Zagoya, J. (2010). Effects of native banana starch supplementation on body weight and insulin sensitivity in obese type 2 diabetics. *Int. J. Environ. Res. Public Health*, 7, 1953-1962.

Chau, CF. & Huang, YL. (2005). Effects of the insoluble fiber derived from Passiflora edulis seed on plasma and hepatic lipids and fecal output. *Mol. Nutr. Food Res.*, 49, 786-790.

Chopra, M., Galbraith, S., & Darnton-Hill, I. (2002). A global response to a global problem: the epidemic of overnutrition. *Bull. World Health Organ.*, 80, 952-958.

Esaki, H., Onozaki, H., Kawakishi, S. & Osawa, T. (1996). New antioxidant isolation from tempeh. *J. Agric. Food Chem.*, 44, 696-700.

Forster, MP., Rodriguez, ER. & Romero, CD. (2002). Differential characteristics in the chemical composition of banana from Tenerife (Canary Islands) and Ecuador. *J. Agric. Food Chem.*, 50, 7586-7592.

Furukawa, S., Fujita, T., Shimabukuro, M., Iwaki, M., Yamada, Y., Nakajima, Y., Nakayama, O., Makishima, M., Matsuda, M. & Shimomura, I. (2004). Increased oxidative stress in obesity and its impact on metabolic syndrome. *J. Clin. Invest.*, 114, 752-761.

Golbitz, P. (1995). Traditional soyfoods: Processing and products. *J. Nitr.*, 125, 570S-572S.

Goodma-Gruen, D. & Kritz-Silveratein, D. (2001). Usual dietary isoflavone intake is associated with cardiovascular risk factors in post menopausal women. *J. Nutr.*, 131, 1202-1206.

Gulcin, I. (2006). Antioxidant and antiradical of L-carnitine. *Life Science*, 78, 803-811.

Heuberger, R. & Wakshiag, J. (2011). The relationship of feeding patterns and obesity in dogs. *J. Anim. Physiol. Anim. Nutr.*, 95, 98-105.

Hogan, S., Canning, C., Sun, S., Sun, X. & Zhou, K. (2010). Effects of grape pomace antioxidant extract on oxidative stress and inflammation in diet induced obese mice. *J. Agric. Food Chem.*, 58, 11250-11256.

"Okara" a New Preparation of Food Material with
Antioxidant Activity and Dietary Fiber from Soybean
325

Iwai, K., Sato, M. & Matsue, H. (2001a). Relationship between antioxidant activity of commercial tea drinks by XYZ-dish method and their concentration of catechins. *Food Sci. Biotechnol.*, 10, 508-512.

Iwai, K., Abe, K., Chung, S. & Matsue, H. (2001b). XYZ-dish method as a new antioxidant activity assay using photon detection. *Food Sci. Biotechnol.*, 10, 513-520.

Jenkins, DJ., Wolever, TM., Spoller, G., Buckley, G., Lam, Y., Jenkins, AL. & Josse, RG. (1989). Hypocholesterolemic effect of vegetable protein in a hypocaloric diet. *Atherosclerosis*, 78, 99-107.

Keaney, JF. Jr., Larson, MG., Vasan, RS., Wilson, PW., Lipinska, I., Corey, D., Massaro, JM., Sutherland, P., Vita, JA. & Benjamin, EJ. (2003). Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham study. *Arterioscler. Thromb. Vasc. Biol.*, 23, 434-439.

Laflamme, DP., (2006). Understanding and managing obesity in dogs and cats. *Veterinary Clinics North America Small Animal Practice.*, 36, 1283-1296.

Lew, EA., (1985). Mortality and weight: insured lives and the American Cancer Sosiety studies. *Ann. Intern. Med.*, 103, 1024-1029.

Maeda, Y. (1992). Development and application of soybean polysaccharides. *Shokuhinn to Kaihatsu* 27, 47-49 (in Japanese).

Matsuo, M. (1989a). Morphological, compositional and physicochemical properties of "Okara" fermented by *Aspergillus oryzae*. *Nippon Nogeikagaku Kaishi* 63, 1765-1770 (in Japanese).

Matsuo, M. (1989b). Morphological and physicochemical properties and composition of "Okara" fermented by *Rhizopus oligosporus*. *Nippon Eiyo Shokuryo Gakkaishi* 42, 173-178 (in Japanese).

Matsuo, M. (1997). *In vivo* antioxidant activity of *Okara Koji*, a fermented Okara, by *Aspergillus oryzae*. *Biosci. Biotech. Biochem.*, 61, 1968-1972.

Matsuo, M. (2005). *In vivo* antioxidant activity of methanol extract from Quinoa fermented with *Rhizopus oligosporus*. *J. Nutr. Sci. Vitaminol.*, 51, 449-452.

Matsumoto, K., Watanabe, Y., & Yokoyama, S. (2007). Okara, soybean residue, prevents obesity in a diet-induced murine obesity model. *Biosci. Biotech. Biochem.*, 71, 720-727.

Mizuno, M., Morimoto, M., Minato, K. & Tsuchida, H. (1998). Polysaccharides from *Agaricus blazei* stimulate lymphocyte T-cell subsets in mice. *Biosci. Biotech. Biochem.*, 62, 434-437.

Mokdad, AH., Ford, ES., Bowman, BA., Dietz, WH., Vinicor, F., Bales, VS. & Marks, JS. (2003). Prevalence of obesity, diabetes, and obesity-related health risk factor, 2001. *JAMA.*, 289, 76-79.

Nakamura, T., Matsugo, S., Uzuka, Y., Matsuo, S. & Kawagishi, H. (2004). Fractionation and anti-tumor activity of the mycelia of liquid-cultured *Phellinus linteus*. *Biosci. Biotech. Biochem.*, 68, 868-872.

Nishi, S., Saito, Y., Koaze, H., Hironaka, K. & Kojima, M. (2007). Anti-obesity effects of seaberry (*Hippophae rhamnoides*) leaf polyphenols (SBLPP) in male mice fed a high-fat diet. *Nippon Shokuhin Kagaku Kogaku Kaishi* 54, 477-481 (in Japanese).

Okubo, K. (2002). Photon emission scavenging reactive oxygen by XYZ system and its application. *Journal of Photon Emission Scavenging Reactive Oxygen by XYZ system*, 1, 3-20 (in Japanese).

Ohno, A. & Shoda, M., (1993). Production of the antifungal peptide antibiotic, iturin by *Bacillus subtilis* NB22 in solid state fermentation. *J. Ferment. Bioeng.*, 75, 23-27.

O'Toole, DK. (1999). Characteristics and use of okara, the soybean residue from soy milk production-a review. *J. Agric. Food Chem.*, 47, 363-371.

Prosky, L., Asp, NG., Schweizer, TF., Devries, JW. & Furda, I. (1988). Determination of insoluble, soluble, and total dietary fiber in foods and food products: interlaboratory. *J. Assoc. Off. Anal. Chem.*, 71, 1017-1023.

Rimm, EB., Ascherio, A., Giovannucci, E., Spiegelman, D., Stampfer, MJ., & Willett, WC. (1996). Vegetable, fruit and cereal intake and risk of coronary heart disease among men. *JAMA.*, 275, 447-451.

Roberts, SB., McCrory, MA. & Saltzman, E. (2002). The influence of dietary composition on energy intake and body weight. *J. Am. Coll. Nutr.*, 21, 140S-145S.

Shen, XH., Tang, QY., Huang, J. & Cai, W. (2010). Vitamin E regulates adipocytokine expression in a rat model of dietary-induced obesity. *Experimental Biology and Medicine*, 235, 47-51.

Slavin, JL. (2005). Dietary fiber and body weight. *Nutrition*, 21, 411-418.

Someya, S., Yoshiki, Y. & Okubo, K. (2003). Chemiluminescence properties of banana extracts. *Journal of Photon Emission Scavenging Reactive Oxygen by XYZ system*, 2, 15-18 (in Japanese).

Stone, R., Bergoff, N., Steiner, JM. & Zoran, D. (2009). Use of a bioelecrical impedence device in obese and lean healthy dogs to estimate body fat percentages. *Veterinary Therapy*, 10, 59-70.

Suruga, K., Akiyama, Y., Kadokura, K., Sekino, Y., Kawagoe, M., Komatsu, Y. & Sugiyama, T. (2007). Synergistic effect on reactive oxygen scavenging activity of fermented okara and banana by XYZ system. *Food Sci. Technol. Res.*, 13, 139-144.

Swaroop, A., Shinba, AK., Chawla, R., Arora, R., Sharma, RK. & Kumar, JK. (2005). Isolation and characterization of 1,3-dicapryloy-2-linoleoylglycerol a novel triglyceride from berries of *Hippophae ehamnoides*. *Chem. Pharm. Bull.*, 53, 1021-1024.

Van Bennekum, AM., Nguyen, DV., Schulthess, G., Hauser, H. & Phillips, MC. (2005). Mechanism of chlesterol-lowering effects of dietary insoluble fiber: relationships with hepatic cholesterol parameters. *Br. J. Nutr.*, 94, 331-337.

Van Horn, L. (1997). Fiber, lipids, and coronary heart disease: a statement for healthcare professionals from the Nutrition Committee, American heart association. *Circulationl*, 95, 2701-2705.

Vincent, HK., Powers, SK., Stewart, DJ., Shanely, RA., Demirel, H. & Naito, H. (1999). Obesity is associated with increased myocardial oxidative stress. *Int. J. Obes.*, 23, 67-74.

Visscher, TLS., Kromhout, D., & Seidell, JC. (1997). Long-term and recent time trends in the prevalence of obesity among Dutch men and women. *Int. J. Obes.*, 26, 1218-1224.

Yang, ZG., Li, HR., Wang, LY., Li, YH., Lu, SG., Wen, XF., Wang, J., Daikonya, A. & Kitanaka, S. (2007). Triterpenoids from *Hippophae ehamnoides* L. and their nitric oxide production-inhibitiory and DPPH radical-scavenging activities. *Chem. Pharm. Bull.*, 55, 15-18.

Yoshiki, Y., Onda, N., & Okubo, K. (2004). Relationship between photon emission and chemopreventive potential of tea. *Food Chemistry*, 87, 269-274.

Soybean: Friend or Foe

Cristina M. Sena and Raquel M. Seiça

Institute of Physiology; IBILI, Faculty of Medicine, University of Coimbra
Portugal

1. Introduction

Soy products have been consumed in Asian countries such as China and Japan for many centuries. Epidemiological studies have shown a lower incidence of several chronic diseases in Asia when compared to Western countries, including cardiovascular diseases and certain types of cancer (Wu et al., 1998; Messina, 1995). These studies have suggested that consumption of a traditional Asian diet high in soy may play a pivotal role in preventing chronic diseases.

In 1999, the Food and Drug Administration (FDA) in USA approved a health claim based on the role of soybean protein in reducing the risk of coronary disease. This claim establishes that soybean protein included in a diet low in saturated fat and cholesterol may reduce the risk of coronary disease (FDA, 1999; Henkel, 2000).

Soybeans (Glycine max) are a species of legume that yields a valuable amount of oil that has a healthy fatty acid profile and high-quality protein that is replete in all the essential amino acids required for sustaining human nourishment (Young, 1991). Soybeans have antihypertensive, anticholesterol, and antioxidant activities, and appear to prevent several types of cancer (Wu et al., 1998; Messina, 1995). However, other studies suggest that soy is linked to health problems. Thus, soy products are best consumed in moderation to obtain the benefits they offer without any of the potential negative consequences.

1.1 Composition

Soybean products have been designated as one of world's healthiest foods due to being an excellent source of high quality protein as well as providing various health benefits. Soybean contains vegetable protein, oligosaccharides, dietary fiber, phytochemicals (especially isoflavones), and minerals (Anderson et al., 1999; Messina, 1999; Liu, 1999) (table I, Fig 1). The association of high-quality protein and phytochemicals, especially isoflavones (genistein and daidzein), is unique among plant-based proteins because isoflavones are not widely distributed in plants other than legumes (Velasquez & Bhathena, 2007).

In addition, large amounts of secondary metabolites are also present in both seeds and leaves of soybean: for example, it has been reported that soybean leaves normally accumulate high levels of several secondary metabolites, including kaempferol and quercetin (Buttery & Buzzell, 1975). The beans also contain significant amounts of phytic acid (Garcia et al., 1997).

Soybean products have low levels of saturated fat (Friedman & Brandon, 2001). The lipid content of soybean is approximately 60% polyunsaturated, 24% monounsaturated (MFA)

and 15% saturated (SFA). Soybean fat stands out for its high content of the polyunsaturated fatty acids, linoleic (C18: 2) and linolenic (C18: 3) acids, both of which are essential fatty acids (Anderson et al., 1999; Garcia et al., 1997). Soybean offers one of the few non-fish sources rich in omega-3 fatty acids, essential for various body functions. Available data suggest that soybean could be a good source to increase the linolenic acid intake in people with a poor ω-3 fatty acids diet.

The protein content of soybean is 32% to 42% (depending on the variety and growth conditions) of which approximately 80% is composed of 2 storage globulins, 7S globulin (β-conglycinin) and 11S globulin (glycinin), having various functional and physicochemical properties (Garcia et al., 1997; Kwon et al., 2002; Kwon et al., 2003). Soybean products are considered a good substitute for animal protein, and their nutritional value is almost equivalent to that of animal protein because soy proteins contain most of the essential amino acids for human nutrition.

Soybeans cannot be eaten raw; the heat-labile antinutrients (e.g. trypsin and chymotrypsin inhibitors) must undergo effective thermal inactivation to improve soy's digestibility and its sulfur amino availability in humans (Damodaran, 1996). Traditional methods of preparing soybeans for consumption include germination, cooking, roasting, and fermenting. Soy can be used as alternative to a variety of dairy and meat products. People who are intolerant of lactose can eat imitation dairy products. Along with providing alternative options of certain foods, soy protein and isoflavones are thought to have many health benefits.

Composition	Soybean seeds
Complex carbohydrates (g)	21
Simple carbohydrates (g)	9
Water (g)	8.5
Vitamin C (mg)	6
Niacin (mg)	1,6
Choline (mg)	115.9
Vitamin E (mg)	0.85
Protein (g)	36
Total fat (g)	19
Saturated fat (g)	2.8
Monounsaturated fat (g)	4.4
Polyunsaturated fat (g)	11.2
Phytosterols (mg)	161
Fiber, total dietary (g)	9.3
Calcium (mg)	276
Magnesium (mg)	280
Potassium (mg)	1.797
Iron (mg)	16
Zinc (mg)	4.8

Table 1. Nutrition profile of soybeans expressed per 100g Dry Matter. Data extracted from the USDA National Nutrient Database for Standard reference (USDA, 2009).

Isoflavones and soy proteins are the two major groups of components that have received the most attention (Xiao, 2008; Friedman & Brandon, 2001; Omoni, 2005). Isoflavones belong to a broad group of plant derived compounds that have structural and functional similarities to

estrogens, and this has led to the term phytoestrogens (Setchell, 1998). The analysis, bioavailability, and health effects of isoflavones have been extensively studied and frequently reviewed (Cassidy et al., 2006; Larkin et al., 2008). Consumption of isoflavones has been suggested to have multiple beneficial effects on certain types of cancer (Adlercreutz, 2002; Gullett et al., 2010; Sarkar, 2003), bone functions (Zhang et al., 2008), and prevention of obesity (Orgaard, 2008; Velasquez & Bhathena, 2007). Soy protein has also been shown to have several beneficial effects, including cardiovascular and others related to obesity and renal functions (Velasquez & Bhathena, 2007; Anderson et al., 1999; Anderson, 2008).

Several studies suggest that soybean products seem to have a positive effect on lipids (Anderson et al., 1995), diabetes (Chandalia et al., 2000), diarrhoea and constipation and as a therapy of irritable bowel syndrome (Bosaeus, 2004); they have also anti- inflammatory and anti-carcinogenic effects on digestive system (Scheppach et al., 2004). Thus, we will focus at describing some of the beneficial effects of soybean products and discuss some controversies.
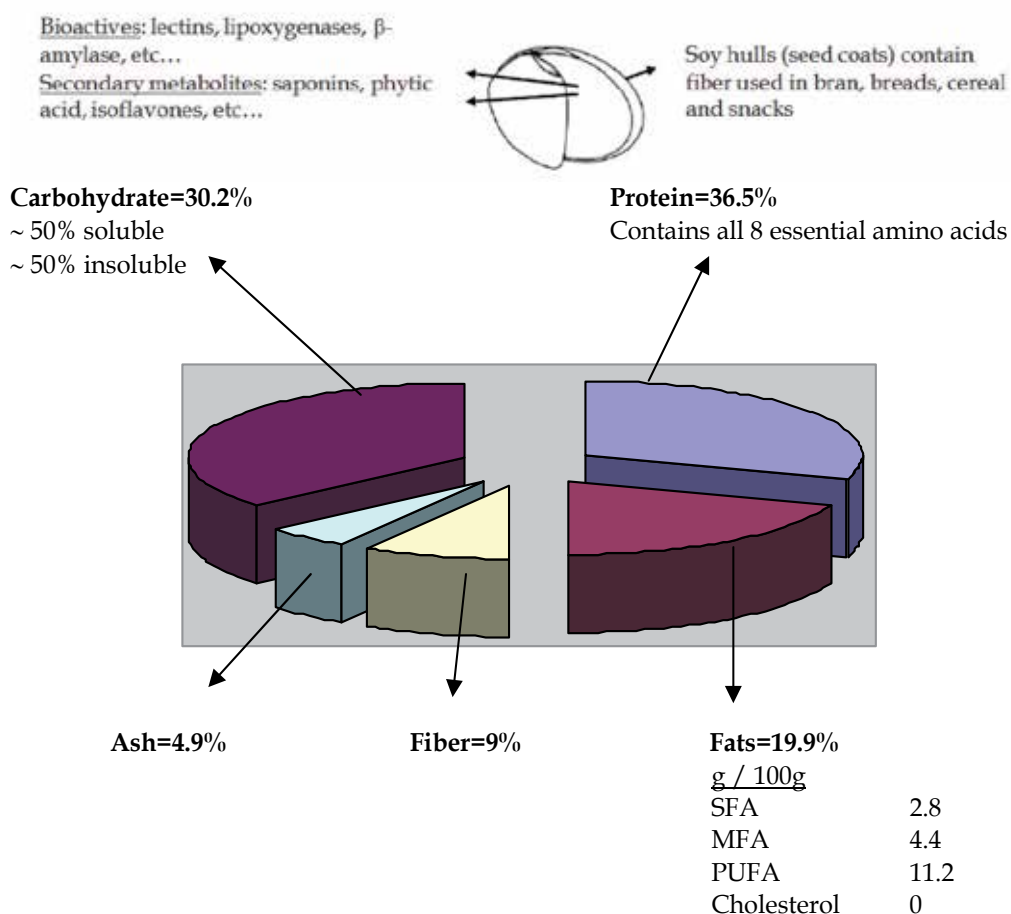
Fig. 1. The percentage nutrient composition of whole soy. Soybean seeds consist of approximately 36% protein, 30% carbohydrate, 20% fat, 9% crude fiber, and 5% ash (Garcia et al., 1997). Data extracted from the USDA National Nutrient Database for Standard reference (USDA, 2009).

2. Beneficial effects of soy products

2.1 Lowering cholesterol/reducing heart disease

The atheroprotective effects of soy-based diets have been attributed to its effect on reducing serum cholesterol levels in animal and human nutrition studies (Anthony et al., 1997; Jenkins et al., 2002; Reynolds et al., 2006). Although, other studies reveal that soybeans have also atheroprotective effects independent of the lipid-lowering activity (Ni et al., 1998; Adams et al., 2002; Nagarajan et al., 2008).

Eating soy protein in particular, has been shown to reduce levels of both cholesterol and blood lipids (Anderson et al., 1995). Extensive clinical and experimental evidence links hypertension and atherosclerotic vascular disease with the accumulation of oxidized LDL and the enhanced generation of reactive oxygen species within the vascular walls (Stocker & Keaney, 2004). We have previously described that soybean oil decreased plasma levels of cholesterol (total and non-HDL) and triglycerides in an aged animal model of type 2 diabetes with hyperlipidemia (Sena et al., 2008a). Thus, the lipid-lowering properties of soy products are very important in this context. Moreover, recent studies in the atherosclerosis-susceptible apolipoprotein E knockout mouse models showed that atherosclerotic lesions are reduced when fed a soy-containing diet despite unchanged serum lipid levels (Ni et al., 1998; Adams et al., 2002; Nagarajan et al., 2008). These studies suggest that dietary soy may inhibit atherosclerotic lesion development by mechanism(s) other than lowering serum lipid levels. Furthermore, other studies have demonstrated that mice fed the β-conglycinin-containing diet, had a pronounced inhibitory effect on the development of atherosclerosis compared to mice fed casein–lactalbumin-based diets (Adams et al., 2002; Adams et al., 2004). These findings suggest an atheroprotective role for the protein components of soy diet or peptides generated from soy protein such as β-conglycinin and glycinin.

Although not completely elucidated there are several components in the soy that have atheroprotective properties. Several studies suggest that soybean dietary fiber also plays a role in the reduction of cholesterol levels in some hyperlipidemic individuals and has a major protective effect on cardiovascular disease (Anderson et al., 1995; Anderson et al., 1999; Reynolds et al., 2006).

The protective effect of soy protein has been investigated in many clinical trials. Several meta-analyses show that the intake of soy protein could lead to a significant mean reduction in serum LDL (Anderson et al., 1995; Reynolds et al., 2006; Weggemans & Trautwein, 2003; Zhan & Ho, 2005; Balk et al., 2005). A significant reduction in triglyceride was also found in two of the studies (Anderson et al., 1995; Balk et al., 2005). Overall, these meta-analyses of individual clinical trials concluded a beneficial effect on serum cholesterol level through the consumption of 25–50 g of soy protein daily (Anderson et al., 1995; Reynolds et al., 2006; Weggemans & Trautwein, 2003; Zhan & Ho, 2005; Balk et al., 2005).

Replacing saturated fat by polyunsaturated fat as the main source of dietary fat intake is effective in reducing serum LDL and preventing atherosclerosis (Rudel et al., 1995; Mensink et al., 1992). A meta-analysis of 60 clinical trials further concluded that polyunsaturated fat from soy oil could increase the concentration of HDL-cholesterol, which is important for cardiovascular health (Mensink et al., 2003).

One randomized, controlled clinical trial found significant reductions in both systolic and diastolic blood pressure for those consuming 40 g of soy protein supplement daily after 12 weeks, when compared with control subjects who received complex carbohydrate (He et al., 2005). The study provided valuable evidence of soy protein as a substitute for carbohydrate, but long-term studies are required to confirm its beneficial effects.

Consumption of isoflavones has also been suggested to have multiple beneficial effects on heart disease (Clair & Anthony, 2005; Clarkson, 2002). Soya products contain significant amounts of the isoflavones genistein and daidzein either in an unconjugated aglycone form or in different glycoside conjugates (Setchell, 1998; Williamson & Manach, 2005). Although there is controversy concerning the benefits of soy isoflavones (Sacks et al., 2006a), it is clear that isoflavones can modulate vascular reactivity and have important anti-inflammatory roles via the activation of estrogen receptors and/or intracellular kinase signalling cascades (Li et al., 2006). The gender specific bioavailability data of isoflavones and its metabolites is very important to formulate food composition/ matrix characteristic of soy-based functional foods. A randomised crossover human trial has examined the effect of age, gender, and influence of the food matrix on the bioavailability of different soy foods (Cassidy et al., 2006), but more convincing human studies are required to make a definite health claim.

A clinical trial evaluating the benefits of soy isoflavones reported increased brachial artery flow-mediated dilation (Squadrito et al., 2003). Clarkson (2002) reported plasma concentrations of genistein and daidzein that range between 50 and 800 ng/mL in adults consuming soy-rich foods, similar to levels found in the Japanese population.

Several human studies during last years are not in agreement with the beneficial effect of soy isoflavones (Sacks et al., 2006b), rather the evidence favours soy protein. However, the American Heart Association Nutrition Committee has not ruled out the possibility that another component could be the active factor (Sacks et al., 2006b).

Discrepancies among clinical studies examining cardiovascular benefits of isoflavones may be influenced not only by the ability of intestinal bacteria to metabolize daidzein to equol but also by an individual's metabolic status. Studies in rodent models (Mahn et al., 2005; Knock et al., 2006; Si & Liu, 2008), have shown that dietary isoflavones supplementation increases antioxidant and eNOS gene expression.

Current evidence from several small studies suggests that omega-3 and soy isoflavone supplementation provides an effective means of reducing arterial stiffness (Pase et al., 2011). There is also a growing body of evidence suggesting that soy food consumption is associated with a significant reduction in ischemic stroke risk (Liang et al., 2007; Liang et al., 2009).

2.2 Reducing cancers

Epidemiological studies suggest that populations consuming high levels of soybean products (Asian countries) have both lower incidences of cancer and lower mortality rates for the major tumor types commonly found in Western countries.

The soluble fiber in soy products is claimed to help protect the body from many digestive related cancers (Scheppach et al., 2004). In vitro studies, animal experiments and epidemiological observations have shown that consumption of soybean products reduce cancer risk and it is associated with overall low mortality rates due to prostate (Jacobsen et al., 1998; Lee et al., 2003; Hwang et al., 2009), breast (Wu et al., 1998; Yamamoto et al., 2003) and endometrial cancers (Goodman et al., 1997). In a study of 59 countries, soy products were found to be significantly protective with an effect size per kilocalorie at least four times greater than that of any dietary factor (Lee et al., 2003).

Soybean contains a variety of phytochemicals with demonstrated anticancer activity (Park et al., 2005). The most widely studied bioactive substances are the isoflavones and the Bowman–Birk protease inhibitor (BBI). The chemopreventive properties of soybean isoflavones have been attributed to different biological activities, mainly to their long-term

estrogenic effects and their antioxidant activity (McCue & Shetty, 2004). BBI works by inhibiting proteases involved in initiation and promotion of carcinogenesis (Kennedy et al., 1998). Its capacity for preventing or suppressing carcinogenic processes has been demonstrated in different cell lines as well as in a wide variety of *in vitro and in vivo* animal model systems (Losso, 2008). It is currently being evaluated in large-scale human trials as an anti-carcinogenic agent in its less pure form BBI concentrate (Losso, 2008).

Other soy proteins, including lectins and the more recently discovered peptide lunasin, may contribute to the role of soy in the prevention and/or treatment of cancer (Hernández-Ledesma et al., 2009). The processing of soy protein into peptides in the gastro-intestinal tract greatly increases their healthful effects by exposing active groups within the amino acid chain. Lunasin, one of the most promising of these peptides, has been shown in recent studies to be an effective anti-cancer agent. Found in a variety of readily available foods, lunasin is an accessible component to healthy living (Hernández-Ledesma et al., 2009).

Consumption of soy may also reduce the risk of colon cancer, possibly due to the presence of sphingolipids (Symolon et al., 2004).

Dietary factors present in soybean foods have also been implicated in the etiology of breast cancer and soy isoflavones has been a candidate for dietary intervention. The majority of the studies describe a benefit of soy products; a few studies do not show benefits. In the breast, soy intake during childhood and adolescence might provide lifelong protection against breast cancer and sensitize for the protective effects of adult soy intake (Korde et al., 2009; Lee et al., 2009; Wu et al 2002).

In 2006 the American Cancer Society (ACS) issued some key information on soy-derived foods indicating they are an excellent food source and a good alternative to meat (Doyle et al., 2006). The ACS recommends that breast cancer survivors should consume only moderate amounts of soy foods as part of a healthy plant-based diet. Although two recent human studies in breast cancer survivors did not indicate adverse effects (Guha et al., 2009; Shu et al., 2009) and suggest a reduction in the risk of recurrence in Asian women consuming soy regularly, more studies are needed to determine whether Western soy products or isoflavone supplements are safe for women diagnosed with breast cancer. Furthermore, the ACS advises these same individuals against the deliberate ingestion of large amounts of soy products in the diet and discourages the consumption of concentrated sources of soy like those found in pills, powders, or supplements containing isolated or concentrated isoflavones.

Inconclusive evidence from epidemiological studies, a small number of prospective studies and errors in the estimation of soy and isoflavone intake, in addition to the existence of hidden sources of soy makes it difficult to address this research accurately. There is a need for more prospective studies—with extensive exposure measurement. To interpret the data, isoflavone dose, forms and sources of isoflavone, timing of isoflavone exposure, and the equol producer status, estrogen-receptor status, and hormonal profile of individuals, need to be considered since they potentially modulate the association between soy intake and cancer risk. Other dietary, environmental, and genetic factors may also modify the association. Future studies need to address these questions by including samples large enough to detect the factors that are capable of modifying the associations between soy and cancer risk.

2.3 Reducing menopausal symptoms

Isoflavones have emerged as alternatives to classical hormonal therapy in menopause, yet there are still conflicting reports on their vascular health benefits. Recent evidence in

postmenopausal women supplemented with genistein for six months reveals a significant improvement in glycemic control and endothelial function compared to a placebo group (Villa et al., 2009). These investigators emphasized that an individual's pre-existing metabolic status may affect responses to isoflavone therapy.

Studies evaluating the effectiveness of soy foods in ameliorating vasomotor and vaginal symptoms have been conducted and have utilized a variety of soy foods containing different amounts of isoflavones. Among these studies the results are diverse, 10 studies published in the last 20 years, some have had negative results and one reported worsening of symptoms in the group consuming soy food. Some studies have assessed only vaginal cytology, one study has assessed vasomotor symptoms and reported a reduction in symptoms. Two studies evaluated both outcomes, with opposite findings. In conclusion, considering the conflicting results provided by a small number of studies, the efficacy of soy foods in improving menopausal symptoms remains unclear [for extensive review see Levis & Griebeler, 2010].

Noteworthy, the beneficial effects of soy are more convincing if soy has been consumed throughout life rather than if the intake starts at menopause (Mardon et al., 2008; Piekarz et al., 2007).

2.4 Improving bone health

Studies in Asia found a link between greater intake of isoflavones and stronger bones. Japanese women have a lower rate of hip fractures than American women, but that might be because of genetics or other factors. Clinical observations have suggested a relationship between osteoarthritis and a changed estrogen metabolism in menopausal women. Moreover phytoestrogens have been shown to ameliorate various menopausal symptoms (Claassen et al., 2008). The effect of phytoestrogens, including genistein, has been studied on articular cartilage matrix metabolism and inflammation. Nevertheless, the data for genistein and osteoarthritis are limited and not consistent to support a beneficial effect of genistein on articular cartilage. Genistein does not affect cartilage metabolism (Claassen et al., 2008; Hooshmand et al., 2007) but could have an anti-inflammatory effect by suppressing COX-2 but not nitric oxide production (Hooshmand et al., 2007). In addition, the consumption of an extract of soy phytoestrogen in animal failed to modify cartilage metabolism in ovariectomised monkey (Ham et al., 2004). Additional experiments are needed to clarify the potential benefit of genistein in articular cartilage metabolism.

More recently, basic scientific research studies and a systematic review and meta-analysis of the available high-quality randomized clinical trials indicate that 300 mg of avocado and soybean unsaponifiables per day (with or without glucosamine and chondroitin sulfate) appears to be beneficial for patients with hip or knee osteoarthritis (Dinubile, 2010).

2.5 Improving diabetic conditions

Animal and human studies have been conducted to investigate antidiabetic effects of soybeans and their actions (Trujillo et al., 2005; Chandalia et al., 2000; Pipe et al., 2009; Azadbakht et al., 2008). Previous observations have shown that soya-containing diets were associated with an improvement in insulin resistance and glycemic control (Kwon et al., 2010). It has also been suggested that the protein and fiber found in soy help to regulate blood glucose levels and kidney filtration, thereby, helping to control diabetic complications along with kidney disease. Hence, soybeans may help prevent type 2 diabetes and delay its progression.

Soybean diet may be a good option in type 2 diabetes individuals due to its effect on hypertension, hypercholesterolemia, atherosclerosis and obesity, which are frequently associated with diabetic disease (Holt et al., 1996).

In addition, substituting animal protein for soybean or other vegetable protein may also decrease renal hyperfiltration, proteinuria, and renal acid load and therefore reduces the risk of renal disease in type 2 diabetes (Jenkins et al., 2003).

It is generally accepted that a high fiber diet, particularly soluble fiber, is useful to control plasma glucose concentration in diabetics. Soybean fiber may be useful because of its insulin-moderated effect. In short- and long-term experiments it has been reported an improvement in blood glucose attributed to fiber intake from soybeans (Messina, 1999; Chandalia et al., 2000). The mechanisms to improve glycemic control during dietary fiber intake seem to be due to the effects of slowing carbohydrate absorption, so that dietary fiber reduces or delays the absorption of carbohydrates. Soybean dietary fiber also increases faecal excretion of bile acid and therefore may cause a low absorption of fat (Chandalia et al., 2000; Jenkins et al., 2003) and reduces the caloric density in some foods (Liu, 1999).

Additionally, an increased consumption of n-3 PUFA coupled with a reduced intake of saturated fat has been suggested to reduce the risk of progression from impaired glucose tolerance to type 2 diabetes in overweight subjects (Nettleton & Katz, 2005). We have previously shown that after 8 weeks of treatment with soybean oil young Goto-Kakizaki (GK) rats had a significant decrease in glycated haemoglobin accompanied by a significant decrement in fasting blood glucose levels (Sena et al., 2008b). The improved diabetic profile is probably due to the soybean oil antioxidant composition, namely α-tocopherol and coenzyme Q. Moreover, it has recently been described that dietary phytoestrogens activate AMP-activated protein kinase with improvement in lipid and glucose metabolism (Cederroth et al., 2008), thus other constituents may be involved.

Isoflavones are structurally and functionally similar to estradiol (Knight & Eden, 1996). Thus, soy isoflavones may improve glucose homeostasis through their estrogenic action. The estrogen receptor α is emerging as a key molecule involved in glucose and lipid metabolism. Isoflavones may have antidiabetic actions through estrogen receptors.

Soybean isoflavones and protein consumption alleviate some of the symptoms associated with type 2 diabetes (Davis et al., 2005; Mezei et al., 2003). However, human clinical trials are contradictory (Sites et al., 2007; González et al., 2007). Some studies report that consumption of isolated isoflavones did not affect insulin sensitivity as assessed by an oral 2-hour glucose tolerance test in a crossover study of postmenopausal women, even though serum ghrelin levels were decreased by the isoflavonoid treatment, indicating some changes in appetite (Nikander et al., 2004). In addition, insulin secretion, visceral fat, total body fat, and lean body mass did not change among postmenopausal women who consumed soy protein for 3 months compared with those that consumed casein protein (Sites et al., 2007). A 6-month randomized controlled trial did not support the hypothesis that soy protein with or without isoflavone supplementation had favorable effects on glycemic control and insulin sensitivity among postmenopausal Chinese women (Liu et al., 2010). A recent meta-analysis of 24 trials and more than 1,500 subjects reported no significant overall effect of soy intake on fasting glucose and insulin concentrations. There were not enough trials to generate sufficient evidence for other glycemic variables, such as HbA1c and 2-h postchallenge glucose and insulin concentrations (Liu et al., 2011). However, some studies have shown positive effects. For instance, postmenopausal women taking dietary supplementation with

phytoestrogens had significantly improved glycemic control, insulin resistance and serum lipoproteins (Jayagopal et al., 2002; González et al., 2007). In a recent meta-analysis of randomized clinical trials, there was a favorable change in fasting glucose concentrations observed in studies that used whole soy foods or a soy diet in the subgroup analysis (Liu et al., 2011).

Recent experiments have shown that isoflavones in soybeans enhance insulin secretion and insulin sensitivity in experimental animal models of diabetes (Lu et al., 2008; Cederroth et al., 2008; Noriega-López et al., 2007), and that soy protein attenuates insulin resistance in male Sprague-Dawley rats (Ronis et al., 2009). Phytoestrogens seem to modulate energy expenditure, adiposity and glucose tolerance in rodents (for review see Cederroth & Nef, 2009). However, the effect of isoflavones and soy protein remains unclear, although several studies have revealed mechanisms by which soy isoflavones may impact glucose metabolism (Ronis et al., 2009).

Soy foods are beneficial for decreasing the risk of onset and progression of insulin resistance and type 2 diabetes and the effectiveness is enhanced by fermentation. Phytoestrogens and proteins in soybeans seem to have beneficial actions, and additional micronutrients such as saponins, phytosterols, trypsin inhibitors, as well as the amino acid and protein composition may have additive or synergistic effects. Fermentation of soybeans leads to structural changes in proteins and phytoestrogen, which may contribute to more beneficial effects on glucose metabolism. Investigating soybeans and fermented soy products have not used standardized formulations, doses, routes of exposure, durations of exposure, and subsequent analyses to evaluate antidiabetic effects and mechanisms of action.

Studies performed in diabetic patients with soybean diets show several potential advantages, but at the moment more work is required to define the exact role of soybean in the control of diabetes mellitus.

3. Opposing viewpoints on the health benefits of soybeans

Currently, there are opposing viewpoints on the health benefits of soy products. Some studies suggest that soy is linked to health problems including: malnutrition, digestive distress, thyroid dysfunction, cognitive decline, reproductive disorders, infertility, birth defect, immune system breakdown, and cancer (Leopald, 1976; Setchell et al., 1987; Doerge, 2002; Helferich et al., 2008; Xiao, 2008; Patisaul & Jefferson, 2010; Cederroth et al., 2010, Bar-El & Reifen, 2010).

Soybeans contain haemagglutinin, a clot-promoting substance. Haemagglutinin and trypsin inhibitors can act as growth inhibitors. Weanling rats fed soy containing these antinutrients fail to grow normally. However, these growth-depressant compounds are deactivated during the process of fermentation (Borchers, 1962).

A very large percentage of soy is genetically modified (more than 99 %) and it also has one of the highest percentages of contamination by pesticides. Recent studies on safety assessment of genetically modified soybeans showed rather contradictory results. Two research groups have been especially active in relation to those investigations. One of them, headed by Dr. Delaney from Pioneer Hi-Bred International, Inc. (Johnston, IA, USA), has reported data showing that various genetically modified soybeans were safe. In contrast, the group headed by Dr. Malatesta from the University of Verona (Verona, Italy) has shown notable concerns (Delaney et al., 2008; Malatesta et al., 2008a; Malatesta et al., 2008b). Certain pesticides are known to increase the incidence of several types of cancers

(Weichenthal et al., 2010). Thus, contamination of soybeans with pesticides may explain the increase risk of certain types of cancer.

The soybean has one of the highest phytate levels of any grain or legume that has been studied (El Tinay, 1989), and the phytates in soy are highly resistant to normal phytate-reducing techniques such as long, slow cooking (Ologhobo et al., 1984). Only a long period of fermentation will significantly reduce the phytate content of soybeans. Phytates can block the uptake of essential minerals - calcium, magnesium, copper, iron and especially zinc - in the intestinal tract. Diets high in phytates contribute to widespread mineral deficiencies in third world countries (Moser et al., 1988; Harland et al., 1988).

When soy products like tofu are consumed with meat, the mineral-blocking effects of the phytates are reduced (Sandström et al., 1989). The Japanese traditionally eat a small amount of tofu or miso as part of a mineral-rich fish broth, followed by a serving of meat or fish. However, vegetarians who consume tofu and bean curd as a substitute for meat and dairy products risk severe mineral deficiencies.

Zinc is needed for the optimal development and functioning of the brain and nervous system (Takeda & Tamano, 2009). It plays a role in protein synthesis and collagen formation; it is involved in the blood-sugar control mechanism and thus protects against diabetes (Jansen et al., 2009); it is also needed for a healthy reproductive system (Eickhoff et al., 2004). Zinc is a key component in numerous vital enzymes and plays a role in the immune system. Phytates found in soy products interfere with zinc absorption more completely than with other minerals (Greger, 1999).

Soy protein isolate (SPI) is the key ingredient in most soy foods that imitate meat and dairy products, including baby formulas and some brands of soy milk. Much of the trypsin inhibitor content can be removed through high-temperature processing, but not all. Trypsin inhibitor content of soy protein isolate can vary as much as fivefold (Rackis et al., 1986). But high-temperature processing has the unfortunate side-effect of so denaturing the other proteins in soy that they are rendered largely ineffective (Wallace et al., 1971a; Wallace et al., 1971b). Thus, animal models feed with soybean diets need aminoacid supplements for normal growth.

In feeding experiments, the use of SPI increased requirements for vitamins E, K, D and B12 and created deficiency symptoms of calcium, magnesium, manganese, molybdenum, copper, iron and zinc (Rackis, 1974). Phytic acid remaining in these soy products greatly inhibits zinc and iron absorption; test animals fed SPI develop enlarged organs, particularly the pancreas and thyroid gland, and increased deposition of fatty acids in the liver (Rackis, 1974).

Soy protein isolate and textured vegetable protein are used extensively in school lunch programs, commercial baked goods, diet beverages and fast food products. "Nutritional Quality of Soy Bean Protein Isolates: Studies in Children of Preschool Age", studied a group of Central American children suffering from malnutrition. Researchers did not use soy products to help the children recover from malnutrition. Instead, a supplement with soy-sugar mixture and nutrients (largely absent in soy products - notably, vitamins A, D and B12, iron, iodine and zinc) was used.

3.1 Isoflavones have harmful side effects

Phytoestrogens tend to have weaker effects than most estrogens, are not stored in the body, and can be easily broken down and eliminated. They are considered to be endocrine disrupting compounds, and have some beneficial effects on health, including reducing the

risk of breast cancer and improving metabolic parameters. However, the supporting evidence that consumption of phytoestrogens is beneficial is indirect and inconsistent. Lifetime exposure to estrogenic substances, especially during critical periods of development, has been associated with formation of malignancies and several anomalies of the reproductive systems (Cederroth et al., 2010). Isoflavones can prevent ovulation and actually stimulate cancer cell growth (Bar-El & Reifen, 2010).

Soy products in western countries are quite different from those consumed in the traditional Asian diet. Most Asian soy products use whole soybeans with or without fermentation. Soy products or second generation soy foods in the US are mostly based on soy protein at different levels of purification or extraction such as texturized vegetable protein (45% protein), soy protein concentrate (70% protein), or isolates (90% protein), each with a different profile of nutrient and non-nutrient compounds, including isoflavones and saponins (Setchell & Cole, 2003; Fang et al., 2004). It is likely that processing of soy foods modulates the profile of isoflavones and modifies their bioaccesibility and bioavailability, but how these differences affect cancer risk and risk of recurrence need to be investigated.

3.2 Soy milk and infants

It has been previously suggested that the highly concentrated phytoestrogens in soy formula might weaken the immune systems of infants. The authors have suggested potential immune, reproductive and endocrine effects in infants or adults as a result of high isoflavone consumption in the soy formulas (Yellayi et al., 2002). The formulas have more of these compounds than soy foods do. There's no evidence that soy formula is unsafe, or that infants drinking it have been harmed (Yellayi et al., 2002). However, breast milk is still the first choice, followed by milk-based formulas. Moreover, only infants allergic to milk should drink soy formula (Setchell et al., 1997).

3.3 Thyroid disease

Soy also contains goitrogens - substances that depress thyroid function. Diffuse goiter and hypothyroidism appeared in some of the subjects consuming soybeans and many complained of constipation, fatigue and lethargy, even though their intake of iodine was adequate (Ishizuki et al., 1991). In 1997, researchers from the FDA's National Center for Toxicological Research reported that the goitrogenic components of soy were the isoflavones (Divi et al., 1997). It has been previously suggested that soy protein supplements can interfere with the absorption of thyroid medications (Drane et al., 1980). One study showed that soy foods may actually interfere with normal thyroid function, perhaps leading to goiter (Kimura et al., 1976).

There's no risk of goiter in healthy people consuming soy that are not deficient in iodine. Strict vegetarians, who eat no iodine-rich fish or dairy products, might be at risk—and eating higher amounts of soy might increase the risk. A healthy balanced diet with the appropriate amounts of soy and iodine intake is essential (Fitzpatrick, 2000).

3.4 Kidney stones

People with higher risk of development of calcium-oxalate kidney stones should limit their intake of soy. Many soy foods are rich in oxalates and thus may promote the formation of such stones in those at risk (Al-Wahsh et al., 2005). On the other hand, soybean is associated with health benefits for patients with gallstones. The mechanism of beneficial effect of

soybean on gallstones is not well known but it may be related to the blood cholesterol lowering effects of soybean protein containing isoflavones (Holt et al., 1996).

4. Conclusion

Soy foods have been consumed for centuries in Asian countries. Many potential benefits have been linked to intake of soy products according to epidemiological investigations. For instance, consumption of soy foods may contribute to lower incidences of coronary heart diseases, atherosclerosis, type 2 diabetes, and decreased risk of certain types of cancer such as breast and prostate cancers as well as better bone health and relief of menopausal symptoms.

The American Dietetic Association's position on vegetarian diets indicates that certain eating patterns may reduce risk of chronic disease, and soy products and phytochemicals are included in the list of influential dietary factors (Nitzke & Freeland-Graves, 2007; Craig & Mangels, 2009). Moreover they also acknowledged that even foods associated with a healthful diet, such as soybeans, should not be viewed oversimplistically as being ideal, or good or bad, but instead their value should be ascertained within the context of the total diet.

Perhaps an effort to investigate whole soy, knowing that more than one component of this food is likely implicated if appreciable health benefits can be attributed to its consumption outside of Asian populations.

5. References

Adams, M.R., Golden, D.L., & Register, T.C., Anthony, M.S., Hodgin, J.B., Maeda, N., Williams, J.K. (2002). The atheroprotective effect of dietary soy isoflavones in apolipoprotein E−/− mice requires the presence of estrogen receptor-alpha. *Arteriosclerosis, Thrombosis and Vascular Biology*, Vol.22, No.11, (November 2002), pp. 1859-1864, ISSN 1079-5642

Adams, M.R., Golden, D.L., Franke, A.A., Potter, S.M., Smith, H.S., & Anthony, M.S. (2004). Dietary soy beta-conglycinin (7S globulin) inhibits atherosclerosis in mice. *Journal of Nutrition*, Vol.134, No.3, (March 2004), pp. 511-516, ISSN 1541-3166

Adlercreutz, H. (2002). Phyto-oestrogens and cancer. *Lancet Oncolology*, Vol.3, No.6, (June 2002), pp. 364–373, ISSN 1470-2045

Al-Wahsh, I.A., Horner, H.T., Palmer, R.G., Reddy, M.B., & Massey, L.K. (2005). Oxalate and phytate of soy foods. *Journal of Agricultural and Food Chemistry*, Vol. 53, No.14, (July 2005), pp. 5670-5674, ISSN 0021-8561

Anderson, J.W. (2008). Beneficial effects of soy protein consumption for renal function. *Asia Pacific Journal of Clinical Nutrition*, Vol.17, Suppl.1, (January 2008), pp.324-328, ISSN 0964-7058

Anderson, J.W., Johnstone, B.M., & Cook, N. (1995). Meta-analysis of the effects of soy protein intake on serum lipids. The *New England Journal of Medicine*, Vol.333, No.5, (August 1995), pp. 276–282, ISSN 0028-4793

Anderson, J.W., Smith, B.M., & Washnock, C.S. (1999). Cardiovascular and renal benefits of dry bean and soybean intake. The *American Journal of Clinical Nutrition*, Vol.70, Suppl.3, (September 1999), pp. 464-474, ISSN 0002-9165

Anthony, M.S., Clarkson, T.B., Bullock, B.C., & Wagner, J.D. (1997). Soy protein versus soy phytoestrogens in the prevention of diet-induced coronary artery atherosclerosis of male cynomolgus monkeys. *Arteriosclerosis, Thrombosis and Vascular Biology*, Vol.17, No.11, (November 1997), pp. 2524–2531, ISSN 1079-5642

Azadbakht, L., Atabak, S., & Esmaillzadeh, A. (2008). Soy protein intake, cardiorenal indices, and C-reactive protein in type 2 diabetes with nephropathy: a longitudinal randomized clinical trial. *Diabetes Care*, Vol.31, No.4, (April 2008), pp. 648-654, ISSN 0149-5992

Balk, E., Chung, M., Chew, P., Ip, S., Raman, G., Kupelnick, B., Tatsioni, A., Sun, Y., Devine, & D., Lau, J. (2005). Effects of soy on health outcomes. *Evidence Report Technology Assessment (Summaries)*, Vol.126, (August 2005), pp. 1-8, ISSN 1530-440X

Bar-El, D.S., & Reifen, R. (2010). Soy as an endocrine disruptor: cause for caution? *Journal of Pediatric Endocrinology and Metabolism*, Vol.23, No.9, (September 2010), pp. 855-861, ISSN 0334-018X

Borchers, R. (1962). Supplementary methionine requirement of wenling rats fed soybean oil meal rations. *Journal of Nutrition*, Vol.77, No.3, (July 1962), pp. 309-311, ISSN 1541-3166

Bosaeus, I. (2004). Fibre effects on intestinal functions (diarrhoea, constipation and irritable bowel syndrome). *Clinical Nutrition Supplements*, Vol.1, No.2, (2004), pp. 33-38, ISSN 1744-1161

Buttery, B.R., & Buzzell, R.I, (1975). Soybean flavonol glycosides: identification and biochemical genetics. *Canadian Journal of Botany*, Vol.53, No.2, (January 1975), pp. 219–224, ISSN 1916-2804

Cassidy, A., Brown, J.E., Hawdon, A., Faughnan, M.S., King, L.J., Millward, J., Zimmer-Nechemias, L., Wolfe, B., & Setchell, K.D. (2006). Factors affecting the bioavailability of soy isoflavones in humans after ingestion of physiologically relevant levels from different soy foods. *Journal of Nutrition*, Vol.136, No.1, (2006), pp. 45-51, ISSN 1541-3166

Cederroth, C.R., & Nef, S. (2009). Soy, phytoestrogens and metabolism: A review. *Molecular Cellular Endocrinology*, Vol.304, No.1, (May 2009), pp. 30–42, ISSN 0303-7207

Cederroth, C.R., Auger, J., Zimmermann, C., Eustache, F., & Nef, S. (2010). Soy, phyto-oestrogens and male reproductive function: a review. *International Journal of Androlology*, Vol.33, No.2, (April 2010), pp. 304-316, ISSN 0105-6263

Cederroth, C.R., Vinciguerra, M., Gjinovci, A., Kühne, F., Klein, M., Cederroth, M., Caille, D., Suter, M., Neumann, D., James, R.W., Doerge, D.R., Wallimann, T., Meda, P., Foti, M., Rohner-Jeanrenaud, F., Vassalli, J.D., & Nef, S. (2008). Dietary phytoestrogens activate AMP-activated protein kinase with improvement in lipid and glucose metabolism. *Diabetes*, Vol.57, No.5, (May 2008), pp. 1176-1185, ISSN 0012-1797

Chandalia, M., Garg, A., Lutjohann, D., Von Bergmann, K., Grundy, S.M., & Brinkley, L.J. (2000). Beneficial effects of high dietary fiber intake in patients with type 2 Diabetes Mellitus. The *New England Journal of Medicine*, Vol.342, No.19, (May 2000), pp. 1392-1398, ISSN 0028-4793

Claassen, H., Briese, V., Manapov, F., Nebe, B., Schunke, M., & Kurz, B. (2008). The phytoestrogens daidzein and genistein enhance the insulin-stimulated sulfate uptake in articular chondrocytes. *Cell and Tissue Research*, Vol.333, No.1, (July 2008), pp. 71-79, ISSN 0302-766X

Clair, R.S, & Anthony, M. (2005). Soy, isoflavones and atherosclerosis. *Handbook of Experimental Pharmacolology*, Vol.170, (2005), pp. 301–323, ISSN 0171-2004

Clarkson, T.B. (2002). Soy, soy phytoestrogens and cardiovascular disease. *Journal of Nutrition*, Vol.132, No.3, (March 2002), pp. 566S–569S, ISSN 1541-3166

Craig, W.J., & Mangels, A.R. (2009). Position of the American Dietetic Association. Vegetarian diets. *Journal of the American Dietetic Association*, Vol.109, No.7, (July 2009), 1266–1282, ISSN 0002-8223

Damodaran S. Amino acids, peptides, and proteins. In: Fennema OW, editor. Food chemistry. 4rd ed. New York: Marcel Dekka, Inc. (September 2007), p.321–429, ISBN: 9780849392726

Davis, J., Iqbal, M.J., Steinle, J., Oitker, J., Higginbotham, D.A., Peterson, R.G., & Banz, W.J. (2005). Soy protein influences the development of the metabolic syndrome in male obese ZDFxSHHF rats. *Hormone and Metabolic Research*, Vol.37, No.5, (May 2005), pp. 316-325, ISSN 0018-5043

Delaney, B., Appenzeller, L.M., Munley, S.M., Hoban, D., Sykes, G.P., Malley, L.A., & Sanders, C. (2008). Subchronic feeding study of high oleic acid soybeans (event DP-3Ø5423-1) in Sprague–Dawley rats. *Food and Chemical Toxicology*, Vol.46, No.12, (December 2008), pp. 3808–3817, ISSN 0278-6915

Dinubile NA. (2010). A potential role for avocado- and soybean-based nutritional supplements in the management of osteoarthritis: a review. *The Physician and Sportsmedicine*, Vol.38, No.2, (June 2010), pp. 71-81, ISSN 0091-3847

Divi, R.L., Chang, H.C., & Doerge, D.R. (1997). Anti-thyroid isoflavones from the soybean. *Biochemical Pharmacology*, Vol.54, No.10, (November 1997), pp. 1087-1096, ISSN 0006-2952

Doerge, D.R. (2002). Goitrogenic and estrogenic activity of soy isoflavones. *Environmental Health Perspectives*,Vol.110, Suppl.3, (June 2002), pp. 349-353, ISSN 0091-6765

Doyle, C., Kushi, L.H., Byers, T., Courneya, K.S., Demark-Wahnefried, W., Grant, B., McTiernan, A., Rock, C.L., Thompson, C., Gansler, T., & Andrews, K.S. (2006). Nutrition and physical activity during and after cancer treatment: an American Cancer Society guide for informed choices. *CA: a Cancer Journal for Clinicians*, Vol.56, No.6, (November-December 2006), pp. 323–353, ISSN 0007-9235

Drane, H.M., Patterson, D.S., Roberts, B.A., & Saba, N. (1980). Oestrogenic activity of soya-bean products. *Food, Cosmetics and Technology,* Vol.18, No.4, (August 1980), pp.425-427, ISSN 0015-6264

Eickhoff, R., Baldauf, C., Koyro, H.W., Wennemuth, G., Suga, Y., & Seitz, J. (2004). Influence of macrophage migration factor (MIF) on the zinc content and redox state of protein-bound sulfhydryl groups in rat sperm: indications for a new role of MIF in sperm maturation, *Molecular Human Reproduction*, Vol.10, No.8, (August 2004), pp. 605–611, ISSN 1360-9947

El Tinay, A.H., Mahgoub, S.O., Mohamed, B.E., & Hamad, M.A. (1989). Proximate Composition and Mineral and Phytate Contents of Legumes Grown in Sudan. *Journal of Food Composition and Analysis*, Vol.2, No.1, (March 1989), pp. 69-78, ISSN 0889-1575

Fang, N., Yu, S., & Badger, T.M. (2004). Comprehensive phytochemical profile of soy protein isolate. *Journal of Agricultural and Food Chemistry*, Vol.52, No.12, (June 2004), pp. 4012–4020, ISSN 0021-8561

Fitzpatrick, M. (2000). Soy formulas and the effects of isoflavones on the thyroid. *The New Zealand Medical Journal*, Vol.113, No.1103, (February 2000), pp. 24-26, ISSN: 0028-8446

Food and Drug Administration (FDA). FDA approves new health claim for soybean protein and coronary heart disease. FDA Talk Paper 1999, [cited 2011 Mar 25], In: www.fda.gov

Friedman, M., & Brandon, D.L. (2001). Nutritional and health benefits of soy proteins. *Journal of Agricultural and Food Chemistry*, Vol.49, No.3, (March 2001), pp. 1069–1086, ISSN 0021-8561

Garcia, M.C., Torre, M., Marina, M.L., & Laborda, F. (1997). Composition and characterization of soybean and related products. *Critical Reviews in Food Science and Nutrition*, Vol.37, No.4, (June 1997), pp. 361-391, ISSN 1040-8398

González, S., Jayagopal, V., Kilpatrick, E.S., Chapman, T., & Atkin, S.L. (2007). Effects of isoflavone dietary supplementation on cardiovascular risk factors in type 2 diabetes. *Diabetes Care,* Vol.30, No.7, (July 2007), pp. 1871-1873, ISSN 0149-5992

Goodman, M.T., Wilkens, L.R., Hankin, J.H., Wu, A.H., & Kolonel, L.N. (1997). Association of soy and fiber consumption with the risk of endometrial cancer. *American Journal of Epidemiology*, Vol.146, No.4, (August 1997), pp.294–306, ISSN 0002-9262

Greger, J.L. (1999) Nondigestible carbohydrates and mineral bioavailability. Journal of Nutrition, Vol.129, Suppl.7, (July 1999), pp. 1434S-1435S, ISSN 1541-3166

Guha, N., Kwan, M.L., Quesenberry, C.P. Jr., Weltzien, E.K., Castillo, A.L., & Caan, B.J. (2009). Soy isoflavones and risk of cancer recurrence in a cohort of breast cancer survivors: the Life After Cancer Epidemiology study. *Breast Cancer Research and Treatment*, Vol.118, No.2, (November 2009), pp. 395–405, ISSN 0167-6806

Gullett, N.P., Ruhul,Amin, A.R., Bayraktar, S., Pezzuto, J.M., Shin, D.M., Khuri, F.R., Aggarwal, B.B., Surh, Y.J., & Kucuk, O. (2010). Cancer prevention with natural compounds. *Seminars in Oncology*, Vol.37, No.3, (June 2010), pp. 258-281, ISSN 0093-7754

Ham, K.D., Oegema, T.R., Loeser, R.F., & Carlson, C.S. (2004). Effects of longterm estrogen replacement therapy on articular cartilage IGFBP-2, IGFBP-3, collagen and proteoglycan levels in ovariectomized cynomolgus monkeys. *Osteoarthritis Cartilage*, Vol.12, No.2, (February 2004), pp. 160-168, ISSN 1063-4584

Harland, B.F., Smith, S.A., Howard, M.P., Ellis, R., & Smith, J.C. Jr. (1988). Nutritional status and phytate: zinc and phytate X calcium: zinc dietary molar ratios of lacto-ovovegetarian Trappist monks: 10 years later. *Journal of the American Dietetic Association*, Vol.88, No.12, (December 1988), pp. 1562-1566, ISSN 0002-8223

He, J., Gu, D., Wu, X., Chen, J., Duan, X., Chen, J., & Whelton, P.K. (2005). Effect of soybean protein on blood pressure: a randomized, controlled trial. *Annals of Internal Medicine*, Vol.143, No.1, (July 2005), pp. 1–9, ISSN 0003--4819

Helferich, W.G., Andrade, J.E., & Hoagland, M.S. (2008). Phytoestrogens and breast cancer: a complex story. *Inflammopharmacology*, Vol.16, No.5, (October 2008), pp. 219–226, ISSN 0925-4692

Henkel, J. (2000). Soybean: Health claims for soybean protein, questions about other components. FDA Consumer 2000, [cited 2011 Mar 25]., In www.cfsan.fda.gov

Hernández-Ledesma, B., Hsieh, C.-C., & de Lumen, B.O. (2009). Lunasin, a novel seed peptide for cancer prevention. *Peptides*, Vol.30, No.2, (February 2009), pp. 426–430, ISSN 0196-9781

Holt, S., Muntyan, I., & Likyer, L. (1996). Soybean-based diets for Diabetes Mellitus. *Alternative & Complementary Therapies*, Vol.2, No.2, (March/April 1996), pp. 79-92, ISSN 1557-9085

Hooshmand, S., Soung do, Y., Lucas, E.A., Madihally, S.V., Levenson, C.W., & Arjmandi, B.H. (2007). Genistein reduces the production of proinflammatory molecules in human chondrocytes. *Journal of Nutrition Biochemistry*, Vol.18, No.9, (September 2007), pp. 609-614, ISSN 0955-2863

Hwang, Y.W., Kim, S.Y., Jee, S.H., Kim, Y.N., & Nam, C.M. (2009). Soy food consumption and risk of prostate cancer: a meta-analysis of observational studies. *Nutrition Cancer*, Vol.61, No.5, (2009), pp.598-606, ISSN 0163-5581

Ishizuki, Y., Hirooka, Y., Murata, Y., & Togashi, K. (1991). The effects on the thyroid gland of soybeans administered experimentally in healthy subjects. Nippon Naibunpi Gakkai Zasshi Vol.767, No.1 (May 1991), pp. 622-629, ISSN 0916-4804

Jacobsen, B.K., Knutsen, S.F., & Fraser, G.E. (1998). Does high soy milk intake reduce prostate cancer incidence? The adventist health study (United States). *Cancer Causes Control*, Vol.9, No.6, (December 1998), pp. 553–557, ISSN 0957-5243

Jansen, J., Karges, W., & Rink, L. (2009). Zinc and diabetes-clinical links and molecular mechanisms. *Journal of Nutrition Biochemistry*, Vol.20, No.6, (June 2009), pp. 399-417, ISSN 0955-2863

Jayagopal, V., Albertazzi, P., Kilpatrick, E.S., Howarth, E.M., Jennings, P.E., Hepburn, D.A., Atkin, S.L. (2002). Beneficial effects of soy phytoestrogen intake in postmenopausal women with type 2 diabetes. *Diabetes Care,* Vol.25, No.10, (October 2002), pp. 1709-1714, ISSN 0149-5992

Jenkins, D.J., Kendall, C.W., Augustin, L.S., Franceschi, S., Hamidi, M., Marchie, A., Jenkins, A.L., & Axelsen, M. (2002). Glycemic index: overview of implications in health and disease. *American Journal of Clinical Nutrition*, Vol.76, No.1, (July 2002), pp. 266S-273S, ISSN 0002-9165

Jenkins, D.J., Kendall, C.W., Marchie, A., Jenkins, A.L., Augustin, L.S., Ludwig, D.S., Barnard, N.D., & Anderson, J.W. (2003). Type 2 diabetes and vegetarian diet. *American Journal of Clinical Nutrition*, Vol.78, Suppl.3, (September 2003), pp. 610S-616S, ISSN 0002-9165

Kennedy, A.R. (1998). The Bowman–Birk inhibitor from soybeans as an anticarcinogenic agent. *American Journal of Clinical Nutrition*, Vol.68, Suppl.6, (December 1998), pp. 1406S–1412S, ISSN 0002-9165

Kimura, S., Suwa, J., Ito, M., & Sato, H. (1976). Development of malignant goiter by defatted soybean with iodine-free diet in rats. *Gann*, Vol.67, No.5, (October 1976), pp. 763-765, ISSN 0016-450X

Knight, D.C., & Eden, J.A. (1996). A review of the clinical effects of phytoestrogens. *Obstetrics and Gynecolology*, Vol.87, No.5, Pt2, (May 1996), pp.897-904, ISSN 0029-7844

Knock, G.A., Mahn, K., Mann, G.E., Ward, J.P., & Aaronson, P.I. (2006). Dietary soy modulates endothelium-dependent relaxation in aged male rats: increased agonist-induced endothelium-derived hyperpolarising factor and basal nitric oxide activity. *Free Radical Biology & Medicine*, Vol.41, No.5, (September 2006), pp.731-739, ISSN 0891-5849

Korde, L.A., Wu, A.H., & Fear, T. (2009). Childhood soy intake and breast cancer risk in Asian-American women. *Cancer Epidemiology, Biomarkers & Prevention*, Vol.18, No.4, (April 2009), pp.1050–1059, ISSN 1055-9965

Kwon, D.Y., Daily, J.W. 3rd., Kim, H.J., & Park, S. (2010). Antidiabetic effects of fermented soybean products on type 2 diabetes. *Nutrition Research*, Vol.30, No.1, (January 2010), pp. 1-13, ISSN 0271-5317

Kwon, D.Y., Kim, S., Kim, H.Y.L., & Kim, K.S. (2003). Changes in physicochemical properties of glycinin due to maleylation. *Food Science & Biotechnology*, Vol.12, No.2, (April 2003), pp.122-127, ISSN 1226-7708

Kwon, D.Y., Oh, S.W., Lee, J.S., Yang, H.J., Lee, S.H., Lee, J.H., Lee, Y.B., Sohn, H.S., & Lee, J.S. (2002). Amino acid substitution of hypocholesterolemic peptide originated from

glycinin hydrolyzate. *Food Science & Biotechnology*, Vol.11, No.1, (February 2002), pp.55-61, ISSN 1226-7708

Larkin, T., Price, W.E., & Astheimer, L. (2008). The key importance of soy isoflavone bioavailability to understanding health benefits. *Critical Reviews of Food Science Nutrition*, Vol.48, No.6, (June 2008), pp.538-552, ISSN 1040-8398

Lee, M.M., Gomez, S.L., Chang, J.S., Wey, M.,Wang, R.T., & Hsing, A.W. (2003). Soy and isoflavone consumption in relation to prostate cancer risk in china. *Cancer Epidemiology Biomarkers & Prevention*, Vol.12, No.7, (July 2003), pp. 665–668, ISSN 1055-9965

Lee, S.A., Shu, X.O., Li, H., Yang, G., Cai, H., Wen, W., Ji, B.T., Gao, J., Gao, Y.T., & Zheng, W. (2009). Adolescent and adult soy food intake and breast cancer risk: results from the Shanghai Women's Health Study. *American Journal of Clinical Nutrition*, Vol.89, No.6, (June 2009), pp. 1920–1926, ISSN 0002-9165

Leopald, A.S. (1976). Phytoestrogens: Adverse effects on reproduction in California Quail. *Science*, Vol.191, No.4222, (January 1976), pp.98-100, ISSN 0036-8075

Levis, S., & Griebeler, M.L. (2010). The role of soy foods in the treatment of menopausal symptoms. *Journal of Nutrition*, Vol.140, No.12, (December 2010), pp. 2318S–2321S, ISSN 1541-3166

Li, M., Liu, R.M., Timblin, C.R., Meyer, S.G., Mossman, B.T., & Fukagawa, N.K. (2006). Age affects ERK1/2 and NRF2 signaling in the regulation of GCLC expression. *Journal of Cell Physiology*, Vol.206, No.2, (February 2006), pp. 518-525, ISSN 0021-9541

Liang, W., Lee, A.H., Binns, C.W., Hu, D., Huang, R., & Tian, H. (2007). Are soy foods protective against ischemic stroke? *Future Neurology*, Vol.2, No. 5, (September 2007), pp. 505-511, ISSN 1479-6708

Liang, W., Lee, A.H., Binns, C.W., Huang, R., Hu, D., & Shao, H. (2009). Soy consumption reduces risk of ischemic stroke: a case-control study in southern China. *Neuroepidemiology*, Vol.33, No.2, (2009), pp. 111-116, ISSN 0251-5350

Liu, K.S. (1999). Chemistry and nutritional value of soybean components. In Soybeans: Chemistry, Technology and Utilization. Aspen Publishers Inc. Gaithersburg, Maryland, USA, 1999, pp. 25-113, ISBN 0834212994

Liu, Z.M., Chen, Y.M., Ho, S.C. (2011). Effects of soy intake on glycemic control: a meta-analysis of randomized controlled trials. *Americam Journal of Clinical Nutrition*, [Epub ahead of print], (2 March 2011), ISSN 0002-9165

Liu, Z.M., Chen, Y.M., Ho, S.C., Ho, Y.P., Woo, J. (2010). Effects of soy protein and isoflavones on glycemic control and insulin sensitivity: a 6-mo double-blind, randomized, placebo-controlled trial in postmenopausal Chinese women with prediabetes or untreated early diabetes. *Americam Journal of Clinical Nutrition*, Vol.91, No.5, (May 2010), pp.1394–1401, ISSN 0002-9165

Losso, J.N. (2008). The biochemical and functional food properties of the Bowman–Birk Inhibitor. *Critical Reviews of Food Science and Nutrition*, Vol.48, No.1, (January 2008), pp.94–118, ISSN 1040-8398

Lu, M.P., Wang, R., Song, X., Chibbar, R., Wang, X., Wu, L., & Meng, Q.H. (2008). Dietary soy isoflavones increase insulin secretion and prevent the development of diabetic cataracts in streptozotocin-induced diabetic rats. *Nutrition Research*, Vol.28, No.7, (July 2008), pp.464-471, ISSN 0271-5317

Mahn, K., Borras, C., Knock, G.A., Taylor, P., Khan, I.Y., Sugden, D., Poston, L., Ward, J.P., Sharpe, R.M., Vina, J., Aaronson, P.I., & Mann, G.E. (2005). Dietary soy isoflavone induced increases in antioxidant and eNOS gene expression lead to improved

endothelial function and reduced blood pressure in vivo. *FASEB Journal*, Vol.19, No.12, (October 2005), pp. 1755-1757, ISSN 0892-6638

Malatesta, M., Boraldi, F., Annovi, G., Baldelli, B., Battistelli, S., Biggiogera, M., & Quaglino, D. (2008a). A long-term study on female mice fed on a genetically modified soybean: effects on liver ageing. *Histochemistry and Cell Biology*, Vol.130, No.5, (November 2008), pp. 967–977, ISSN 0948-6143

Malatesta, M., Perdoni, F., Santin, G., Battistelli, S., Muller, S., & Biggiogera, M. (2008b). Hepatoma tissue culture (HTC) cells as a model for investigating the effects of low concentrations of herbicide on cell structure and function. *Toxicolology In Vitro*, Vol. 22, No. 8, (December 2008), pp. 1853–1860, ISSN 0887-2333

Mardon, J., Mathey, J., Kati-Coulibaly, S., Puel, C., Davicco, M.J., Lebecque, P., Horcajada, M.N., & Coxam, V. (2008). Influence of lifelong soy isoflavones consumption on bone mass in the rat. *Experimental Biology and Medicine (Maywood)*, Vol.233, No.2, (February 2008), pp. 229–237, ISSN 1535-3702

McCue, P., & Shetty, K. (2004). Health benefits of soy isoflavonoids and strategies for enhancement: a review. *Critical Reviews in Food Science and Nutrition*, Vol.44, No.5, (2004), pp. 361–367, ISSN 1040-8398

Mensink, R.P., & Katan, M.B. (1992). Effect of dietary fatty acids on serum lipids and lipoproteins. A meta-analysis of 27 trials. *Arteriosclerosis and Thrombosis*, Vol.12, No.8, (August 1992), pp. 911–919, ISSN 1049-8834

Mensink, R.P., Zock, P.L., Kester, A.D., & Katan, M.B. (2003). Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *Americam Journal of Clinical Nutrition*, Vol.77, No.5, (May 2003), pp.1146–1155, ISSN 0002-9165

Messina, M. (1995). Modern applications for an ancient bean: soybeans and the prevention and treatment of chronic disease. *Journal of Nutrition*, Vol. 125, Suppl.3, (March 1995), pp. 567S–569S, ISSN 1541-3166

Messina, M.J. (1999). Legumes and soybeans: overview of their nutritional profiles and health effects. *American Journal of Clinical Nutrition*, Vol. 70, No.3, (September 1999), pp. 439-450, ISSN 0002-9165

Mezei, O., Banz, W.J., Steger, R.W., Peluso, M.R., Winters, T.A., & Shay, N. (2003). Soy isoflavones exert antidiabetic and hypolipidemic effects through the PPAR pathways in obese Zucker rats and murine RAW 264.7 cells. *Journal of Nutrition*, Vol. 133, No.5, (May 2003), pp. 1238-1243, ISSN 1541-3166

Moser, P.B., Reynolds, R.D., Acharya, S., Howard, M.P., Andon, M.B., & Lewis, S.A. (1988). Copper, iron, zinc and selenium dietary intake and status of Nepalese lactating women and their breastfed infants. *American Journal of Clinical Nutrition*, Vol.47, No.4, (April 1988), pp. 729-734, ISSN 0002-9165

Nagarajan, S., Burris, R.L., Stewart, B.W., Wilkerson, J.E., & Badger, T.M. (2008). Dietary soy protein isolate ameliorates atherosclerotic lesions in apolipoprotein E-deficient mice potentially by inhibiting monocyte chemoattractant protein-1 expression. *Journal of Nutrition*, Vol.138, No.2, (February 2008), pp. 332–337, ISSN 1541-3166

Nettleton, J.A., & Katz, R. (2005). n-3 long-chain polyunsaturated fatty acids in type 2 diabetes: a review. *Journal of the American Dietetic Association*, Vol.105, No.3, (March 2005), pp. 428–440, ISSN 0002-8223

Ni, W., Tsuda, Y., Sakono, M., & Imaizumi, K. (1998). Dietary soy protein isolate, compared with casein, reduces atherosclerotic lesion area in apolipoprotein E-deficient mice. *Journal of Nutrition*, 1998;128:1884–1889, ISSN 1541-3166

Nikander, E., Tiitinen, A., Laitinen, K., Tikkanen, M., & Ylikorkala, O. (2004). Effects of isolated isoflavonoids on lipids, lipoproteins, insulin sensitivity, and ghrelin in postmenopausal women. *Journal of Clinical Endocrinolology and Metabolism*, Vol.89, No.7, (July 2004), pp. 3567-3572, ISSN 0021-972X

Nitzke, S., & Freeland-Graves, J. (2007). Position of the American Dietetic Association. Total diet approach to communicating food and nutrition information. *Journal of the American Dietetic Association*, Vol.107, No.7, (July 2007), 1224–1232, ISSN 0002-8223

Noriega-López, L., Tovar, A.R., Gonzalez-Granillo, M., Hernández-Pando, R., Escalante, B., Santillán-Doherty, P., & Torres, N. (2007). Pancreatic insulin secretion in rats fed a soy protein high fat diet depends on the interaction between the amino acid pattern and isoflavones. *Journal of Biological Chemistry*, Vol.282, No.28, (July 2007), pp. 20657-20666, ISSN 0021-9258

Ologhobo, A.D., & Fetuga, B.L. (1984). Distribution of phosphorus and phytate in some Nigerian varieties of legumes and some effects of processing. *Journal of Food Science*, Vol.49, No.1, (January 1984), pp. 199-201, ISSN 1750-3841

Omoni, A.O., & Aluko, R.E. (2005). Soybean foods and their benefits: potential mechanisms of action. *Nutrition Reviews*, Vol.63, No.8, (August 2005), pp. 272–283, ISSN 0029-6643

Orgaard, A., & Jensen, L. (2008). The effects of soy isoflavones on obesity. *Experimental Biology and Medicine (Maywood)*, Vol.233, No.9, (September 2008), pp. 1066–1080, ISSN 1535-3702

Park, J.H., Jeong, H.J., & de Lumen, B.O. (2005). Contents and bioactivities of lunasin, Bowman–Birk Inhibitor, and isoflavones in soybean seed. *Journal of Agricultural and Food Chemistry*, Vol.53, No.20, (October 2005), pp.7686–7690, ISSN 0021-8561

Pase, M.P., Grima, N.A., & Sarris, J. (2011). The effects of dietary and nutrient interventions on arterial stiffness: a systematic review. *American Journal of Clinical Nutrition*, Vol.93, No.2, (February 2011), pp.446-454, ISSN 0002-9165

Patisaul, H.B., & Jefferson, W. (2010). The pros and cons of phytoestrogens. *Frontiers in Neuroendocrinology*, Vol.31, No.4, (October 2010), pp. 400–419, ISSN 0091-3022

Piekarz, A.V., & Ward, W.E. (2007) Effect of neonatal exposure to genistein on bone metabolism in mice at adulthood. *Pediatric Research,* Vol.61, No.1, (January 2007), pp. 48-53, ISSN 0031-3998

Pipe, E.A., Gobert, C.P., Capes, S.E., Darlington, G.A., Lampe, J.W., & Duncan, A.M. (2009). Soy protein reduces serum LDL cholesterol and the LDL cholesterol:HDL cholesterol and apolipoprotein B:apolipoprotein A-I ratios in adults with type 2 diabetes. *Journal of Nutrition*, Vol.139, No.9, (September 2009), 1700-1706, ISSN 1541-3166

Rackis, J.J. (1974). Biological and Physiological Factors in Soybeans. *Journal of the American Oil Chemists' Society*, Vol. 51, No.1, (January 1974), pp. 161A-170A, ISSN 0003-021X

Rackis, J.J., Wolf, W.J., & Baker, E.C. (1986). Protease inhibitors in plant foods: content and inactivation. *Advances in Experimental Medicine and Biology*, Vol.199, (1986), pp. 299-347, ISSN 0065-2598

Reynolds, K., Chin, A., Lees, K.A., Nguyen, A., Bujnowski, D., & He, J. (2006). A Meta-Analysis of the Effect of Soybean Protein Supplementation on Serum Lipids. *American Journal of Cardiolology*, Vol.98, No.5, (September 2006), pp.633-640, ISSN 0002-9149

Ronis, M.J., Chen, Y., Badeaux, J., & Badger, T.M. (2009). Dietary soy protein isolate attenuates metabolic syndrome in rats via effects on PPAR, LXR, and SREBP

signaling. *Journal of Nutrition*, Vol.139, No.8, (August 2009), pp.1431-1438, ISSN 1541-3166

Rudel, L.L., Parks, J.S., & Sawyer, J.K. (1995). Compared with dietary monounsaturated and saturated fat, polyunsaturated fat protects African green monkeys from coronary artery atherosclerosis. *Arteriosclerosis Thrombosis Vascular Biology*, Vol.15, No.12, (December 1995), pp. 2101–2110, ISSN 1079-5642

Sacks, F.M., Lichtenstein, A., Van Horn, L., Harris, W., Kris-Etherton, P., & Winston, M. (2006b). Soy protein, isoflavones, and cardiovascular health: A summary of a statement for professionals from the American Heart Association Nutrition Committee. *Arteriosclerosis Thrombosis Vascular Biology*, Vol.26, No.8, (August 2006), pp. 1689-1692, ISSN 1079-5642

Sacks, F.M., Lichtenstein, A., Van Horn, L., Harris, W., Kris-Etherton, P., & Winston, M. (2006a). Soy protein, isoflavones, and cardiovascular health: An American Heart Association Science Advisory for professionals from the Nutrition Committee. *Circulation* Vol.113, No.7, (February 2006), pp. 1034–1044, ISSN 0009-7322

Sandström, B., Almgren, A., Kivistö, B., & Cederblad, A. (1989). Effect of protein level and protein source on zinc absorption in humans. *Journal of Nutrition* Vol.119, No.1, (January 1989), pp. 48-53, ISSN 1541-3166

Sarkar, F.H., & Li, Y. (2003). Soy isoflavones and cancer prevention. *Cancer Investigation*, Vol.21, No.5, (January 2003), pp.744–757, ISSN 0735-7907

Scheppach, W., Luethrs, H., Melcher, R., Gostner, A., Schauber, J., Kudlich, T., Weiler, F., & Menzel, T. (2004). Antiinflammatory and anticarcinogenic effects of dietary fibre. *Clinical Nutrition Supplements*, Vol. 1, No.2, (2004), pp. 51-58, ISSN 0261-5614

Sena, C.M., Nunes, E., Louro, T., Proença, T., Fernandes, R., Boarder, M.R., & Seiça, R.M. (2008a). Effects of alpha-lipoic acid on endothelial function in aged diabetic and high-fat fed rats. *British Journal of Pharmacology*, Vol, 153, No.5, (March 2008), pp. 894-906, ISSN 0007-1188

Sena, C.M., Proença, T., Nunes, E., Santos, M.S., & Seiça, R.M. (2008b). The effect of soybean oil on glycaemic control in Goto-kakizaki rats, an animal model of type 2 diabetes. *Medicinal Chemistry*, Vol.4, No.3, (May 2008), pp. 293-297, ISSN 1573-4064

Setchell, K.D. (1998). Phytoestrogens: the biochemistry, physiology, and implications for human health of soy isoflavones. *Americam Journal Clinical Nutrition*, Vol. 68, Suppl.6, (December 1998), pp. 1333S–1346S, ISSN 0002-9165

Setchell, K.D., & Cole, S.J. (2003). Variations in isoflavone levels in soy foods and soy protein isolates and issues related to isoflavone databases and food labeling. *Journal of Agricultural and Food Chemistry*, Vol.51, No.14, (July 2003), pp. 4146–4155, ISSN 0021-8561

Setchell, K.D., Gosselin, S.J., Welsh, M.B., Johnston, J.O., Balistreri, W.F., Kramer, L.W., Dresser, B.L., & Tarr, M.J. (1987). Dietary oestrogens - a probable cause of infertility and liver disease in captive cheetahs. *Gastroenterology*, Vol.93, No.2, (August 1987), pp. 225-233, ISSN 0016-5085

Setchell, K.D., Zimmer-Nechemias, L., Cai, J., & Heubi, J.E. (1997). Exposure of infants to phyto-oestrogens from soy-based infant formula. *Lancet*, Vol 350, No.9070, (July 1997),pp. 23-27, ISSN 0140-6736

Shu, X.O., Zheng, Y., Cai, H., Gu, K., Chen, Z., Zheng, W., & Lu, W. (2009). Soy food intake and breast cancer survival. *The Journal of the American Medical Association*, Vol.302, No.22, (December 2009), pp. 2437–2443, ISSN 0087-7484

Si, H., & Liu, D. (2008). Genistein, a soy phytoestrogen, upregulates the expression of human endothelial nitric oxide synthase and lowers blood pressure in spontaneously

hypertensive rats. *Journal of Nutrition*, Vol.138, No.2, (February 2008), pp. 297-304, ISSN 1541-3166

Sites, C.K., Cooper, B.C., Toth, M.J., Gastaldelli, A., Arabshahi, A., & Barnes, S. (2007). Effect of a daily supplement of soy protein on body composition and insulin secretion in postmenopausal women. *Fertility and Sterility*, Vol.88, No.6, (December 2007), pp. 1609-1617, ISSN 0015-0282

Squadrito, F., Altavilla, D., Crisafulli, A., Saitta, A., Cucinotta, D., Morabito, N., D'Anna, R., Corrado, F., Ruggeri, P., Frisina, N., & Squadrito, G. (2003). Effect of genistein on endothelial function in postmenopausal women: A randomized, double-blind, controlled study. *The Americam Journal of Medicine*, Vol.114, No.6, (April 2003), pp. 470–476, ISSN 0002-9343

Stocker, R., & Keaney, J.F., Jr. (2004). Role of oxidative modifications in atherosclerosis. *Physiological Reviews*, Vol.84, No.4, (October 2004), pp. 1381–1478, ISSN 0031-9333

Symolon, H., Schmelz, E., Dillehay, D., & Merrill, A. (2004). Dietary soy sphingolipids suppress tumorigenesis and gene expression in 1,2-dimethylhydrazine-treated CF1 mice and ApcMin/+ mice. *Journal of Nutrition*, Vol.134, No.5, (May 2004), pp. 1157–1161, ISSN 1541-3166

Takeda, A., & Tamano, H. (2009). Insight into zinc signaling from dietary zinc deficiency. *Brain Research Reviews*, Vol.62, No.1, (December 2009), pp. 33-44, ISSN 0165-0173

Trujillo, J., Ramírez, V., Pérez, J., Torre-Villalvazo, I., Torres, N., Tovar, A.R., Muñoz, R.M., Uribe, N., Gamba, G., & Bobadilla, N.A. (2005). Renal protection by a soy diet in obese Zucker rats is associated with restoration of nitric oxide generation. *American Journal of Physiology. Renal Physiology*, Vol.288, No.1, (January 2005), pp. F108-F116, ISSN 0363-6127

USDA National Nutrient Database for Standard Reference. Release 23. Nutrient Data Laboratory home page, USDA, Agricultural Research Service; 2010 [cited 2011 Mar 25]. Available from: http://www.ars.usda. gov/ba/bhnrc/ndl.

Velasquez, M.T., & Bhathena, S.J. (2007). Role of dietary soy protein in obesity. *International Journal of Medical Sciences*, Vol.4, No.2, (February 2007), pp. 72-82, ISSN 1449-1907

Villa, P., Costantini, B., Suriano, R., Perri, C., Macri, F., Ricciardi, L., Panunzi, S., & Lanzone, A. (2009). The differential effect of the phytoestrogen genistein on cardiovascular risk factors in postmenopausal women: relationship with the metabolic status. *The Journal of Clinical Endocrinology and Metabolism*, Vol.94, No.2, (February 2009), pp. 552-558, ISSN 0021-972X

Wallace, G.M., Bannaryne, W.R., Khaleque, A. (1971a). Studies on the processing and properties of soymilk: II.—Effect of processing conditions on the trypsin inhibitor activity and the digestibility *in vitro* of proteins in various soy milk preparations. *Journal of the Science of Food and Agriculture*, Vol.22, No.10, (October 1971), pp. 526-531, ISSN 1097-0010

Wallace, G.M., Bannaryne, W.R., Khaleque, A. (1971b). Studies on the processing and properties of soymilk: III.—Factors affecting concentration of soymilk and its stability during heat sterilisation. *Journal of the Science of Food and Agriculture*, Vol.22, No.10, (October 1971), pp. 531-535, ISSN 1097-0010

Weggemans, R.M., & Trautwein, E.A. (2003). Relation between soy-associated isoflavones and LDL and HDL cholesterol concentrations in humans: a meta-analysis. *European Journal of Clinical Nutrition*, Vol.57, No.8, (August 2003), pp.940–946, ISSN 0954-3007

Weichenthal, S., Moase, C., & Chan, P. (2010). A review of pesticide exposure and cancer incidence in the Agricultural Health Study cohort. *Environmental Health Perspectives*, Vol.118, No.8, (August 2010), pp. 1117-1125, ISSN 0091-6765

Williamson, G., & Manach, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. *American Journal of Clinical Nutrition*, Vol.81, Suppl.1, (January 2005), pp. 243S-255S, ISSN 0002-9165

Wu, A.H., Wan, P., Hankin, J., Tseng, C.C., Yu, M.C., & Pike, M.C. (2002). Adolescent and adult soy intake and risk of breast cancer in Asian-Americans. *Carcinogenesis*, Vol.23, No.9, (September 2002), pp. 1491–1496, ISSN 0143-3334

Wu, A.H., Ziegler, R.G., Nomura, A.M., West, D.W., Kolonel, L.N., Horn-Ross, P.L., Hoover, R.N., & Pike, M.C. (1998). Soy intake and risk of breast cancer in Asians and Asian Americans. *Americam Journal Clinical Nutrition*, Vol.68, Suppl.6, (December 1998), pp. 1437S–1443S, ISSN 0002-9165

Xiao, C.W. (2008). Health effects of soy protein and isoflavones in humans. *Journal of Nutrition*, Vol.138, No.6, (June 2008), pp. 1244S–1249S, ISSN 1541-3166

Yamamoto, S., Sobue, T., Kobayashi, M., Sasaki, S., & Tsugane, S. (2003). Soy, isoflavones, and breast cancer risk in Japan. *Journal of the National Cancer Institute*, Vol.95, No.12, (June 2003), pp. 906–913, ISSN 0027-8874

Yellayi, S., Naaz, A., Szewczykowski, M.A., Sato, T., Woods, J.A., Chang, J., Segre, M., Allred, C.D., Helferich, W.G., & Cooke, P.S. (2002). The phytoestrogen genistein induces thymic and immune changes: a human health concern? *Proceedings of the National Academy of Sciences of the United States of America*, Vol.99, No.11, (May 2002), pp. 7616-7621, ISSN 0027-8424

Young, V.R. (1991). Soy protein in relation to human protein and amino acid nutrition. *Journal of the American Dietetic Association* , Vol.91, No.7, (July 1991), pp. 828-835, ISSN 0002-8223

Zhan, S., & Ho, S.C. (2005). Meta-analysis of the effects of soy protein containing isoflavones on the lipid profile. *Americam Journal Clinical Nutrition*, Vol.81, No.2, (February 2005), pp.397–408, ISSN 0002-9165

Zhang, Y, Chen, W.F., Lai, W.P., & Wong, M.S. (2008). Soy isoflavones and their bone protective effects. *Inflammopharmacology*, Vol.16, No.5, (October 2008), pp. 213–215, ISSN 0925-4692

Metabolism of α- Linolenic Acid (ALA) in Meat Animals

Pilar Teresa Garcia
Area Bioquímica y Nutrición
Instituto Tecnología Alimentos, ITA
Centro de Investigación en Agroindustria, CIA
Instituto Nacional de Tecnnología Agropecuaria, INTA
INTA Castelar, CC77(B1708WAB) Moron, Pcia Buenos Aires
Argentina

1. Introduction

A key function of ALA (C18:3 n-3) is as substrate for the synthesis of longer-chain omega 3 fatty acid found in fish EPA (C20:5 n-3) and DHA (C22:6 n-3) which play an important role in the regulation of inflammatory immune reactions and blood pressure, brain development, cognitive function, etc. (Sirot et al., 2008).

The American Heart Association (AHA) recommends to increase the consumption of n-3 fatty acids and to reach a low omega 6/omega 3 ratio for reach a healthy status (Russo, 2009). Fat is an important component of the human diet, but current levels of intake are considered too high and the overall fatty acid composition imbalanced. There is an excessive intake of saturated fatty acids (SFA) relative to polyunsaturated fatty acids (PUFA), expressed usually as the P/S ratio, and the consumption of n-6 PUFA is too high relative to n-3 PUFA. The ratio of n-6/n-3 PUFA is a risk factor in cancers and coronary heart disease, especially the formation of blood clots leading to a heart attack. More recently, nutritionists have focused on the type of PUFA and the balance in the diet between n-3 PUFA formed from C18:3 n-3 and the n-6 PUFA from C18:2 n-6 (Willians, 2001)

ALA and LNA (C18:2 n-6) serve as the precursor molecules from which the rest of fatty acids belonging to the n-3 and n-6 fatty acid family can be synthesized through a series of elongation and desaturation reactions. All the reactions are catalyzed by an enzymatic system consisting in fatty acyl-CoA synthetases Δ-6 and Δ-5 desaturases and respective elongases. These two fatty acid families not only share these enzymes, but they also compete for the same enzymes (Brenner, 1989).

There are two basic metabolic fates for ALA. First it is subjected to β-oxidation and extensive carbon recycling. Second, it is converted into longer fatty acids via elongation and desaturation. The predominant fate of ALA is catabolism (Demar et al. 2005) and carbon recycling to acetate (Cunnane et al., 1997, 2003). In rodents, only 16% of an ALA dose is found in rat tissues, mainly adipose, and 6% was elongated /desaturated (Kaduce et al., 2008). Increasing the ALA content of the maternal diet of sucking rats led to increased of ALA, EPA and DPA (C22:5 n-3) in the whole body, skin epididymal fat pads and but there was no effect on the DHA content of these tissues nor on the brain or muscles (Bordoni et

al., 1996). For studies on ALA metabolism, the main focus is to establish if it is converted in sufficient quantities to maintain adequate tissue levels of DHA. Although less importance has been given to the EPA and DPH accumulation.

The conversion of ALA from vegetal oils in long-chain fatty acids EPA (C20:5 n-3), DPA (C22:5 n-3) and DHA (C22:6 n-3) is a hot point in this moment because many studies considere that its conversion is not important. There is little doubt regarding the essential nature of ALA, yet the capacity of dietary ALA to maintain adequate issue levels of long-chain fatty acids remains quite controversial (Barcelo-Coblijn & Murphy, 2009).

The metabolization of ALA to long-chain seems to be affected for several factors as amounts of other fatty acids in the diet as LA, sex, animal species, etc. Several reviews concerning the ALA metabolism to EPA, DPA and DHA have appeared recently (Brenna, 2002; Burdge & Calder. 2005)

EPA and DHA present different effects on several functions of leukocytes, insulin secreting cells, and endothelial cells. These differences are associated with their effects on membrane physicochemical, intracellular signalling pathways and gene expression (Gorjao et al., 2009). The marked differences between the effects of EPA and DHA indicate that it not possible to generalize the effects of omega 3 fatty acids on cell function. Substantial improvement in the therapeutic usage of n-3 fatty acids will be possible with the discovery of the different mechanisms of actions of DHA and EPA.

Meat, fish, fish oils and eggs are the only significant sources of long-chain n-3 PUFA for man. Although meat has lower concentrations of these FA compared to oily fish, it is a very significant source for many people, since fish consumption is low. The low PUFA concentration and the high concentrations of saturated FA in ruminant tissues result from the biohydrogenation of dietary PUFA in the rumen. (Harfoot &Hazlewood, 1988). Ruminal micro-organisms in vitro did not hydrogenate EPA and DHA to any significant extent (Ashes et al., 1992). Several studies have been published covering studies describing manipulation of the fatty acid composition of animal meat but paying less attention to long chain PUFA.

Soybean oil is one of the few plant sources providing ample amounts of both essential fatty acids C 18:2 n-6 and C18:3 n-3. The fatty acid content of soy foods is often unrecognized by health professionals, perhaps because there is so much focus on soy proteins. The major fatty acids in soy oil are the essential fatty acid linoleic (C18:2 n-6) (54%), oleic acid (C22%), palmitic acid (C16:0) and the essential omega 3 fatty acid alfa- linolenic (C18:3 n-3) (8%). Soybeans are used in cattle, poultry and pigs diets and could be a more important source of ALA for animal nutrition and also increase ALA and its fatty acids metabolites in meats. Canola oil is the other important source in commercial oils that contain the precursor alfa linolenic acid (ALA). The main sources of ALA are presented in Table 1.

Genomics, specifically marker assisted plant breeding combined with recombinant DNA technology, provided powerful means for modifying the composition of oilseeds to improve their nutritional value and provide the functional properties required for various food oils (Owen & Sing, 2005).

Transgenic canola oil was obtained that contains >23% of SDA (C18:4 n-3) . In a clinical study (James et al., 2003) observed that SDA was superior to ALA as a precursor by a factor of 3.6 in producing EPA, DHA and DPA (C22:5 n-3).

Modern plant husbandry, either through selective breeding or genetic modifications, affords the opportunity to alter the fatty acid profile of plants. The result was the development of soybean plants, traditionally rich in PUFA, which are high in MUFA, and rapeseed plants traditionally rich in MUFA, which are high in PUFA (Hazebrock, 2000).

Common name	Scientific name	A LA/100g material
Flaxseed	Linum usitatissimum	22.8a
Flaxseed oil		53.3a
Perilla	Perilla frutescens	58.0
Chia seed	Salvia hispanica	17.6a
Camelin oil	Camelina Sativa	38.0c
Canola oil	Brassica campestris	9.1a
Soybeen oil	Glycine max	6.8a
Soybeen green raw		0.4
Walnuts	Juglans regia	9.1a
Cloudberry	Rubus chamaemorus	1.2b
Blueberry	Vaccine corymbosum	0.8b
Cowberry	Vaccinium vitis-idaea	0.2b

a) Agricultural Research Service USA 2009

b) Bere E. , 2007

c) Karvonen et al., 2002

Table 1. Main natural sources of ALA

2. Conversion of ALA into long-chain n-3 PUFA in meat animals

The potential use of livestock products as vehicles to deliver n-3 fatty acids has been the subject of intensive research (Moghadasian, 2008).

The lipid composition of animal body tissues largely depends on the feeding background of the meat producing non-ruminant animals. There has been an increased interest in the substitution of animal fat sources with vegetable oils in animal nutrition.

Vegetable oils have been attributed with reducing the level of saturation in animal fat tissue due to their unsaturated fatty acid concentration when compared with animal fat.

Since some meats naturally have a P/S ratio of around 0.1, meat has been implicated in causing the imbalanced fatty acid intake of today's consumers. Thus the recommended ratio should be increased to above 0.4. In addition, some vegetable oils are rich in n-3 PUFA, mostly C18:3 n-3.

Increasing the n-3 content in animal meats can be achieved by including fish oil or fish meal in the diet, rich in EPA and DHA or vegetable oils rich in ALA. Diet rich in ALA results in an increased level of ALA, EPA, and DPA in the meat, while in most cases no effect on DHA level was observed.

Several reviews have been published covering studies describing manipulation of animal meat but paying less attention to long chain PUFA. All data should be presented as g/100g of total fatty acids to obtain a better comparison of results originated from studies with large differences in fat content. Fatty acid of neutral fraction was characterized by a high proportion of SFA and MUFA, whereas the PL fraction showed a high proportion of PUFA (Raes, De Smet & Demeyer, 2004).

Fish oil and other marine raw materials are limited, expensive and the quality varies.

EPA and DHA, and to a lesser extent DPA, are mainly found in marine products, and fish oil additions to pig diets have been evaluated in several experiments with different inclusion levels. Overland et al. (1996) fed pigs with 1% and 3% dietary fish and found a dose-dependent increase in long-chain n-3 PUFA in fat and muscle.

From a general stand point, fish oil supplementation seems to be the most effective way to increase tissue deposition on DHA, whereas the dietary inclusion of ingredients, flaxseed and flaxseed oil, containing its precursor, ALA, results only in a small increase in DHA, probably due to the limited conversion of DPA to DHA (Riley et al 2000, Raes & De Smet, 2004, Portolesi, Powell & Gibson, 2007; Pawlosky et al., 2003).

Microalgae, the original source of DHA in the marine food chain (Abril et al, 2003) have been included in animal feeds to improve the DHA level of foods of animal origin.

In Tables 2, 3, 4 and 5 are present the effect of different feeding in the proportions of ALA, EPA, DPA and DHA in pork, poultry, beef and lamb meats.

	ALA	EPA	DPA	DHA	Reference
Grass silage only (GS)	1.88	1.00	1.52	0.24	Faucitano et al. (2008)
Growing: GS+4% Soybean Finish: GS+ 4% barley	0.83	0.39	0.85	0.13	Faucitano et al. (2008)
Growing: GS+8% soybean Finish: GS+ 8% barley	0.72	0.31	0.63	0.09	Faucitano et al. (2008)
C German Holstein bulls	0.34	0.14	0.36	0.09	Nuernberg et al. (2005)
Grass German Holstein bulls	1.67	0.58	0.80	0.15	Nuernberg et al. (2005
C German Simental bulls	0.46	0.08	0.29	0.05	Nuernberg et al. (2005
Grass German simental bulls	2.22	0.94	1.32	0.16	Nuernberg et al. (2005
Pasture only	1.30	0.52	0.71	0.43	Garcia et al. (2008)
Pasture+0.7% corn grain	0.89	0.31	0.51	0.18	Garcia et al. (2008)
Pasture+1.0% corn grain	0.74	0.26	0.49	0.14	Garcia et al. (2008)
Concentrate	0.28	0.12	0.30	0.16	Garcia et al. (2008)
Grass + 4 kg C	0.71	0.20	ND		Woods et al. 2011
8 kg concentrate+1 kg hay	0.72	0.12	ND		Woods et al. 2011
6 kg grass+5 kg C	0.87	0.27	ND		Woods et al. 2011
12 kg grass+2.5 kg C	1.01	0.24	ND		Woods et al. 2011
22 kg grass	1.13	0.23	ND		Woods et al. 2011
Feedlot	0.48	0.47	0.91	0.11	Alfaia et al. 2009
Pasture+ 4 month C	0.84	0.77	1.04	0.12	Alfaia et al. 2009
Pasture+ 2 month C	1.96	1.28	1.48	0.14	Alfaia et al. 2009
Pasture only	5.53	2.13	2.56	0.20	Alfaia et al. 2009

C concentrate

Table 2. Effect of dietary source of ALA on the long-chain n-3 PUFA of the beef Longissimus muscle (% of total fatty acids)

	ALA	EPA	DPA	DHA	Reference
Pasture LD	2.64	1.07	0.91	0.41	Garcia et al. (2008)
Pasture Leg muscles	2.89	1.37	1.23	0.41	Garcia et al. (2008)
Extruded linseed-high.oleic rapessed	2.20	0.58	0.75	0.17	Berthelot et al. (2010)
Extruded linseed normal rapeseed	2.38	0.60	0.79	0.20	Berthelot et al. (2010)
Extruded linseed-soybean	2.58	0.75	0.97	0.22	Berthelot et al. (2010)
Grazing *T. subterraneum*	1.97	0.48	0.92	0.64	Chiofalo et al. (2010)
Grazing *L.Multiflorum*	1.52	0.47	0,90	0.64	Chiofalo et al. (2010)
Control (C)High concentrate diet	4.18	0.07	0.06	0.31	Radunz et al. (2009)
Soy bean + linseed oil (2:1)	4.90	0.04	0,02	0.32	Radunz et al. (2009)
Grass hay	2.27	1.23	0.77	0.38	Demirel et al. (2006)
Concentrate	0.72	0.60	0.31	0.17	Demirel et al. (2006)
Soy oil replaced with 0%	0.93	0.19	0.46	0.14	Jeronimo et al (2009)
Soy oil replaced with 33.3%	1.57	0.29	0.54	0.15	Jeronimo et al (2009)
Soy oil replaced with 66.6%	2.62	0.51	0.62	0.19	Jeronimo et al (2009)
Soy oil replaced with 100%	3.05	0.50	0.54	0.20	Jeronimo et al (2009)

Table 3. Effect of dietary source of ALA on the long-chain n-3 PUFA of lamb Longissimus muscle (% of total fatty acids)

2.1 Conversion of ALA in humans

If humans receive an ALA- enriched source there are a generally increase in ALA, EPA and DPA in plasma, in red blood cells and in mononuclear cells (Goyens et al., 2006). More controversial and less consistent are found in regards of the conversion and accumulation of DHA from dietary ALA.

Several reviews concerning the issue of ALA metabolism to EPA, DPA and DHA have appeared recently (Brenna,2002;Burdge et al.2005). Recently Brenna et al. 2009 concluded that there is a little doubt regarding the essential nature of ALA, yet the capacity of dietary ALA to maintain adequate tissue levels of long-chain n-3 fatty acids remains quite controversial.

2.2 Conversion of ALA in pigs

Different factors determine the fatty acid composition in pig's carcasses (Wiseman et al., 2000). In non-ruminants, the fatty acid pattern of dietary lipids is reflected in the fatty acid composition of tissues. Dietary strategies used to customize FA composition of pig fat have been proven to be very effective because dietary fatty acids can be incorporated into pig fat with little modifications (Bee, Jacot, Guex & Biolley, 2008).

	ALA	EPA	DPA	DHA	Reference
6% crushed linseed 20 days	2.77	0.68	0.90	0.44	Kouba et al. (2003)
6% crushed linseed 60 days	3.00	0.77	0.82	0.21	Kouba et al. (2003)
6% crushed linseed 100 days	2.19	0.44	0.63	0.11	Kouba et al. (2003)
Control LD	0.49	0.13	0.34	0.15	Huang et al. (2008)
Linseed (10%) 30 days LD	2.46	0.46	0.56	0.15	Huang et al. (2008)
Linseed (10%) 60 days LD	3.32	0.61	0.65	0.16	Huang et al. (2008)
No fat	0.47	ND	0.12	0.06	Duran et al. (2010)
Tallow (T)	0.93	0.03	0.21	0.11	Duran et al. (2010)
High oleic sunflowe oil (SFHO)	0.72	ND	0.14	0.11	Duran et al. (2010)
Sunflower oil (SFO9	0.70	ND	0.17	0.15	Duran et al. (2010)
Linseed oil (LO)	14.18	0.27	0.43	0.10	Duran et al. (2010)
T(55%)+SFHO (35%) + LO (15%)	5.42	0.13	0.36	0.14	Duran et al. (2010)
Fish oil(40%) +LO (60%)	8.86	0.93	1.02	3.11	Duran et al. (2010)
Palm kernel oil LD	0.63	ND	0.48	0.34	Teye et al. (2010)
Palm oil LD	0.57	ND	0.54	0.36	Teye et al. (2010)
Soybean oil LD	0.87	ND	0.47	0.27	Teye et al. (2010)
Corn+WCR+0% Soybean oil LD	0.33	0.06	0.23	0.16	Wang et al. (2011)
Corn+WCR+8% Soybean oil LD	0.52	0.08	0.29	0.19	Wang et al. (2011)
Corn+WCR+12% Soybean oil LD	0.68	0.11	0.27	0.21	Wang et al. (2011)
Corn+WCR+16% Soybean oil LD	0.72	0.08	0.29	0.15	Wang et al. (2011)
Basal+ 3 % Rapessed oil LD	3.03		0.15	0.11	Tikk et al. (2007)
Basal +3% Palm oil LD	1.17		0.09	0.08	Tikk et al. (2007)
Basal LD	0.55	0.22	0.47	0.14	Haak et al. (2008)
Linseed crushed 3% LD	1.24	0.54	0.75	0.18	Haak et al. (2008)
Fish oil 10% LD	0.47	1.37	0.82	1.02	Haak et al. (2008)

Table 4. Effect of dietary source of ALA on the Long-chain n-3 PUFA of pork muscles (% of total fatty acids)

Different studies agree in that saturated fatty acids are deposited to a higher extent than unsaturated fatty acids (Leyton et al., 1987) and that the degree of deposition of fatty acids increases as fatty acid length increases. In relation to unsaturated fatty acids it seems that as unsaturation increases, deposition decreases (DeLany et al., 2000). However, differences in the relationships between intake and deposition exist, and these relate to whether the specific FA can be synthesized in vivo (Enser et al., 2000). Duran-Monge et al. (2010) found that deposition rates where between 65 and 73 % for diets rich in LA and between 63 and 64% for diets rich in ALA.

	ALA	EPA	DPA	DHA	Reference
Breast					
Control +3% rapessed oil	2.33	0.07	0.57	0.32	Kitessa & Young (2009)
Control + 3% Echium oil	6.50	0.24	1.09	0.37	Kitessa & Young (2009)
Thigh					
Control +3% rapessed oil	2.84	0.03	0.12	0.03	Kitessa & Young (2009)
Control + 3% Echium oil	6.40	0.17	0.33	0.08	Kitessa & Young (2009)
Control Breast	1.76	0.10	Traces	0.01	Rondelli et al. (2007)
Control+5% chia seeds	6.73	0.65	Traces	0.86	Rondelli et al. (2007)
Control+10% chia seeds	9.26	0.68	Traces	1.01	Rondelli et al. (2007)
Control Tigh	1.44	Tr	Tr	Tr	Rondelli et al. (2007)
Control+5% chia seeds	6.56	0.29	Tr	Tr	Rondelli et al. (2007)
Control+10% chia seeds	9.56	0.21	Tr	Tr	Rondelli et al. (2007)
Breast					
15% Sunflower high oleic (SFHO)	2.03	0.13	0.82	0.79	Lozupone at al. (2009)
15 % Flax seed + SFHO	7.91	0.79	1.93	1.46	Lozupone at al. (2009)
15% Canola + SFHO	2.79	0.16	0.92	0.86	Lozupone at al. (2009)
15% Chia+SFHO	10.82	0.61	1.51	1.02	Lozupone at al. (2009)
15% Chia expeller +SFHO	8.73	0.42	1.19	0.95	Lozupone at al. (2009)
Breast					
Concentrate +Soybean	1.71	0.13	0.79	0.75	Azcona et al. (2008)
Concentrate + soybean pasture	1.61	0.27	0.65	0.87	Azcona et al. (2008)
Linseed+SFHO+pasture	4.32	0.39	1.22	1.45	Azcona et al. (2008)
Basal	1.29	0.07	0.38	0.14	Crespo et al. (2002)
Tallow	1.26	0.05	0.51	0.25	Crespo et al. (2002)
Olive oil	1.14	0.05	0.32	0.17	Crespo et al. (2002)
Sunflower oil	0.98	nd	0.03	0.18	Crespo et al. (2002)
Linseed oil	35.28	1.01	0.91	0.35	Crespo et al. (2002)
Control (Corn-soybean)	3.19	0.11	0.57	0.47	Azcona et al. (2008)
Flaxseed (15%)	10.03	0.55	119	0.85	Azcona et al. (2008)
Rapeseed (15%)	3.69	0.16	0.70	0.60	Azcona et al. (2008)
Chia seed (15%)	11.73	0.92	1.23	0.72	Azcona et al. (2008)
Chia meal (15%)	8.82	0.32	1.16	0.89	Azcona et al. (2008)

Table 5. Effect of dietary source of ALA on the Long-chain n-3 PUFA of poultry meat (% of total fatty acids)

The deposition rate for long-chain PUFA was 33.6%, 47, 9% and 48.9% for ARA (C20:4 n-6), EPA an DHA respectively. When no EPA and DHA were added to the diet, high linolenic acid content s in the diet only can increase EPA contents in the whole animal but not DHA. Mathematical relationships between the dietary concentration of PUFA and the fatty acid composition of back fat and intramuscular fat of swine have been established (Nguyen et al., 2003).

Poumes-Ballihaut et al. (2001) have shown that the tissue concentrations of the long-chain n-3 PUFA, particularly DHA, are lower in an ALA-based diet than one in which the performed long-chain n-3 PUFA are presented. Barcelo-Coblijn et al. (2005) found in guinea pigs, both the brain and retina DHA levels were greater when a diet containing 1% of ALA and 1.8% of DHA was fed relative to one with only 7.1% ALA. When guinea pigs were fed a high level of ALA, many tissues had very substantial increases in ALA, EPA and DHA but comparatively little increase in DHA.

Pork normally has a high C18:2 content, producing a high P/S ratio, but an unfavourable n-6/n-3 ratio. A major aim of the feeding strategy was to improve the n-6/n-3 ratio, whilst maintaining a beneficially high P/S ratio.The n-3 PUFA level can be increased in pork by feeding fat sources as linseed, which contains abundance of C18:3 n-3, C18:3 n-3 might elongate in pig tissues to produce long chain n-3 PUFA (Wood & Enser 1997). Conversely, a higher proportion of long chain n-6 PUFA derived from linoleic acid results in a pro-inflammatory status. The ratio of n-6/n-3 PUFA is a risk factor in cancers and coronary heart disease, especially the formation of blood clots leading to a heart attack (Enser, 2001). More recently, nutritionists have focused on the type of PUFA and the balance in the diet between n-3 PUFA formed from C18:3 n-3 and the n-6 PUFA from C18:2 n-6 (Willians 2001)

According to Canadians standards, enrichments of n-3 PUFA, can be obtained in pork products when relatively low levels of back fat from pigs fed flaxseed are included. with pig diets supplemented with vegetable oils such as soybean oil, sunflower oil, and corn oil, contain a high percentage of PUFA and should lead to healthy products for consumers.

Flax is a rich oilseed source of ALA and feeding flax to pigs has been used to increase levels of n-3 PUFA in pork, as reviewed by Nuijens et al. (2003).The effect of feeding flaxseed on tissue deposition on n-3 PUFA in pigs has been found to be quite variable. Feeding extracted flaxseed oil likely provides the most digestible form of ALA, but would be relatively expensive for inclusion in swine diets. Huang et al. (2008) found 9,72% n-3 PUFA in back fat fatty acids when feeding 10% flax to pigs for close to 13 weeks.

Several authors have reported a lack of effect of flax diets on tissue levels of DHA and only a few studies have reported increased levels of DHA after feeding a relative low level (2-2.5%) of dietary flax (Corino, Musella & Mourot, 2008; Enser et al.,2000). The lack of effect on DHA may be explained by competition for Δ6 desaturase activity between ALA and the precursor for DHA (i.e. 24:5 n-3), when the dietary concentration of ALA is high (Cameron et al., 2000).The addition of a 50:50 mix of extruded flax/peas to pig diets provided a highly available source of ALA yielding n-3 fatty acid enrichments in back fat comparable to reports when feeding supplemental flax seed oil (Juarez et al., 2010). Kouba et al. (2003) studied the FA composition of longissimus muscle from pigs fed a 6%crushed linseed diet for 20, 60, or 100 days. The ALA proportions were 2.77, 3.00 and 2,19% respectively, the EPA 0.68, 0.77 and 0.44 respectively, the DPA 0.90, 0.82 and 0.63 respectively and the DPA 0.44, 0.21 and 0.11 % respectively. The reason of the absence of effect of linseed–supplemented diets on the proportion of the quantity of DHA in pig's tissues is not clear. It is either

directly due to the low capacity of the pig to synthesize DHA from EPA, or it is due to a rapid utilization of DHA in pig tissues (Kouba & Mourt, 2011)

The supplementation of pigs fed with whole crop rice with soy bean- oil at levels of 0, 8, 12 or 16% affected the fatty acid composition of back fat and *Longissimus* muscle. The concentration of SFA and MUFA in back fat and in *Longissimus dorsi* muscle decreased significantly with the inclusion of soybean oil in the diet (p<0.05). The n-3 and n-6 PUFA were significantly higher in back fat and in *Longissimus dorsi* muscle of pigs fed diet with soybean oil. The ratios P/S were significantly higher and lower in n-6/n-3 in both tissues of pigs fed with soybean oil (Wang et al. 2010). Feeding 3% of soybean oil to seventy –to crossbreed barrows increased the contents of C18:3 and C18:2 in the neutral lipids and phospholipids in both Longissimus and biceps brachii muscles (Ping et al., 2008).

Male and female pigs were fed diets containing palm kernel oil to fish oil in ratios given as % inclusion 4.1:0.0, 3.9:0.3, 3.6:0.5, and 3.4:0.7.The levels of EPA, DPA and DHA in M. Longissimus increased in a dose-dependent manner when dietary fish oil increased up to 0.7%. The high levels of DPA compared to a markedly lower percentage of EPA than seen in the diet, suggests a strong activity of EPA elongation. The decrease in the ratio between DPA: EPA with increased inclusion of fish oil further suggests a tendency of saturation of this activity (Hallenstvedt et al., 2010).

In pigs the duration and time of feeding a specific fat source on the muscle FA is dependent on the FA considered. For the deposition of ALA and its conversion to long chain metabolites in muscle after the supply of linseed, only the last phase before slaughter was determinant. When fish oil was used, the greatest EPA and DHA proportions were found in case of a continuous supply throughout the fattening period, and levels of DHA but not EPA were lower when fish oil was fed during the first fattening phase followed by linseed feeding before slaughter (Haak, et al., (2008).

2.3 Conversion in poultry

There are opposite deposition patterns between broilers fed fish products and those fed flaxseed and suggests that n-3 fatty acid tissue deposition is source dependent. Fish products are a source of EPA and DHA. whereas crop such as flaxseed provide ALA. Long chain n-3 were either not detected or were detected in very low concentrations in white and dark poultry meats tissues.

Several authors have shown that poultry meat can also be enriched with EPA an DHA by supplementing their diets with marine-based n-3 PUFA sources (Rymer & Givens, 2005).

The incorporation of dehydrated leguminous- based forage in the diet for broiler chicks results in more favourable polyunsaturated fatty acid/ saturated fatty acids and n-6/n-3 nutritional ratios for animals slaughtered at early stages of grow (Ponte et al., 2008).

Addition of 0. 5 and 10% of chia seed to poultry diets increased in broiler breast lipids the ALA proportions (1.76; 6.73 and 9.26 % respectively), the EPA proportions (0.10, 0.65, and 0.68% respectively) and   DHA proportions (0.0, 0.86 and 1.01% respectively (Rondelli et al.,2007)

Broilers fed rapeseed, flaxseed, chia seed and chia meal increase the ALA, long-chain n-3 and total PUFA n-3, contents of white and dark meats. Chia seed gave the highest total PUFA n-3 increasing, yielding 157 and 200%increases for dark and white meat, respectively (Ayerza et al., 2002; Azcona et al., 2008).

Birds fed *Echium* oil-supplemented rations had more than double of ALA, SDA, EPA and DPA in tissues than those fed similar rations supplemented with rapeseed oil. *Echium* oil

was more effective than rapeseed oil in changing the EPA levels in chicken meat, but the two vegetable oil sources were similar in that they both had no impact on the amount of DHA (Kitessa & Young, 2009).

2.4 Conversion in cattle

Meat from pasture- finished beef has greater amounts of n-3 PUFA compared to concentrate-based diets (French et al., 2000; 2003; Garcia et al., 2008). Similar results were observed by Lorenz et al. (2002) in pasture finished bulls compared to concentrate fed. The types of forage, crop variety, cutting, season, year, etc, affects the fatty acid composition of forage crops for grazing forage beef production (Preston, 2005; Garcia et al., 2007)

The forage-based diets increases ALA in LD muscle as compared with feeding concentrates, agreeing with previous studies with beef cattle comparing alfalfa silage (Mandell et al.,1998) or pasture (French et al., 2000, 2003).

The most common method of enhancing the CLA (cis-9, trans-11 C18:2 isomer) and TVA (trans-11 C18:1) content of ruminant meat and dairy products is to provide the animal with additional dietary unsaturated fatty acids, usually from plants oils such as soybean oil (SBO), for use as substrates for ruminal biohydrogenation (Mir et al., 2003). Steers fed a corn-based diet supplemented with SBO may enhance TVA without impacting CLA, while reducing the MUFA content of lean beef (Ludden et al., 2009).

Bulls grazing on pasture and finished on a diet containing linseed accumulated two or three fold higher concentrations of total n-3 PUFA in their muscles compared to those fed concentrate. The increased concentrations of EPA, DPA and DHA in muscle of animals fed on grass suggests that the high availability of ALA in the diet has resulted in an enhanced synthesis of these n-3 long- chain PUFA (Nuernberg et al., 2005)

Supplementation to Friesian bull calves with a high forage fattening diet with soybean oil or extruded full fat soybeans, at a level of 33 g added oil per kg of diet , increases de level of ALA in intramuscular lipids (Aharaoni et al. , 2005).

Concentrates enriched with linseed oil-enriched concentrates results in a favourable n-6/n-3 PUFA ratio. The fatty acid proportion of polar lipids of fat from Longissimus dorsi presented similar proportions of ALA and EPA but less DPA and DHA compared with beef heifers offered pasture only (Noci et al., 2007).

2.5 Conversion in lamb and sheep

All n-3 polyunsaturated fatty acids were higher in muscle from lambs fed dried grass- based diet than from lambs fed concentrate-based diets (Demirel, 2006)

Meat from Patagonian lamb raised on natural grasses showed high concentrations of ALA and long-chain fatty acids, EPA, DPA and DHA compared with lipids from other lamb production systems (Garcia et al., 2008).

The feeding regime, herbage or concentrate affected the total n-3 PUFA in *Longissimus dorsi* muscle of lambs. The herbage regime presented more ALA and EPA but no significant changes in DHA (Vasta et al. 2010).

Berthelot & Schmidely (2010) used sixty male lambs in two trials to study the efficiency of transfer and elongation of ALA in muscle and caudal adipose tissue. In experimental 1 diet lambs were fed a control diet or extruded linseed diet either with wheat or corn and in experiment 2 lambs were fed with normal rapessed or hig-oleic rapessed, or soybean. In experiment 1 linseed increased ALA proportion and total n-3 PUFA in muscle and adipose tissue. In experiment 2 increasing LA intake increased LA proportion in muscle and adipose tissue but did not modify ALA proportion. They concluded that in agreement with

published results, feeding extruded linseed increased the proportion of ALA and long-chain n-3 PUFA in the muscle and adipose tissue of intensively–reared lambs fed high starch concentrates. The type of cereal grains was not a major factor of transfer of ALA in carcasses. Increasing LA intake in lambs fed linseed did not affect ALA transfer or elongation and desaturation of long- chain n-3 PUFA in the muscle.

Several strategies have been tested to improve the nutritional value of meat of intensively-reared lambs, kept indoors and fed high- concentrate diets rich in LA and poor in ALA. Incorporating linseed rich in lipid and ALA in the diet has been advocated by several research (Wachira et al., 2002; Bas et al., 2007) because it induced a high ALA in tissue content and an increase in long-chain PUFA n-3.

The effect of grazing on *Trifolium subterraneum* and *lolium mltiflorum* on lamb meat was studied (Chiofalo et al., 2010). The grazed forage species has influenced the fatty acid composition of lamb meat. The grazing on *T. subterraneum* as monoculture and associated with *L. multiflorum* in the proportion T/L =66/33 has increased the linolenic acid of L.dorsi muscle.

Supplementation of linseed and soybean oils as a source of PUFA en lambs finishing diets had only modest effects on fatty acid composition on muscle and adipose tissues (Radunz et al., 2009). Similar studies have come to the same conclusion based on effects of feeding unsaturated oil supplementation in high–concentrate diets (Beaulieu et al. ,2002; Engle et al., 2000; Rizzi et al., 2001; Santos–Silva et al., 2003).

The dietary replacement of sunflower oil with linseed oil increases significantly the n-3 PUFA in lamb meat. However, the synthesis of EPA and DHA from dietary C18:3 n-3 seems to be limited, and thus the EPA and DHA enriched lamb meat contributes only in a small amount to the recommended daily intake for humans diet (Jeronimo et al. (2009).

Lambs initially fed with concentrate showed a lower proportion of most of n-3 PUFA than lambs initially fed with lucern in intramuscular fat from Longissimus muscles (Bessa et al., 2008).

Peng et al. (2010) investigated the effects of supplemental oilseeds in the diet (sunflower seed, saflower seed, rapeseed and linseed), on fatty acid composition in different ewes tissues. Beneficial fatty acid content of tissue can be increased by oilseed supplementation, but the magnitude of increase varies according to tissue.

The dietary replacement of sunflower oil with linseed oil increased significantly the n-3 PUFA in lamb meats, with the highest value of n-3 long-chain PUFA achieved with 78% of sunflower oil with linseed oil replacement. However, the synthesis of EPA and DHA from dietary ALA seems to be limited, and thus the EPA and DHA enriched lamb meat contributes only in a small amount to the recommended daily intake for human diet (Jeronimo et al., 2009).

3. New sources of long chain PUFA n-3

It is know that global fish stocks are in danger, so, fish production may decrease in the future. In addition of this, some fishes, especially marine fishes like salmon, sardine, tuna, anchovy, mackerel or hake, are sometimes contaminated with heavy metals as copper or mercury, and organic pollutants as PCBs or dioxins, which have a toxic effect for human health (Domingo et al., 2007). Worm & Barbier (2006) predicted that sea food resources would face total collapse mid this century.

For that reasons, several alternative sources for omega-3 PUFA have been proposed, as marine microalgaes, algaes or transgenic plants (Lichenstein et al., 2006).

Marine microalgae, provide the food base that supports the entire animal population in open seas. Cardoso et al., (2007) have reviewed the most recent research on microalgae production of high-value compound having relevance in food science, pharmacology or human health, as PUFA. Marine microalgae are one of the primary producers of long-chain PUFA, and these are capable of converting LNA and ALA to ARA, EPA and DHA, by a series of aerobic desaturations and elongations.

Both LNA and ALA are found in many crop plants such as canola, linseed and soybean and provide a good stating point for transgenic conversion to long-chain PUFA. The transgenic aerobic long-chain PUFA production begins with either a Δ6-desaturation or a Δ9-elongation as the first commited steps of two separate pathways that lead to long -chain PUFA. These enzymes can often act on ALA and LNA equally well, resulting in parallel pathways, which yield both omega-3 PUFA and less desirable omega-6 PUFA product including ARA. ARA will only be converted to EPA, and thus DHA, by a Δ17-desaturation (Wu et al. 2005).

Petrie et al. (2010) have identified and characterised a probable acyl-CoA Δ6-desaturase with strong omega-3 preference from the marine microalga *M. Pusilla*. They have used this enzyme in a highly productive pathway in *N. benthamiana* that culminated with the accumulation of 26% EPA in TAG and have confirmed strong omega 3 preference in transgenic *Arabidopsis*.

Plants have the capacity to serve as a sustainable source of omega -3 fish oils. In order to investigate the impact of different genes on accumulation of n-3 long chain n-3 PUFA, plants were transformed with a number of recombinant binary plasmids, expressing a range of different genes from a variety of organism under control of seed-specific promoters.

During the last 10 years, genes encoding the primary enzymes involved in biostnthesis of these fatty acids have been succefully isolated from a range of VLC-PUFA-synthesising organisms with a number of these being heterologously expressed, syngle or in combination, in oil-seed crops (Sayanova et al., 2004; Napier, 2007)   Depending of the combination of genes, Ruiz et al., 2010 could identify in Arabidopsis plants lines with high EPA and/or DHA content, and with/ without the accumulation of intermediates. They showed the practical feasibility of large-scale production of these important n-3 PUFA.

Although the biosynthesis of ARA, EPA and to some extent to DHA has been demostrated using different approaches in transgenic plants, the resu
ltant fatty acid composition and levels are not equivalent to that found in fish oil. In most current examples such transgenic plants also contain high levels of n-6 and n-3 metabolic intermediaries (Venegas-Calderon, Sayanova & Napier, 2010). Fish oils are almost free of omega- 6 fatty acids such as GLA and DHGLA.

Some early results in enriching plant with n-3 PUFA through transgenesis have been reported in Arabidopsis, soybean and rapeseed (Robert et al., 2005; Sato et al., 2004; Ursin, 2003). The profile and relative profile concentrations of fatty acids in corn and soybeen seed from transgenic and isogenic crops was reported by Jimenez et al., 2009.

4. Conclusions

The conversion of ALA from vegetal oils in long-chain fatty acids EPA (C20:5 n-3), DPA (C22:5 n-3) and DHA (C22:6 n-3) as a results of many studies with both ruminants and non-ruminants is not important, resulting in only a small increase in the deposition of EPA and DPA. The capacity of dietary ALA to maintain adequate issue levels of DHA in meat lipids

seems to be very low. The nutritional importance of increased ALA concentration is not clear since ALA is not as bioactive as longer chain n-3 PUFA such as EPA, DPA and DHA.

5. References

Abril, R., Garret, J., Zeller, S.G., Sander, W.J.& Mast, R.W. (2003). Safety assesment of DHA-rich microalgae from Schizochytrium sp: Part V. Target animal safety/toxicity in growing swine. *Regul.Toxicol. Pharmacol*, 7:73-82.

Aharoni, Y, Orlow, A., Brosh, A., Granit, R.& Kanner, J. (2005). Effects of soybean supplementation of high forage fattening diet on fatty acid profiles in lipid depots of fattening bull calves, and their levels of blood vitamin E. *Animal Feed Science and Technology,* 119:191-202.

Alfaia, C.P.M., Alves, S.P., Martins, S.I.V., Costa, A.S.H., Fontes, C.M.G.A.,Lemos, J.P.C., Bessa, R.J.B. & Prates, J.A.M. (2009). Effect of the feeding system on intramuscular fatty acids and conjugated linoleic acid isomers of beef cattle, with emphasis on their nutritional value and discriminatory ability. *Food Chemistry*, 114:939-946.

Ashes, J. L., Siebert, B.D., Gulati, S.K., Cuthbertson, A.Z. & Scott, T.W. (1992). Incorporation of n-3 fatty acids of fish oil into tissue and serum lipids of ruminants. *Lipids*, 27:629-631.

Ayerza, R., Coates, W. & Lauria, M. (2002). Chia seed (Salvia hispanica l.) as an w-3 fatty acid source for broilers: Influence on fatty acid composition, cholesterol and fat content of white and dark meats, growth performance, and sensory characteristics. *Poultry Science*, 81: 826 -837.

Azcona, J.O., Garcia, P.T., Cossu, M.E., Iglesias, B.F., Picallo, N.A., Perez, C., Gallinger, C.I., Shang,M.J. & Canet, Z.E. (2008). Meat quality of Agentinean Camperos chicken enhanced in omega 3 and omega 9 fatty acids. *Meat Science*, 79:437-443.

Azcona, J.O., Schang, M., Garcia, P.T., , C., Gallinger, C.I., Ayerza, R. & Coates, W., (2008) Omega -3 enriched broiler meat: The influence of dietary α-linolenic-ω-3 sources on growth, performance and meat fatty acid composition. *Canadian Journal of Animal Science,* 88:257-269.

Barcelo-Coblijn, G.& Murphy, E.J. (2009). Alpha-linolenic acid and its conversion to longer chain n-3acids: benefits for human health and a role in maintaining tissue n-3 fatty acid levels. *Progress in Lipid Research,* 48:355-374.

Bas, P., Berthelot, V., Pottier, E.& Normand, J.,(2007) Effect of linseed on fatty acid composition of muscles and adipose tissues of lamb with emphasis on trans fatty acids. *Meat Science,* 77:678-688.

Bauchart , D., Gladine, C., Gruffat, D.,Leloutre, L., & Durand, D. (2005) Effects of diets supplementd with oil seeds and vitamin E on specific fatty acids of Rectus abdominis muscle in Charolais fattening bulls. In *J.F. Hocquette & S.Gigli (Eds.). Indicators of milk and beef quality 112:431-436 EAAP Publ., Wageningen Academic Publishers*.

Beaulieu, A.D., Drackley, J. K. & Merchen, N.R. (2002) Concentrations of conjugated linoleic acid (cis-9, trans-11-octadecadienoic acid) are not increased in tissue lipids of cattle

fed a high concentrate diet supplement with soybean oil. *Journal Animal Science*, 80: 847-861.

Bee, G., Jacot, S., Guex, G.& Biolley, C. (2008). Effects of two supplementation levels of linseed combined with CLA or tallow on meat quality trits and fatty acid profile of adipose and different muscle tissues in slaughter pigs. *Animal,* 2:800-801.

Bere, E. (2007). Wild berries: a good source of omega 3. *Eur.Journal Clinical Nutrition,* 61:431-433.

Berthelot, V., Bas, P.& Schmidely, P. (2010). Utilization of extruded linseed to modify fatty acid composition of intensively-reared lamb meat: Effect of associated cereals (wheat vs. corn) and linoleic acid content of th diet. *Meat Science,* 84:114-124.

Bessa, R.J.B., Lourenco, M., Portugal, P.V.& Santos-Silva, J. (2008). Effects of previous diet and duration of soybean oil supplementation on ligh lamb carcass composition, meat quality and fatty acid composition. *Meat Science,* 80:1100-1105.

Bordoni, A, Lopez Jimenez, J.A.,Spano, C., Biagi, P., Horrobin, D.F.& Hrelia, S. (1996). Metabolism of linoleic and alpha- linolenic in cultured cardiomyocytes: effect of different n-6 y n-3 fatty acid supplementation.*Mol. Cell. Biochem,* 157:217-222.

Brenna, J.T., Salem Jr., N., Sinclair, A.J.& Cunnana, S.C., (2009). Alfa-linolenic acid supplementation and conversion to n-3 long-chain polyunsaturated fatty acids in humans. *Prostaglandins, Leukotrienes and Essential Fatty Acids,* 80:85-91.

Brenna, J.T. (2002). Efficiency of conversion of α-linolenic to long chain n-3 fatty acids in man. *Curr.Opin. Clin.Nutr.Metab.Care,* 5:127-132.

Brenner, R.R. (1989). Factors influencing fatty acid chain elongation and desaturation. *In A.J. Vergroesen & M. Crawford (Eds.) The role of fat in humannutrition. London: Academic press.*

Burdge, G.C.& Calder, P.C. (2005). Conversion of α-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. *Reprod. Nutr. Dev,* 45:581-597.

Cameron, N.D., Wood, J.D., Enser, M.,Wihittington, F.M., Penman J.C.& Robinson, A.M. (2000). Sensitivity of pig genotypes to short-term manipulation of plasma fatty acids by feeding linseed. *Meat Science,* 56:379-386.

Cardoso, K.H.M., Guaratini, T., Barros, M.T., Falcao, V.R., Tonon, A.P., Lopez, A.P. et al. (2007). Metabolites from algae with economical impact . *Comparative Biochemistry and Physiology,* Part C, 146:60-78.

Chiofalo, B., Simonella, S., Di Grigoli, A., Liotta, L.,Frenda, A.S., Lo Presti, V., Bonanno, A. & Chiofalo, V.(2010). Chemical and acidic compostion of longissimus dorsi of Comisana lambs fed with trifolium subterraneum and Lolium multiflorum. *Small Ruminant Research,* 88:89-96.

Corino, C., Musella, M.& Mourot, J. (2008). Influence of extruded linseed on growth , carcass composition, and meat quality of slaughtered pigs at one hundred ten and one hundred sixty kg of liveweight. *Journal Animal Science*, 86:1850-1860.

Crespo, N.& Esteve-Garcia,E. (2002). Dietary polyunsaturated fatty acids decreasefta deposition in separable fat depot but no in the remainder carcass. *Poultry Science,* 81:512-518.

Cunnane, S.C: & Anderson, M.J.,(1997). The mayority of dietary linoleato in growing rats is beta-oxidized or stored in visceral fat. *Journal of Nutrition,* 127:146-152.

Cunane, S.C.(2003). Problems with essential fatty acids:time for a new paradigm? *Progress Lipid Research*, 42:544-568.

DeLany, J.P., Windhauser, M.M., Champagne, C.M.& Bray, G..A (2000). Differential oxidation of individual dietary fatty acids in humans. *American Journal Clinical Nutrition,* 72:905-911.

Demar, J.C., Ma, K., Chang, I.,Bell, J.M. & Rapoport, S.I. (2005). Alpha linolenic acid does not contribute appreciably to docosahexaenoic acid within brain phospholipids of adult rats fed a diet enriched in docosahexaenoic acid. *Journal Neurology,* 94:1063-1076.

Demirel, G., Ozpinar, H., Nazli, B. & Keser, O. (2006). Fatty acid of lamb meat from to breeds fed different forage: concentrate ratio. *Meat Science*, 72:229-235.

Domingo, J.L.,Bocio, A., Falco, G. & Llobet, J.M. (2007).Benefits and risks of fish consumption I. A quantitative analysis of the intake of omega -3 fatty acids and chemical contaminants. *Toxicology,* 230:219-226.

Duran-Montge,P., Realini,C.E., Barroeta,A.C., Lizardo, R.G.& Esteve-Garcia, E. (2010). De novo fatty acid synthesis and balance of fatty acids of pigs fed different fat sources. *Livestock Science,* 132: 157-164.

Engle,T.E., Spears, J.W., Fellner, V. & Odle, J. (2000). Effects of dietary soybean oil and dietary copper on ruminal and tissue lipid metabolism in finishing steers. *Journal Animal Science,* 78:2713-2721.

Enser, M., Richardson, R. I., Wood, J.D.,Gill, B.P.& Sheard, P.R. (2000).Feeding linseed to increase the n-3 PUFA of pork: Fatty acid compositionof muscle, adipose tissue, liver and sausages. *Meat Science,* 55: 201-212.

Faucitano,l., Chouinard, P.Y., Fortin, J.J., Mandell, I.B., Lafreniere, C.& Girard, C.L., Berthiaume, R. (2008). Comparison of alternative beef production systems based on forage finishing or grain-forage diets with or without growth promotans: 2. Meat quality, fatty acid composition, and overall palatability. *Journal Animal Science*, 86:1678-1689.

French, P., Stanton, C., Lawless, F., O`Riordan, E.G., Monahan, F.J., Caffrey, P.J.& Moloney, A.P. (2000). Fatty acid composition , including conjugated linoleic acid, of intramuscular fat from steers offered grazed grass, grass silage or concentrate – based diets. *Journal Animal Science,* 78.2849-2855.

French, P., O'Riordan, E. G.,Monahan, F. J., Caffrey, P.J. & Moloney, A.P. (2003). Fatty acid composition of intramuscular triacylglycerols of fed autumn grass and concentrates. Livestock Production Science, 81:307-317.

García, P. T., Pordomingo, A., Perez, C. A., Rios, M.D.& Casal, J.J. (2007). Influence of cutting and season on the fatty acid composition of forage crops for grazing forage beef production. *Proceedings 53nd International Congress Meat Science & Technology*. 97-98.

Garcia, P.T., Pensel, N.A, Sancho, A.M., Latimori, N.J., Kloster, A. M. , Amigone, M.A.& Casal, J.J. (2008). Beef lipids in relation to animal breed and nutrition in Argentina. *Meat Science,* 79:500-508.

Garcia, P.T:, Casal, J:J:, Fianuchi, S., Magaldi, J.J., Rodriguez, F.J.& Nancucheo, J.A. (2008). Conjugated linolenic acid (CLA) and poliunsaturated fatty acids in muscle lipids of lamb from the Patagonian area of Argentina. *Meat Science,* 79:541-548.

Gorjao, R., Acevedo-Martins,A.K.,Gomez Rodrigues, H., Abdulkader, F., Arcisio-Miranda, M., Procopio, J.& Curi, R. (2009). Comparative effects of DHA and EPA on cell function. *Pharmacologia &Therapeutics,* 122: 56-64.

Goyens, P.LL.,Spilker, M.E., Zock, P.L., Katan, M.B.& Mensink, R.P. (2006). Conversion of α-linolenic acid in humans is influenced by the absolute amounts of α-linolenic acid and linoleic acid in the diet and not by their ratio. *American Journal Clinical Nutrition,* 84:44-53.

Haak, L., De Smet, S., Fremaut, D., Van Walleghem, K.& Raes, K. (2008). Fatty acid profile and oxidative stability of pork as influenced by duration and time of dietary linseed or fish oil supplementation. *Journal Animal Science,* 86:1418-1425.

Hallenstvedt , E., Kjos, N.P., Rehnberg, A. C., Overland, M.&Thomassen, M. (2010). Fish oil in feeds for entire male and female pigs: changes in muscle fatty acid composition and stability of sensory quality. *Meat Science,* 85:182-190.

Harfoot, C.G. & Hazelwood, G.P. (1988). Lipid metabolism in the rumen. *In The Rumen Microbial Ecosystem, 2nd ed. P.N. Hobson, ed. Elservier Science Publishing Co., Inc., New York, NY.*

Huang , F.R., Zhan, Z.P., Luo, J.& Peng, J. (2008). Duration of dietary linseed feeding affects the intramuscular fat, muscle mass and fatty acid compositionin pig muscle. *Livestock Science,* 118:132-139.

James, M.J., Ursin, V.M.& Cleland, L.G. (2003). Metabolism of stearidonic acid in human subbjects: comparison with the metabolism of other n-3 fatty acids. *American Journal of Clinical Nutrition,* 77:1140-1145.

Jeronimo,E., Alves, S.P., Prates, J.A.M., Santos-Silva, J.& Bessa, R.J.B. (2009). Effect of dietary replacement of sunflower oil with linseed oil on intramuscular fatty acids of lamb meat. *Meat Science,* 84: 499-505.

Jimenez, J.J., Bernal, J.L., Nozal, M.J., Toribio, L.& Bernal, J. (2009). Profile and relative concentrations of fatty acids in corn and soybean seed from transgenic and isogenic crops. Journal of Chromatography ,A. 1216: 7288-7295.

Juarez, M., Dugan, M.E.R., Aldai, N., Aalhus, J.L., Patience, J.F., Zijlstra, R.T.& Beaulieu, A.D.(2010). Increasing omega-3 levels throgh dietary co-extruded flaxseed supplementation negatively affects pork palatability. *Food Chemistry,* 126:1716-1723.

Kitessa, S.M. & Young, P. (2009) Echium oil is better than rapeseed oil in enriching poultry with n-3 PUFA , including eicosapentaenoic acid and docosapentaenoic acid. *British Journal of Nutrition,* 101: 709-715.

Kaduce, T.L., Chen, Y.,Hell, J.W. & Spector, A.A.(2008). Docosahexaenoic acid synthesis from n-3 fatty acid precursors in rat hippocampal neurons. *Journal Neurochem,* 2800.

Karvonen, H.M., Aro, A., Tapola, N.S., Salminen, I., Uusitupa, M.I. &.Sarkkinen, E.S. (2002).Effect of alpha linolenic acid –rich Carmelina sativa oil on serum fatty acid composition and serum lipids in hypercholesterolemic subjects. *Metabolism,* 51:1253-1260.

Kouba, M., Enser, M.,Whittington, F.M., Nute, G.R.& Wood, J.D. (2003). Effect of a high-linolenic diet on lipogenic enzymes activities, fatty acid compostion, and meat quality in the growing pig. *Journal Animal Science,* 81:1967-1979.

Kouba, M.& Mourot, J. (2011). A review of nutritional effects on fat composition of animal products with special emphasis on n-3 polyunsaturated fatty acids. *Biochimie,* 93:13-17.

Leyton, J., Drury, P.J.& Crawford, M.A., (1987). Differential oxidation of saturated and unsaturated fatty acids in vivo in the rat. *British Journal of Nutrition,* 57:383-393.

Lichenstein, A.H., Matthan, N. R., Jalbert, S.M.,Resteghini, N.A., Schaefer, E.J. & Ausman, L.M. (2006). Novel soybean oils with different fatty acid profiles alter cardiovascular disease risk factors in moderately hyperlipidemic subjects. *The American Journal of Clinical Nutrition*, 84:497-504.

Lorenz, S., Buttner, A., Ender , K., Nuernberg, G., Papstein, H.J., Schieberle, P.& Nuernberg, K.(2002). Influence of keeping system on fatty acid composition in the longissimus muscle of bulls and odorants formed after pressure-cooking . *Eur. Food Res Technol.,* 214:112-118.

Lozupone, A., Azcona, J.O., Sancho, A. M., Casal, J.J.& Garcia, P.T. (2009). Efecto del sexo en la composicion de los acidos grasos de los lipidos intramusculares de pollos. *XII Congreso Argentino de Ciencia y Tecnologia de Alimentos. Concordia. Entre Rios 7-9 octubre 2009.*

Ludden, P.A., Kucuk, O., Rule, D.C.& Hess,B.W. (2009). Growth and carcass fatty acid composition of beef steers fed soybean oil for increasing duration before slaughter. (2009). *Meat Science,* 82: 185-192.

Madron, M.S., Peterson, D.G.,Dwyer, D.A., Corl,B.A., Baumgard,L.H., Beermann, D.H.& Bauman,d.E. (2002). Effect of extruded full-fat soybeans on cojugated linoleic acid content of intramuscular, intermuscular, and subcutaneous fat in beef steers. *Journal Animal Science,* 80: 1135-1143.

Mandell, I.B., Buchanan-Smith, J.G., Holub, B.J.& Campbell, C.P. (1998) Effects of fish meal in beef cattle diets on growth performance, carcass characteristics, and fatty acid composition of longissimus Muscle. *Journal Animal Science,* 75:910-919.

Mir,P.S., McAllister, T.A., Zaman, S., Jones, S.D.M., He, M.L., Aalhus,J.L., Jeremiah, L.E., Goonewardene, L.A.-, Weselake, R..J.& Mir, Z. (2003).Effect of dietary sunflower oil and vitamin E on beef cattle performance, carcass characteristics and meat quality. *Canadian Journal Animal Science*, 83:53-66.

Moghadasian, M.H., (2008). Advances in dietary enrichmente with n-3 fatty acids, *Critical Reviews in Food Science and Nutrition,* 48:402-410.

Noci, F., French, P., Monahan, F.J.& Moloney, A.P., (2007). The fatty acid composition of muscle fat and subcutaneous adipose tissue of grazing heifers supplemented with plant oil-enriched concentrates.*Journal Animal Science* , 85:1062-1073.

Napier, J.A. (2007). The production of unusual fatty acids in transgenic plants. *Annu. Rev. Plant. Biol.,* 58:295-319.

Nguyen, L.Q., Nuijens, M.C.G.A., Everts,H., Salde, N.& Beynen, A.C. (2003). Mathematical relationships between the intake of n-6 and n-3 polyunsaturated fatty acids and thir content in adipose tissue of growing pigs. *Meat Science*, 65:1399-1406.

Nuernberg, K., Dannenbrger, D., Nuernberg, G., Ender, K., Voigt, J., Scollan, N.D., Wood, J.D., Nute, G.R.& Richardson, R.I. (2005). Effect of grass-based and a concentrate feeding system on meat quality characteristics and fatty acid composition of longissimus muscle in different cattle breeds. *Livesstock Production Science,* 94:137-147.

Overland,M., Taugbol, O. ,Haug, A.& Sundstol, E. (1996). Effect of fish oil on growth performance, carcass charactristics, sensory parameters, and fatty acid composition in pigs. Acta Agriculturae Scandinavica Section A –*Animal Sciene,* 46:11-17.

Owen, P.W.& Singh, A. (2005). Omega 3/6 fatty acids: Alternative source of production. *Process Biochemistry,* 40:3627-3652.

Pawlosky, R. J., Hibbeln, Lin, Y., Goodson, S., Riggs, P., Sebring, N., Brown, G.L. & Salem, N. Jr (2003). Effects of beef and fish based diets on the kinetics of n-3 fatty acid metabolism in human subjects. *American Journal Clinical Nutrition*, 7: 565-572.

Petrie, J.R.,Shrestha, P., Mansour, M.P.& Nichols, D.N.(2010). Metabolic engineering of omega-3 long-chain polyunsaturated fatty acids in plants using an acyl-CoA Δ6 desaturase with w3-preference from the marine microalga Micromonas pusilla. *Metabolic Engineering* ,12:233-240.

Ping,L.,Zhang,L.Y., Yin, J.D., Everts, A.K.R.& Li, De.F. (2008). Effects of soybean oil and linseed oil on fatty acid composition of muscle lipids and cooked pork flavour. *Meat Science,* 80: 910-918.

Ponte, P.I.P, Alves, S. P. , Bessa, R.J.B., Ferreira, L.M.A., Gama, L.T., Bras, J.L.A., Fontes, C. M. G. A.& Prates, J.A.M. (2008). Influence of pasture intake on the fatty acid composition, and cholesterol, tocopherols, and tocotrienols content in meat from free-range broilers. *Poultry Science*, 87:80-88.

Portolesi, R., Powell, B.C.& Gibson, R.A. (2007). Competition between 24:5n-3 and ALA for Δ6 desaturase may limit the accumulation of DHA in HepG2 cell membranes. *Journal of Lipid Research,* 48: 1592-

Poumes-Ballihaut , C., Langelier, B., Houlier, F., Alessandri, J.M. Durand, G., Latge, C.& Guesnet, P., (2001). Comparative bioavailability of dietary alpha-linolenic and docosahexanoic acids in the growing rat. *Lipids,* 36: 793-780.

Preston, R.L. (2004).Typical composition of feeds for cattle and sheep. *Beef Mag. http//www.beefmagazine.com/mag/beef_typical_composition_feed/index.html.*

Raes, K., De Smet , D. & Demeyer, D. (2004). Effect of dietary fatty acids on incorporation of long chain polyunsaturated fatty acids and conjugated linoleic acid in lamb,beef and pork meat: A review. *Animal* Feed Science and Technology, 113:199-221.

Radunz A.E., Wickersham, L.A., Loerch, S.C., Fluharty, F.L., Reynolds, C.K. & Zerby, H.N. (2009).Effects of dietary polyunsaturated fatty acid composition in muscle and subcutaneous adipose tissue of lamb. *Journal Animal Science*, 87:4082-4091.,

Riley, P.A., Enser, M., Nute, G.R.& Wood, J.D. (2000). Effects of dietary linseed on nutritional value and other quality aspects of pig muscle an adipose tissue. *Animal Science,* 71:483-500.

Rymer, C.& Givens, D.I.,(2005). N-3 fatty acids enrichment of edible tissue of poultry: a review. *Lipids,* 40:121-130.

Rizzi, L., Simioli, M., Sardi, L.& Monetti, P.G. ((2001). Carcass quality, meat chemical and fatty acid composition of lambs fed diets containing extruded soybeans and sunflower seeds.*Animal Feed Science and Tecnology,* 97:103-114.

Robert,S.S, Singgh,S., Zhou, X.R.,Petrie,J.R.,Blackburn, S., Mansour, P.M.,Nichols, P.D., Liu, Q.& Green, A.(2005) .Metabolic engineering of Arabidopsis to produce nutritionally important DHA in seed oil. *Funct.Plant Biol.,* 32:473-479.

Rondelli, S.B. , Martinez, O., Casal, J.J.& Garcia, P.T. (2007). Fatty acid composition of body lipids of broiler fed with different levels of dietary chia seeds. *Proceeding 53rd International Congress Meat Science &Tecnology, 101-102.*

Ruiz-Lopez, N., Haslam, R., Sayanova, O.& Napier, J. (2010). The production of omega-3 very long-chain polyunsaturated fatty acids in transgenic plants: a sustainable source of omega-3 fish oils.(2010). *Chemistry and Physics of Lipids,* 163S Abstract SO27.

Russo, G.L. (2009). Dietary n-6 and n-3 polyunsaturated fatty acids: From biochemistry to clinical implications in cardiovascular prevention. *Biochemical Pharmacology,* 77: 937-946.

Santos-Silva, J. , Bessa, R. J. B. & Mendes, I. A. (2003). The effect of supplementation with expanded sunflower seed on carcass and meat quality of lambs raised on pasture. *Meat Science*, 65:1301-1308.

Sato, S., Xing, A., Ye, X., Schweiger, B., Kinney, A., Graef, G.& Clement, T. (2004). Production of λ-linolenic acid and stearidonic acid in seeds of marker-free transgenic soybean. *Crop Science*, 44:646-652.

Sayanova , O.V.& Napier, J.A. (2004). Eicosapentaenoic acid: biosynthetic routes and the potential for synthesis in trnsgenic plants. *Phytochemistry* ,65:147-158.

Sirot, V., Oseredzuk, M., Bemrah-Aouachria, N.,Volatier, J.L& Leblanc, J.C. (2008). Lipid and fatty acid composition of fish and seafood consumed in France: CALIPSO study. *Journal of Food Composition and Analysis,* 21:8-16.

Ursin, V.M.,(2003).Modification of plant lipids for human health:development of funtional land-based omega-3 fatty acids. *Journal of Nutrition,* 133: 4271-4274.

Teye, G.A., Sheard, P.R., Whittington, F.M., Nute, G.R., Stewart, A. & Wood, J.D.(2006). Influence of dietary oil and protein level on pork quality. 1. Effects on muscle fatty acid composition, carcass, meat and eating quality. *Meat Science*, 73:157-165.

Tikk, K.,Tikk, M.,Aaslyng, M.D., Karlsson, A.H., Lindahl, G.& Andersen, A.H. (2007). Significancia of fat supplemented diets on pork quality- Connections between specific fatty acids and sensory attributes of pork. *Meat Science* 77:275-286.

Vasta, V., Priolo, A., Scerra M., Hallet, K.G. , Wood, J.D. & Doran, O. (2009). Δ9 desaturase protein expression and fatty acid composition of longissimus dorsi muscle in lamb fed green herbage or concentrate with or without added tannins. *Meat Science* , 82: 357-364.

Venegas-Caleron, M. Sayanova, O.& Napier, J.A. (2010). An alternative to fish oils: Metabolic engineering of oil-seed crops to produce omega-3 long chain polyunsaturated fatty acids. *Progress in Lipid Research* , 49:108-109.

Wachira, A.M., Sinclair, L.A., Wilkinson, R.G., Enser, M., Wood, J.D.& Fisher, A.V. (2002). Effects of dietary fat source and breed on the carcass composition, n-3

polyunsaturated fatty acid and conjugated linoleic acid content of sheep and adipose tissue. *Journal of Nutrition,* 88:697-709.

Wang, H.F., Ye, J.A., Li, C.Y., Liu, J.X.& Wu, Y.M. (2011). Effects of feeding whole crop rice combined with soybean oil on growth performance, carcass quality characteristics, and fatty acids profile of Longissimus   muscle and adipose tissue of pigs. *Livestock Science* 136: 64-71.

Wiseman, J., Redshaw, M.S.,Jagger, S., Nute, G.R.& Wood, J.D. (2000). Influence of type and dietary rate of inclusion of oil on meat quality of finnishing pigs. *Animal Science,* 70: 307-315.

Williard, D.E., Harmon, S.D., Kaduce, T.L., Preuss, M., Moore, S.A., Robbins, M.E. et al. (2001). Docosahexaenoic acid synthesis from n-3 PUFA in differentiated rat brain astrocytes. *Journal Lipid Research,* 42: 1368-1376.

Worm, B.& Barbier, E.B. (2006). Response to comments on "Impacts of biodiversity loss on ocean ecosystem service. Science, 316:1285d

Wood , J.D. & Enser, M. (1997).factors influencing fatty acid composition in meat and the role of antioxidants in improving meat quality. *British Journal of Nutrition*, 78:S49-50.

Woods, V.B.& Fearon, A.M. (2009). Dietary sources of unsaturated fatty acids for animals and their transfer into meat, milk and eggs. A review. *Livestock Science* , 126:1-20.

Wu, G., Truksa, M., Datla, N., Vrinten, P., Bauer, J., Zank, T.,Cirpus, P., Heinz, E. & Qiu , X. (2005). Stepwise engineering to produce high yields of very long-chain PUFA in plants. *Nat. Biothecnol.*, 23:1013-1017.

Soya Bean Meal and Its Extensive Use in Livestock Feeding and Nutrition

Yulong Yin[1], Andrew A. Fatufe[1,2] and Francois Blachier[3]
[1]Research Center for Healthy Breeding of Livestock and Poultry and Key Laboratory for Agro-ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, the Chinese Academy of Sciences, 410125 Hunan
[2]Department of Animal sciences, Obafemi Awolowo University, Ile-Ife, Laboratory of Animal Nutrition and Health and Key Laboratory of Subtropical Agro- ecology, Institute of Subtropical Agriculture, The Chinese Academy of Sciences, Changsha, Hunan
[3]INRA, CNRH-IdF, AgroParisTech, UMR 914 Nutrition Physiology and Ingestive Behavior, 16 rue Claude Bernard, Paris,
[1]China
[2]Nigeria
[3]France

1. Introduction

Soya bean (*Glycine max*) is an economic crop used for both human and animal feeding. Oil seed meals are important sources of protein and energy for both human and livestock. The most recent country by country estimate of global production of soya bean as at 2007 by the United States Department of Agriculture was 206.4 million tons with the US, Brazil, Argentina, China and India taking the global lead in descending order of production. Soya bean meal is mainly used as animal feed in most countries, but there is high consumption of soya bean meal as tofu in Asian countries. Soya bean has also assumed a place of importance in the production of biodiesel aimed at supplementing the world's complete dependence on fossil fuel, which is a finite resource. Soya bean meal is one of the most researched ingredients in animal nutrition and one of the most important feed ingredients used for animal feeding. There is a high demand for SBM for livestock production globally, which is driven by improvement in quality of life, a change of taste and a shift from dependence on vegetable protein to animal protein as a result of the emergence of the middle class in Asia especially in China. The recent ban on all terrestrial feed ingredients such as fish meal and bone and meat meal in pig and poultry feeding in the European Union has further tended to heavy dependence on soya bean meal as sole ingredient for non-ruminant feeding. Other oil seed legumes such as peas, field beans, rapeseed seed and cotton seed meal are all measured against soya bean meal in terms of their nutritive values. The proximate and amino acid compositions of SBM and other oil seed legume are shown in Table 1. These feedstuffs are often used to substitute part of soya bean meal for animal feeding, but not to serve as complete replacement if optimum biological performance is to be attained. Most nutrients needed for the nourishment of livestock for sustenance of life,

growth, health and reproduction are contained in feedstuff which they consume. The usefulness of a particular feed ingredient for animal feeding is not only determined by the amount of nutrient contained in it but also by the amount of 'utilisable' nutrient contained in this feed ingredient. This is often referred to as bioavailability, which is the true amount of nutrient available to the animal for productive purposes. Not all the nutrients contained in feed ingredients are available to the animal for metabolic functions. The recovery or extraction of nutrients from feed ingredients in the gastro-intestinal tract is not 100% as some of the nutrients are often lost in faeces. It is the high recovery of nutrients of soya bean

	Jack bean meal	Field bean meal	Mung bean meal	Soya bean meal	Rape seed meal	Peanut meal	Cotton seed meal	Canola meal
Dry matter	940	927	930	891	893.0	830	936	932
Organic matter	943	965	944	942	-	-	-	-
Crude fat	13	16	10	180	-	-	-	-
Crude ash	44	39	44	51	79.2	76	73	68
Calcium	1.7	1.7	1.7	3.2	-	-	-	-
Phosphorus	4.8	4.5	3.5	6.2	-	-	-	-
Gross energy	17.7	18.3	17.9	22.6	20.73	20.75	18.79	20.0
NDF	383	450	352	216	577.4	258	603	
ADF	121	140	139	88	415.0	92	243	
ADL	29	40	38	28	289.4	26	98	
Snsp[2]	27			22	19	3.6	27	
ISnsp[3]	163			167	153	106	231	
Crude protein	205	272	286	412	414.7	500	379	390
Threonine	6.7	6.1	6.3	11.8	12.3	16.3	11.8	14.1
Valine	12.4	12.1	12.9	17.4	17.2	15.7	15.7	16.6
Methionine	4.7	2.6	5.8	2.3	9.9	4.7	3.3	4.9
Isoleucine	10.9	12.8	10.1	14.8	19.0	19.4	10.4	14.3
Leucine	14.9	17.2	17.0	21.2	23.9	31.9	17.6	27.3
Phenyalanine	15.2	15.7	19.1	14.6	28.4	23.0	20.5	14.7
Histidine	7.0	7.9	6.8	7.4	11.3	15.4	10.3	8.5
Lysine	13.0	12.9	9.4	20.4	17.8	20.5	12.9	18.1
Arginine	16.4	21.5	15.6	17.8	20.4	76.6	37.2	21.9
Aspartic acid	19.6	22.7	23.1	30.6	19.1	67.7	25.3	21.6
Serine	6.7	7.0	8.4	14.4	11.0	25.7	14.4	14.0
Glutamic acid	27.1	35.0	36.6	45.5	62.4	114.9	54.5	60.1
Glycine	7.3	8.5	8.2	16.4	15.1	14.6	8.3	8.4
Proline	1.8	2.6	36.6	16.9	3.2	4.9	15.9	20.5
Alanine	7.9	9.1	9.0	15.8	14.3	34.0		
Tyrosine	-	-	-		6.7	27.6	13.8	17.9

[1] Results from our laboratory

[2] Soluble non-starch polysaccharides

[3] Insoluble non-starch polysaccharides

Table 1. Chemical composition of some plant protein feedstuffs (g/kg DM) [1]

meal that makes it the number one protein ingredient for pig and poultry feeding. Corn and soya bean meal are the industrial standard for feeding pigs and poultry and presumably other monogastric animal world wide. They are choice ingredients for monogastric feeding because of high level of bioavailable nutrient inherent in these feedstuffs. Most estimation of nutrient requirement based on practical type diets employed these two feed ingredients for their derivation. This high level of bioavailable nutrients in soya bean is very crucial for reducing the emission of nitrogen and phosphorus to the environment. When highly digestible nutrient are fed to pig and poultry, less nutrient are excreted and there is less concern about contamination of the environment from manure generated from livestock operations. Soya bean meal remains the single largest protein resource used for monogastric animal feeding and as such, there is the need to continually study and re-evaluate this feed ingredient, developed new SBM products, improve upon existing processing techniques and develop new methods of improving its utilisation for livestock feeding.

2. Processing techniques and soya bean meal products

There are different types of soya bean meal products used for animal feeding and these include solvent extracted, mechanically extracted, extruded, full fat, soya protein isolate, soya protein concentrate, soya oligosaccharides and fermented soya among others. The idea behind the various type of treatment applied to soya bean seed is to neutralise the effect of anti-nutrients present in these seeds and to improve the palatability, flavour and digestibility of soya bean meal products for animal consumption and utilisation. An in-depth discussion on the various anti-nutritional factors present in soya bean seed and their effect on digestion and nutrient utilisation will be undertaken later in this chapter. Soya bean meal can be processed by dry heating, moist heating (toasting) or without heat. Proper processing (heating) of SBM is very important because under-processing will cause poor digestibility of protein and its amino acids and improper detoxification of heat labile anti-nutritional factors (e.g. lectins), while over heating will also result in poor protein quality from the reaction of reactive amino group of amino acids and with carbonyl group of reducing sugar also known as Maillard reaction. Lysine and cystine are the most susceptible during overheating processing of SBM and other oil seed legume seeds.

The methods of processing soya bean seed is varied depending on the intended product, level of oil expected in the product and the class of targeted livestock. Before solvent extraction, soya bean seed is cleaned, cracked, de-hulled by aspiration, heated and flaked into chips (Anonymous, 2004). Oil extraction is very efficient using hexane as the organic solvent and fat content is reduced to less than 1.0%. The defatted-flake produced during the oil extraction process can be used as the starting material for other products such as soya protein concentrate (SPC) and soya protein isolate (SPI). SPC is produced by the removal of a large proportion of the 20% soluble carbohydrate fraction present in defatted soya bean flakes through leaching with water, alcohol or acid extraction. The soluble carbohydrate fraction of SBM comprise mainly of starchyose and raffinose, which are known to be responsible for off flavour taste and flatulence in man and low energy output in farm animals. The removal of the soluble carbohydrate fraction in SBM usually leads to higher crude protein (62- 69%) and crude fibre contents (3.4-4.8). To produce soya protein isolate, protein from soya flakes is made soluble by sodium hydroxide or other alkali solution and protein removed from solution by centrifugation, concentrated and spray dried (Lusas and Riaz, 1995).

Soya bean seeds could also be extruded under pressure with steam application and under this processing method, sufficient heat for detoxification of anti-nutrient is generated. No oil is extracted and full fat soya bean meal is formed. Mechanical extraction or expeller method involved the pre-treatment of seeds such as cleaning, cracking and drying. The beans are later forced through expeller press and the oil extracted. This method have been adapted to produce improved patented product for soya bean for ruminant feeding known as SoyPLUS® which have a higher level of bypass protein of about 60% compared with the conventional production method of 35-45%. By pass protein are protected proteins that are not degraded by the microbes in the rumen during extensive fermentation process, but are digested and absorbed directly in the small intestine. This type of protein are more beneficial to the ruminant animal, because the balance protein in soya bean meal will be used directly by the animals and not first diverted to microbial protein production, which is usually high in nucleic acid and not as closely related to the amino acid requirement of ruminant as soya bean protein. Solvent extracted SBM contained less oil usually less than 2%, while mechanically extracted soya bean meal usually contained more residual oil (3-5%). Full fat SBM contains more oil as the name suggest and usually not less than 18%. This variable oil composition also has inverse relationship with the crude protein contents of the different soya bean products and less oily product usually contained more crude protein and less metabolisable or digestible energy and vice versa. Soya bean meal is usually standardized commercially to contain 44% or 48% crude protein by addition of the hull to produce commercial low and high protein soya bean meal respectively (Pond et al., 2005). Full fat soya bean meal usually contains less crude protein of about 38-42% and a crude fibre of 3.5-4.0%.

Dry heating can reduce up to 85% of antinutritional protein present in soya bean meal and also the reduction of these anti-nutrient proteins by extrusion-expeller technique may be comparable, but the efficiency of feed utilisation of broilers fed extruded soya bean meal was found to be higher than that of dry-heated soya bean meal (Vasconcelos et al., 2009). The value of extruded-expelled soya bean meal without hulls or with hulls has also be found to be similar to that of solvent extracted soya bean meal with or without hull added, but was found to be higher than that of extruded-expelled soya bean meal produced for ruminant feeding with high level of by pass protein (Woodworth et al., 2001). The soya bean meal with high by pass protein was less efficiently utilised when fed to young pigs and was characterised with poorer average daily gain and gain to feed ratio. This goes further to buttress the fact that, the choice of which soya bean product to feed should be determined by the class of animals to which product is to be fed and the prevailing prices of other soya bean meal product in the market. Another factor that may affect the feeding value of soya bean meal for farm animals is the location of production, because yield/hectare is known to be inversely related to protein content in soya bean seeds. The proximate and amino acid compositions of the different SBM products are shown in Table 2.

Purified products from soya bean seeds such as oligosaccharides and water soluble polysaccharides have been reported to have health benefits when fed to poultry and pigs. Soya bean seeds contain oligosaccharides in form of sucrose, raffinose and stachyose, which are non-reducing sugars made up of fructose, glucose and galactose and may account for up to 5% of the dry weight of the seed (Espinosa-Martos and Rupérez, 2006). Raffinose and stachyose are α-linked oligosaccharides (galactooligosaccharides) which are non-digestible carbohydrate for pigs and poultry and therefore transit through the gastrointestinal tract to large intestine (colon), where they are metabolised by microbes that are capable of producing α-galactosidase

needed for galactose metabolism. The metabolism of these oligosaccharides results in production of methane, carbon dioxide, hydrogen and volatile fatty acids and therefore are said to function as prebiotics. Reports from animal experiments have shown that these isolated products from soya bean seeds are effective as prebiotics and these products have also found wider application in human nutrition and are known as functional foods. It was observed in broilers that, soya bean meal oligosaccharides and water soluble polysaccharides promoted the growth of lactic acid bacteria communities in the caecum and had competitive exclusion function when chicken was infected with *Eimera tenella* (Lan et al., 2004). Other interesting compound in soya bean flour includes isoflavones or phytoestrogen, which are anti-oxidants (polyphenols) which have shown promise in preventing the spread of breast and prostrate cancer in human.

	Raw SBM	Heated SBM[2]	Steamed SBM[3]	Solvent extracted SBM	Protein isolate[4]	Protein concentrate[4]
Dry matter	895	960	890	894.7	920	900
Ash	57	58	57	75.3	-	-
Gross energy	22.59	22.60	22.98	21.2		-
ADF	88	-	-	115.5	-	-
NDF	216	-	-	352.0	-	-
ADL	28	-	-	42.0	-	-
Crude protein	412	413	411	496.3	858	640
Threonine	11.8	11.7	11.6	20.3	31.7	28.0
Valine	17.4	17.5	17.3	24.8	42.1	34.0
Methionine	2.0	2.0	1.9	5.1	10.1	9.0
Isoleucine	14.8	14.7	14.9	20.0	42.5	33.0
Leucine	21.2	21.2	21.1	33.8	66.4	53.0
Phenyalanine	14.6	14.7	14.5	25.0	43.4	34.0
Histidine	7.4	7.4	7.3	15.7	22.5	18.0
Lysine	20.4	20.5	20.3	30.3	52.6	42.0
Arginine	17.8	17.7	17.8	37.1	68.7	57.9
Aspartic acid	30.6	30.7	30.5	61.2	-	-
Serine	14.4	14.4	14.4	24.2	-	-
Glutamic acid	45.5	45.5	45.0	62.7	-	-
Proline	16.9	16.7	16.6	21.1	-	-
Glycine	16.4	16.4	16.0	23.3	-	-
Alanine	15.8	15.9	15.7	22.9	-	-
Tyrosine	7.4	7.5	7.2	12.8	25.0	18.2

[1] Results from our laboratory

[2] Subjected to 105°C for 30 min in an oven.

[3] Steamed at 0.1 MPa for 30 min in an autoclave.

[4] Adapted from NRC 1998 (SPI and SPC)

Table 2. Chemical compositions of soya bean meal products[1]

3. Amino acid composition and digestibility of soya bean meal

Soya bean meal contained highly digestible amino acid content for pig and poultry. Various terminologies are often employed in the description of digestibility in non-ruminant animal

nutrition. These terminologies are often related to site at which measurement was made and whether correction has been made for endogenous contribution or not. Not all the protein and other nutrients found in faeces and urine are from the feed, but some are from digestive secretions and sloughing off of the gastro-intestinal tissues (from digestive tract itself). Two endogenous sources are known at present which are those caused by the level of dry matter intake and animal factors known as basal endogenous loss and the one caused by the feed component which is known as specific endogenous losses. Due to difficulty involved in the measurement of specific endogenous losses (by isotope dilution technique), only basal endogenous losses is mostly measured in feed ingredient for pigs and poultry. When correction is made for basal and specific endogenous sources, digestibility values are said to be true (standardized) and real respectively, whereas when no correction has been made, digestibility values are said to be apparent.

There are also considerable microbial activities in the hind gut of poultry and pig (more activity in pig than poultry) due to microbial fermentation. These microbial activities usually lead to microbial protein synthesis, production of volatile fatty acids and other metabolites which are not available to the non-ruminant animals for absorption because this region has gone past the region of the small intestine where most of the nutrient absorption takes place. Therefore most animal nutritionists are of the opinion that the measurement of protein in faeces may be misleading and may not be a true picture of what was absorbed and the idea is therefore to make measurement at the end of the small intestine (distal ileum), where absorption is thought to be completed. Both ileal and faecal measurements can be described as apparent and true digestibility depending on if correction was made for endogenous contribution or not. True digestibility is often higher than apparent digestibility especially in high protein ingredients used commonly for pig and poultry feeding and similarly, ileal measurements are often higher than measurements made in the faeces for the same set of ingredients. The apparent ileal amino acid digestibility for swine is above 75% for amino acids lysine, methionine, threonine and tryptophan. These are very important amino acids because, these are the amino acid likely to be deficient in the ration of pigs and poultry based on common ingredients (corn-soya bean) used for poultry and swine ration formulation. The true faecal amino acid digestibility of the essential amino acids contained in soya bean meal for poultry is on the average higher than 85%. We have extensively characterised soya bean products and other protein sources commonly used for swine and poultry feeding for their chemical composition and digestible nutrient contents using the various digestibility techniques in our laboratory (Yin et al., 1991; Yin et al., 1993a; Yin et al., 1993b; Yin 1994; Yin et al., 1994; Yin et al., 2008). The apparent and standardized ileal digestibility coefficients of SBM products and other plant protein sources are presented in Tables 3-6. However, soya bean meal is deficient in sulphur amino acids (methionine and cysteine) and this is the first limiting amino acid in corn-soya bean meal for poultry. Synthetic methionine source (DL- methionine or methionine hydroxy analogue) is usually added to poultry diet to alleviate this deficiency of sulphur amino acids. The order of limiting amino acids in corn-soya bean meal diet for pigs is lysine, methionine, threonine and tryptophan, while that of poultry is methionine, lysine, threonine and tryptophan.

4. Anti-nutritional factors in soya bean meal

Soya bean meal like most other oil seed meal contain antinutritional factors such as protease inhibitors, phytic acid, allergens, lectins, saponins, antivitamins and phytoestrogens, which are

chemical substances used by plants for self defence against invasion of disease causing or foreign organism. These anti-nutritional factors mediate their effect in farm animals by reducing protein digestibility, toxicity, binding with nutrients and causing precipitations or producing un-absorbable chemical forms (unusable) and may negatively impair the development of the gastro-intestinal tract in young animals. Protease inhibitors (trypsin inhibitor) and lectin (haemagglutinin) have a negative effect on digestion, absorption, efficiency of utilisation and metabolism of protein and growth rate in simple stomach farm animals fed on raw or poorly processed (undercooked) soya bean meal. The presence of protease inhibitors often results in hypertrophy of the pancreas from over stimulation of this organ to increase gastric secretions. The protease inhibitors which can constitute as much as 6% of protein in soya bean seed can be classified into the heat labile Kunitz trypsin fraction and Bowman-Birk heat stable protease inhibitor fraction (Francis et al., 2001). The heat labile fraction is readily inactivated and its influence drastically reduced when soya bean meal is subjected to heat treatment. Their influences are more prominent, when raw unprocessed soya bean are fed to pigs and poultry. Moist heat treatments such cooking and steaming are most effective in inactivation or destruction of protease inhibitors and lectin in soya bean seeds.

	Dry matter	Organic matter	Gross energy	ADF	NDF	ADL	Crude protein
Raw SBM	81	85	80	31	52	-	59
Heated SBM	86	88	85	-	-	-	67
Steamed SBM	86	88	87	-	-	-	68
SBM	88	90	91	-	-	-	87
RSM	84	85	82	32	41	22	66
PNM	87	88	88	40	44	26	87
CSM	74	77	76	38	46	26	73
Jack bean[2]	84	85	85	16	64	9	54
Jack bean[3]	84	84	84	-	-	-	56
Field beans[2]	88	89	88	15	66	14	83
Field beans[3]	82	84		-	-	-	71
Peas[2]	90	91	90	35	73	29	85
Peas[3]	83	85	85	-	-	-	70
Mung bean[2]	86	88	86	25	64	32	74
Mung bean[3]	90	92	89	-	-	-	80

[1] Results from our laboratory
[2] Raw form
[3] Steamed at 0.1 MPa for 30 min in an autoclave.

Table 3. Apparent Ileal digestibility of soya bean meal and other plant proteins feedstuffs in pigs (proximate constituents) [1]

Evidence from previous studies with chicks revealed that about 15% of growth depression from protease inhibitors is from the effect of lectin (Douglas et al., 1999). This deduction was made on the basis that raw lectin-free soya bean meal had a higher nutritive value than raw soya bean meal, but less value compared kunitz trypsin inhibitors free soya bean meal, implying that trypsin inhibitor may be more toxic than the lectins, but the nutritive value of raw lectin-free and kunitz-trypsin free SBM is lower to that of heat treated SBM (Batal and Parson, 2003). Soya bean meal samples with less trypsin inhibitor content and higher crude protein content have been reported to result in better digestibility of gross energy, nitrogen and amino acids in young broilers (Coca-Sinova et al., 2008). Phytate and antivitamins (e.g.

antivitamins A, D, E, K, thiamine, riboflavin, pyridoxine and B12) affect mineral and vitamin utilisation in non-ruminant animals fed untreated soya bean meal. A large proportion of the phosphorus present in plant (60 - 80%) is in a complex form of myo-inositol hexaphosphate (Phytate). Pigs, poultry and other non-ruminant animals do not possess the digestive enzyme (phytase) in their gastrointestinal tracts that is needed to break down phytate and invariably most of the phosphorus in soya bean meal passes through the tract unused and is excreted in the manure. As a consequence of the poor digestibility, supplemental phosphorus was usually added to the diets of non-ruminant animals to meet the dietary requirements for optimal growth rate. There has been an outcry from the society to reduce phosphorus and nitrogen to the environment from livestock industry in the past years due to the adverse effect on the environment such as eutrophication of water bodies and contamination of ground water by excessive application of animal manure as fertiliser. The runoff from this fertiliser application has been implicated in destruction of aquatic ecosystem through algal blooms; depletion of oxygen and death of aquatic species. Swine manure has the largest concentration of phosphorus because of the addition of inorganic phosphorus in an attempt to supplement the non-digestible phytate.

	Thr	Val	Met	Iso	Leu	Phe	His	Lys	Arg	Asp	Ser	Glu	Pro	Gly	Ala	Tyr
Raw SBM	58	61	68	62	58	56	66	57	67	64	62	68	57	63	60	55
Heated SBM	76	66	62	76	70	73	75	77	89	83	81	83	84	70	70	75
Steamed SBM	79	78	69	73	80	80	76	80	90	84	84	87	66	76	74	82
SBM	83	85	85	87	87	87	88	86	94	89	85	89	84	83	82	82
SPI[2]	90	86	-	90	92	88	88	88	91	-	-	-	-	-	-	89
SPC[2]	85	91	91	93	93	94	95	93	97	-	-	-	-	-	-	93
RSM	72	73	83	76	76	74	80	72	78	72	74	82	68	72	70	66
PNM	79	60	75	75	73	80	80	76	92	89	85	90	47	84	76	86
CSM	66	67	72	68	70	79	70	50	86	71	69	91	69	68	62	69
Jack bean[3]	52	33	36	31	41	34	54	54	35	52	53	58	34	34	34	-
Jack bean[4]	66	58	60	62	61	70	57	72	75	69	74	71	59	57	54	-
Field beans[3]	74	75	65	79	81	71	86	85	89	84	81	88	84	65	74	-
Field beans[4]	71	72	-	65	77	66	58	80	91	82	77	86	-	66	71	64
Mung bean[3]	78	77	63	69	80	76	76	73	70	82	86	86	67	63	73	-
Mung bean[4]	77	79	77	74	80	85	82	89	88	86	86	89	79	80	76	74
Peas[3]	81	89	77	81	83	81	82	81	87	88	88	92	77	77	84	83
Peas[4]	80	82	78	73	80	70	71	86	93	83	83	91	70	75	81	88

[1] Results from our laboratory
[2] Adapted from NRC 1998
[3] Raw form
[4] Steamed at 0.1 MPa for 30 min in an autoclave.

Table 4. Apparent Ileal digestibility of soya bean meal products and selected plant proteins feedstuffs in pigs (amino acids) [1]

	CP (PF)	CP (EHC)	Arg (PF)	Arg (EHC)	His (PF)	His (EHC)	Ile (PF)	Ile (EHC)	Leu (PF)	Leu (EHC)
Raw SBM	61	63	68	69	68	69	64	65	60	61
Heated SBM	69	71	90	91	77	78	78	79	72	73
Steamed SBM	70	72	91	92	78	79	75	76	82	83
Soya-bean meal I (501 g kg-1 DM)	84	85	82	82	86	87	83	83	80	81
Soya-bean meal II (477 g kg-1 DM)	NA	NA	95	95	89	89	91	92	89	89
Soya-bean meal III (511 g kg-1 DM)	89	90	95	96	89	90	86	87	88	90
Jack bean[2]	59	62	37	38	56	57	34	35	43	44
Jack bean[3]	61	64	77	78	59	60	65	66	63	65
Field beans[2]	87	89	90	91	88	89	82	82	83	85
Field beans[3]	75	77	92	93	60	61	68	68	79	81
Mung bean[2]	78	79	72	73	78	80	72	73	82	84
Mung bean[3]	84	85	90	91	84	86	77	78	82	84
Peas[2]	89	91	88	89	85	87	83	84	86	87
Peas[3]	74	76	95	97	74	76	75	76	83	84

[1] Results from our laboratory

[2] Raw form

[3] Steamed at 0.1 MPa for 30 min in an autoclave.

Table 5. Standardized essential amino acid ileal digestibility of soya bean products and other plant protein sources determined by protein-free (PF) or enzyme-hydrolyzed casein (EHC) methods in growing pigs[1]

The advance in breeding techniques has led to identification of the gene responsible for phytate in soya bean and this has led to the breeding of low phytate soya bean by blocking the synthesis of phytate through the use of mutant genes without any loss in yield and the amount of phosphorus present in soya bean. Phytate can be rendered more digestible through heat treatment or through the supplementation of phytase in feed of non-ruminant animals. The anti-vitamins interfere with the absorption and/or metabolism of their respective vitamins, but most are readily destroyed by moist heat treatment used for processing of soya bean meal. Sucrose, raffinose and stachyose are the main α-linked oligosaccharides in soya bean meal. These oligosaccharides and other water soluble polysaccharides are also known to be anti-nutrient when present in soya bean meals

products, because they cause flatulence, muscular cramps, diarrhoea, reduce the true metabolisable energy and fibre digestion in non-ruminant animals, whereas isolated products of these polysaccharides have also found application in functional food in animal and human nutrition as a prebiotics.

	Lys (PF)	Lys (EHC)	Met (PF)	Met (EHC)	Phe (PF)	Phe (EHC)	Thr (PF)	Thr (EHC)	Val (PF)	Val (EHC)
Raw SBM	59	60	73	81	58	59	62	64	63	65
Heated SBM	79	80	67	75	75	76	80	82	68	70
Steamed SBM	82	83	74	82	82	83	83	85	80	82
Soya-bean meal I	86	87	78	82	80	81	80	81	78	79
Soya-bean meal II	87	88	91	95	88	89	88	89	91	92
Soya-bean meal III	87	88	81	91	88	89	83	84	81	82
Jack bean[2]	56	58	47	67	36	37	59	62	59	76
Jack bean[3]	74	76	70	88	72	73	73	76	88	107
Field beans[2]	88	89	68	75	72	73	83	86	78	81
Field beans[3]	83	84	NA	NA	68	69	80	84	76	78
Mung bean[2]	77	79	65	67	77	78	86	90	80	82
Mung bean[3]	93	95	79	81	86	87	86	90	82	84
Peas[2]	84	85	83	94	83	85	89	92	94	97
Peas[3]	89	91	84	96	72	74	88	92	87	90

[1] Results from our laboratory

[2] Raw

[3] Steamed at 0.1 MPa for 30 min in an autoclave.

Table 5. continued. Standardized ileal digestibility of crude protein and essential amino acid of soya bean products and other plant protein sources determined by protein-free (PF) or enzyme-hydrolyzed casein (EHC) methods in growing pigs[1]

	Lys	Met	Thr	Trp
Amino acid (%)	2.98	0.69	1.89	0.61
Digestibility coefficient	91	93	90	-
Digestible amino acid (%)	2.71	0.64	1.70	-

[1] Adapted from Aminodat 1.1, Degussa AG, Germany.

Table 6. Standardized ileal digestibility of soya bean meal (48% CP) in poultry[1]

These antinutritional factors have similar effect when they are found in notable amounts in other feedstuffs especially oil seed legumes and are not limited to soya bean seeds alone. Readers are referred to the reviews on antinutritional factors in oil seed meal of Francis et al.

(2001) and Tacon (1997) for in-depth study of the subject matter. The effect of moist heat treatment on trypsin inhibitors, urease activity and FDNB- available in soya bean is presented in Table 7.

Time (minutes)	0 min.	5 min.	10 min.	15 min.	25 min.
Urease index	2.18	0.00	0.00	0.00	0.00
Trypsin inhibitors (mg/g)	20.04	2.19	2.28	1.58	1.52
FDNB-available lysine[2]	27.80	23.53	19.52	17.68	18.10

[1] From our laboratory
[2] 2, 4-dinitro-fluorobenzene and the free-amino acid group of lysine

Table 7. Influence of autoclaving time at 125C and 0.1MPa pressure on the urease activity, trypsin inhibitors and FDNB-available lysine content (g/kg DM) in raw SBM[1]

As shown in Table 1, the beans list in the table all of them contains non-starch polysaccharide (NSP). Recently, we found that the N in ileal digesta or feces have been found mainly from the endogenous microorganisms N   (Fig. 1). Endogenous N losses, especially those related to microorganisms, in ileal digesta and feces were found to be the major reasons of low efficiency of dietary protein and environmental pollution of N in the pig industry. Further studies we have found that the soluble NSP, rather than the insoluble NSP, is the main factor that causes endogenous N secretion. However, using of the soluble NSP enzyme can improves pig performance through the degradation of soluble NSP in the intestine of pigs and by reducing the loss of endogenous AAs in the small intestine in pigs (Yin et al., 2000a,b; Yin et al., 2001a,b ;Yin and Tan, 2010).
Yin YL and Tan BE, Manipulation of dietary nitrogen, amino acids and phosphorus to reduce environmental impact of swine production and enhance animal health. Journal of Food, Agriculture & Environment. 8: 447-462 (2010).

5. In vitro quality assessment of soya bean meal products for livestock feeding

Three standard methods are routinely used for the in-vitro assessment of the protein quality of SBM for livestock feeding. These include urease test, protein dispersibility index (PDI) and protein solubility methods. Protein dispersibility index and protein solubility are hinged on the solubility of SBM in water and alkali respectively. Soya bean meal product is usually added to water or potassium hydroxide solution and centrifuged over a period of time and the amount of dissolved nitrogen determined in the supernatant. PDI or protein solubility is then expressed as the percentage of the protein present in the initial dry soya bean product. Over-heating of SBM during processing is known to depress the values of

both indices. Urease index which is used to determine residual activity of antinutritional factors in heat treated SBM by observing pH increase. This is used as an indicator for the detoxification of heat labile anti-nutritional factors in processed SBM especially trypsin inhibitor and lectins. This is based on the fact that these heat labile antinutritional factors are more readily inactivated by heat than urease, which is more heat tolerant, but its use may be limited in detecting overcooking in SBM products. The solubility of soya protein in potassium hydroxide for adequately processed SBM product ranged from 70-85%, while higher solubility often indicate lack of or inadequate heat processing and values below the lower threshold often portends excessive heat processing (Araba and Dale, 1990a&b; Lusas and Riaz, 1995; Batal et al., 2000).

6. Use of soya bean meal in non-ruminant animal

Corn-soya bean meal is the industrial standard for feeding pigs and poultry world wide. The inclusion level of soya bean meal and the type of soya bean meal product fed to non-ruminant animal varied, depending on the breeding objective of the livestock enterprise. The inclusion rate of soya bean product in the feed of various categories of non-ruminant animals will vary depending on the crude protein requirement of the target specie. Starting broilers have a crude protein requirement of 23%, while growing pigs have crude protein requirement of 18-20%, therefore theoretically, since soya bean meal can supply all the protein need along with corn, the inclusion level of soya bean meal in the diet of broiler should be higher than that of growing pigs. It is a common practice for soya bean meal to be used as the sole source of protein alongside with corn especially in diets of young non-ruminant animals. The choice of product to be fed is also dependent on the rearing objective. For instance, full fat soya is a good choice of feeding broilers and weanling pigs due to their high energy requirements, but may not be a choice product for feeding laying hen and gilts due to its high fat content and the tendency for these animals to deposit extra energy in form of fat that may interfere with their reproductive performance. It is a well known fact that fat deposition is directly related the amount of fat present in the diet. However, the decision on how much of a particular ingredient to include in the ration of animal is not only a biological decision, but also an economic decision, because the objective of farming enterprise is foremost to maximise profit, and the level of biological performance at which profit can be maximised may not necessarily be the highest level of performance. Therefore the level of inclusion of these products in non-ruminant feeding is not static, but is driven by cost of other ingredients available for ration formulation, which can serve as partial replacement for these soya bean meal products, and the level at which animals are expected to perform or the intended target weight or performance level expected from a stock. This is known as least cost ration formulation for livestock, in which farm animals are furnished with the required nutrient at the most economic rate using a combination of ingredients to achieve this. Technically, farm animals have no need for any specific ingredient, but can thrive on a combination of ingredients provided this is skilfully combined to provide the needed nutrients based on the knowledge of nutrient requirement of the animal for the particular set of nutrients, bioavailability of nutrients from the sources combined, price of various ingredients and the biology of the animal in question. Soya bean meal however, has a comparative

advantage over other protein ingredient for farm animal feeding due to its amino acid composition and high level of bioavailability of its amino acid.

Use in pre-ruminant and ruminant feeding
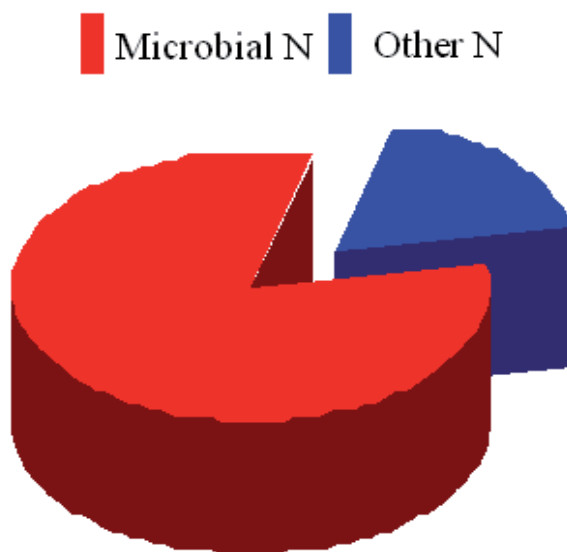
Fig. 1. Faeces N compistion

Soya bean meal is known to contain allergenic or antigenic factors such as α-conglycinin, and β-conglycinin which are known to trigger specific and non-specific immune response in several farm animals, but especially in a large proportion of pre-ruminant calves (Sisson, 1982). The feeding of soya bean products in some pre-ruminant calves were linked with intolerance, reduction in digesta transit time, vomiting and diarrhoea, failure of immuno-defence mechanism of the gut, low secretion of immunoglobulins such IgA and IgM against soya bean antigens, and rapid loss of weight. The presence of *β*-conglycinin in soya bean meal depressed digestibility in young calves and has more potent depressive effect on protein digestibility than trypsin inhibitor (Lalles et al., 1995). However, these immunologically active globulins were not detected in soya protein concentrate produced by leaching with aqueous alcohol, implying their inactivation. Similarly, pre-ruminant calves just like their non-ruminant counterparts have no digestive enzymes for soya

oligosaccharides sucrose, raffinose and starchyose and these oligosaccharides are known to produce similar effect in pre-ruminant calves as they do in non-ruminant counterparts. However, these oligosaccharides are eliminated during the leaching process for the production of SPI and this makes SPI the most suitable SBM product for feeding pre-ruminant calves.

7. Conclusion

Due to the high demand for soya bean meal and its products for livestock feeding, which is set to continue in the future, there is therefore the need to continue to research many ways of improving the efficiency of nutrients utilization in SBM. The area of interest for animal nutritionists will how to improve processing methods and to develop new products that will be suitable for various classes of livestock, reduce the indigestible carbohydrate content, minimise residual anti-nutritional factors and improve nutrient digestibility, which is the main determinant of nutrient availability to livestock species.

8. References

Anonymus 2004. Soybean meal processing http://www.soyplus.com/bulletins.asp accessed on 22/11/2010

Araba, M., N. M. Dale (1990a). Evaluation of protein solubility as an indicator of overprocessing soybean meal. *Poult. Sci*. 69, 76–83.

Araba, M. and N. M. Dale (1990b). Evaluation of protein solubility as an indicator of underprocessing of soybean meal. *Poult. Sci*. 69, 1749–1752.

Batal A. B. and C. M. Parsons (2003). Utilization of different soy products as affected by age in chicks. *Poult Sci*. 82:454–462

de Coca-Sinova A., D. G. Valencia,E. Jiménez-Moreno,R. Lázaro and G. G. Mateos (2008). Apparent Ileal Digestibility of Energy, Nitrogen, and Amino Acids of Soybean Meals of Different Origin in Broilers Poultry Science 87:2613–2623 doi:10.3382/ps.2008-00182

Douglas, M. W., C. M. Parsons and T. Hymowitz (1999). Nutritional evaluation of lectin-free soybeans for poultry. *Poult. Sci*. 78:91–95.

Espinosa-Martos I. and P. Rupérez (2006). Soybean oligosaccharides. Potential as new ingredients in functional food. *Nutr Hosp*. 21(1):92-6

Francis G., H. P.S. Makkar, K. Becker (2001). Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish *Aquaculture* 199: 197–227

Lan Y., B.A. Williams, M.W.A. Verstegen, R. Patterson and S. Tamminga (2007). Soy oligosaccharides *in vitro* fermentation characteristics and its effect on caecal microorganisms of young broiler chickens *Animal Feed Science and Technology* 133: 286–297

Lan, Y., S. Xun, S. Tamminga, B. A. Williams, M. W. A. Verstegen, and G. Erdi (2004). Real-Time PCR detection of lactic acid bacteria in cecal contents of *Eimeria tenella*-Infected broilers fed soybean oligosaccharides and soluble soybean polysaccharides. *Poultry Science* 83:1696–1702

Lalles J.P., H.M. Tukur, R. Toullec and B.G. Miller (1996). Analytical criteria for predicting apparent digestibility of soybean protein in preruminant calves. *Journal of Dairy Science* 79: 475-482 Abstract access this article

Lusas E. W. and M. N. Riaz (1995). Soy Protein Products: Processing and use J. Nutr. 125: 573S-580S

Pond WG, DC Church, KR Pond et al. (2005). Basic animal nutrition and feeding. Fifth edition. John Wiley & Sons, Inc, Danvers, USA.

Sisson J. W. (1982). Effects of soya-bean products on digestive processes in the gastrointestinal tract of preruminant calves. *Proc. Nutr.* Soc., 41, 53

Tacon, A.G.J., 1997. Fishmeal replacers: review of antinutrients within oilseeds and pulses — a limiting factor for the aquafeed Green Revolution? In: Tacon, A., Basurco, B. ŽEds.., Feeding Tomorrow's Fish. Proceedings of the Workshop of the CIHEAM Network on Technology of Aquaculture in the Mediterranean ŽTECAM., Jointly Organised by CIHEAM, FAO and IEO, 24–26 June 1996, Mazarron, Spain. Cahiers-Options-Mediterraneennes, vol. 22, pp. 153–182.

Vasconcelos I. M., I. C. F. Brasil, J. T. A. Oliveira, C. C. Campello, F. M. M. Maia, M. V. M. Campello, D. F. Farias and A. F. U. Carvalho (2009). Combination of Chemical Analyses and Animal Feeding Trials as Reliable Procedures to Assess the Safety of Heat Processed Soybean Seeds. *J. Agric. Food Chem.* 57: 4668–4673 DOI: 10.1021/jf803903h Abstract

Woodworth J. C., M. D. Tokach, R. D. Goodband, J. L. Nelssen, P. R. O'Quinn, D. A. Knabe, and N. W. Said (2001). Apparent ileal digestibility of amino acids and the digestible and metabolizable energy content of dry extruded-expelled soybean meal and its effects on growth performance of pig. *J. Anim. Sci.* 79:1280–1287

Yin Y. L., Huang R. L., Zhong H. Y., Chen C. M., and Dai H. (1991). Influence of different cannulation techniques on the pre-cecal digestibility of protein, amino acids and cell wall constituents from diets, containing different protein meal, in pigs. *Animal Feed Science and Technology* 35:271-281

Yin Y. L., Huang R., and Zhong H. Y. (1993a). Comparison of the ileorectal anastomsis and conventional method for the measurement of ileal digestibility of protein sources and mixed diets in growing pigs. *Animal Feed Science and Technology* 42: 297-308

Yin Y. L., Zhong H. Y., Huang R. L., Chen C. M., Li T. J., and Pai Y. F. (1993b). Nutritive value of feedstuffs and diets for pigs. I. Chemical composition, apparent ileal and fecal digestibility. *Animal Feed Science and Technology* 44:1-27.

Yin Y. L. (1994). Nutritive value of feedstuffs and diets for pigs, II. Apparent post-ileal digestibility and interrelationship between dietary constituents and fecal and ileal digestibility. *Animal Feed Science and Technology* 45:243-255

Yin Y. L., Chen C. M., Zhong H. Y. and Huang R. L. (1994). Digestibility of energy, cell wall constituents, crude protein and amino acids of the Chinese oil seed meals for pigs. *Animal Feed Science and Technology* 45: 283-298

Yin Y. L., Li T. J. and Huang R. L. (2008). Evaluating standardized ileal digestibility of amino acids in growing pigs. *Animal feed Science and Technology* 140:385-401

Soybean and Allergy

Keiji Sugiura and Mariko Sugiura

Department of Environmental Dermatology and Allergology, Daiichi Clinic
Japan

1. Introduction

Food allergies can affect both people and animals, and the number of food allergies not only in human but also in animals has increased. Food allergies can affect a patient's quality of life and lead to a loss of pleasure in eating. There are many kinds of food in the world, and it is possible for all people to have food allergies. The kinds of food allergy differ depending on country, nationality, age, and religion. For example, peanut allergy is common in western countries, but in Asia, it does not occur as frequently. And the incidence of egg allergy in children is higher than that in adults. Not all food allergies in childhood continue into adulthood; in some cases, the symptoms often improve with age, so-called allergy march. In case where food is restricted by religion, the number of its foods allergy must be rare. Food allergies of childhood have close relation to growth and development; this is a serious problem for not only patients but also their families and educational institutions. In some countries, information regarding and measures against food allergy are inadequate. Because religion, economy, education, and nationalities are different, addressing these inadequacies will require large amounts of time and effort. Overcoming food allergy is difficult, but food allergy of childhood often improve with grow up. There are some puzzling aspects of food allergy in childhood; some contaminated foods, plural food allergies and foods that are impossible or difficult to avoid (ex. flour). It needs to measure for people with food allergy, especially, in case of disaster and war. Regardless of religion, economy, education, or nationality, children cannot easily avoid allergens. Their families and other related people must maintain their living environment, and it is important to prepare some kinds of foods without allergens in these cases. Food allergies in adults often appear as a result of occupational problems. Our study showed that some cases of occupational disease were caused by foods; these included a patient working in a bakery with a flour allergy, a green grocer with a vegetable and fruit allergy, a noodle shop worker with a flour allergy, a cook with a fruit allergy, etc (Sugiura K & Sugiura M. 2010a). Occupational food allergies affect a patient's life and occupation. The avoidance of foods that cause allergic reactions during only meals can not resolve the problem of occupational food allergy; if other workers in the same office do not understand the allergy, a patient's symptoms are going to progress, finally food allergy causing them to resign from their work. Economic and productivity loss due to food allergy are huge; a response is therefore needed to problems of allergy in the office and on social ground (Sugiura K & Sugiura M. 2010a).

Recently, the number of pets with food allergies has been increasing. Basically, food allergy in pets is similar to that in humans with regard to symptoms, examinations, treatment, and safety measures. Because animals do not speak, it is often difficult to diagnose animal food allergy. Most of the food allergies in animals show as red spots, scratching, runny nose,

congestion, wheezing, and so on. A previous report showed that dogs with adverse reactions to foods suffer from dermatological symptoms (Proverbio D,et al. 2010).   If pets repeatedly show some symptoms or do not recover from these symptoms, they should be examined in a veterinary clinic.

Soybean is one of the most consumed grains in the world, and soybean allergy is a very important problem in the medical and food fields. Soybean allergy is a subject that has been investigated by many scientists. However, soy sauce allergy without soy and flour allergy have not been discussed. We have previously reported four cases of soy sauce allergy (Sugiura K & Sugiura M. 2010b), and at that time we recognized that the topic of soy sauce allergy must be studied. Our theme is soybean and allergy. We explored this theme here-in based on three categories: general information about allergies, allergies to soybean products, and soy sauce allergy. We also approached and provided general information about allergies and food allergy, including the different kinds of allergy, the causes of allergic reactions, the diagnosis of allergies and treatment, soy products allergy and cases of soybean allergy and soy sauce allergy, including a discussion of examination tests, treatment, and safety measures. The globalization of manufacturing and production of materials that include soybean products has grown, and consumption of soy will continue to increase all over the world, because soybean products are generally healthy and have good effects on the human body; people are interested in these products. In the case of production and manufacturing occurring in different countries, the problems of food allergies are complicated. The reasons for this complication are, the kinds of food allergy, the ratio of people with food allergies and the different criteria regarding the listing of ingredients on packaging, all being different depending on the country, people, and religion. Many food companies use foreign factories for manufacture because of cutting personnel costs. These companies need to educate the foreign people working at these factories about issues regarding to food allergy. An international standard for the labeling of ingredients could be useful for patients, letting people know whether it is safe to eat foods in foreign countries. The methods or procedures for treating food allergies are different depending on the physician, institute and country. International criteria for the labeling of ingredients, not only soybeans but also other foods products, on package, as well as for the diagnosis and treatment of food allergies are needed, because tradition, culture and customs related to food are different. First, we describe the basics of allergy and food allergy, then discuss soy sauce and soy sauce allergy.

2. Allergy

2.1 Introduction

The term 'allergy', coined in 1906, derives from two Greek words: *allos,* meaning "foreign" or "strange", and *ergo*, meaning "to act" (von Pirquet, C. 1906). Allergies are complicated immunological reactions and appear as morbid symptoms in individuals who suffer from them. Allergic symptoms are shock, urticaria, skin rash, contact dermatitis, asthma, nephritis, etc. The mechanisms of allergy have been studied, but they have not been completely analyzed and defined. Here, we described the mechanisms of allergy as they have been illuminated thus far. Allergies are caused by many cells and mediators, and the main trigger of allergies is exposure to allergens. An allergen is a specific material which induces an allergic reaction in some individuals, and varies depending on the case. Allergic reactions are often caused not by just one allergen but by plural allergens. All materials in

the world have the potential to be allergens; generally, the higher frequently of contact or exposure to the materials, the more likely they are to become allergens. Allergic reactions often affect quality of life, childhood growth and development, occupation, economy, morale, etc. Economic loss due to occupational allergy is a huge issue; more attention needs to be given to allergy-preventive measures (Sugiura K & Sugiura M. 2010a).

2.2 Mechanisms of allergies

Allergic reactions have been divided into five types based on pathophysiology. Type I allergies is mediated by IgE antibody (immediate allergy). Type II is antibody-mediated (cytotoxic) allergy. Type III is immune complex allergy. Type IV is cell-mediated allergy (delayed-type allergy). Type V allergies is mediated by antibody against receptors. Type I allergy is often identified as food allergy. This immediate-type allergy is described in a later section. First, we present immunoglobulin II, III, IV and V type allergies. There are various factors essential to allergic reactions; here, we discuss the involvement of immunoglobulin, antigen, eosinophils, macrophages, basophiles, Langerhans cells, lymphocytes, immuno-complex, etc., in the various allergic processes.

2.2.1 Immunoglobulin (antibodies)

Immunoglobulin play essential roles in allergic reactions and immune responses to infections by viruses, bacteria, microbes, etc. When vertebrates are exposed allergens, bacteria, microbes, etc., they produce an antibody, which is a glycoprotein, to protect themselves. These antibodies are always present in the tissue; if we are exposed to or infected with these foreign materials, they respond to the invasion. Antibody has three functions: agglutination, opsonization and neutralization. Antibodies bind allergens (antigens) to form antigen-antibody complexes (agglutination). Macrophages easily recognize and phagocyte these complex via the Fc region of the antibody (opsonization), because macrophages understand these complexes as non-self. The macrophages present the antigen's information to the T cells, and further immunological reactions develop. T cells present the antigen's information to B cells, which produce new, specific antibodies. Therefore, antibodies activate complements, the activated complements bind antigens and assist in further opsonization. When bacteria infect tissue, the activated complements destroy the bacterial cell membranes. The binding of bacteria with activated complements results in bacteriolysis and loss of infectious properties. Antibodies play some essential roles in immunological or allergic reactions. In the case of venom (ex. insect, snake), venom-binding antibodies do not exercise toxicity and do not invade tissue or cells (neutralizing).

There are five kinds of antibodies: IgA, IgD, IgE, IgG and IgM. Antibodies have a basic y-shaped structure, composed of Fc and Fab regions (Figure 1). Fc is the crystallizable region. Some cells (ex. mast cells, macrophages) have Fc receptors; these cells recognize and phagocyte via this region. The Fc region play roles in the immunological systems; this region is essential for the three functions of antibodies, i.e., agglutination, opsonization and neutralization. Fab is the antigen-binding region, which binds to the allergen. Antibody can also be divided into a variable and constant region. The variable region differs depending on the target antigen; because the arrangement of amino acid in the Fab region can change, B cells can produce an antibody against each antigen. The constant region is consistent

across all immunoglobulins. The structures of the five antibody types are shown in Figure 2: IgD, IgE and IgG are monomers, IgA is a dimer, and IgM is a pentamer.

Immunoglobulin A (IgA) plays important roles in the immune systems of mucous membranes, its molecular weight is 170 kDa. This antibody has two sub-classes, and this antibody comprises about 15 % of immunoglobulins. The half-life of this antibody is 7 days. Secretary type IgA transits via the colostrum (first mother's milk after birth), and protects the digestive tracts of newborns from bacteria, viruses, etc. Giving colostrum to newborns is important from a medical viewpoint. The structure of IgA is a dimer of immunoglobulin (Figure 2).
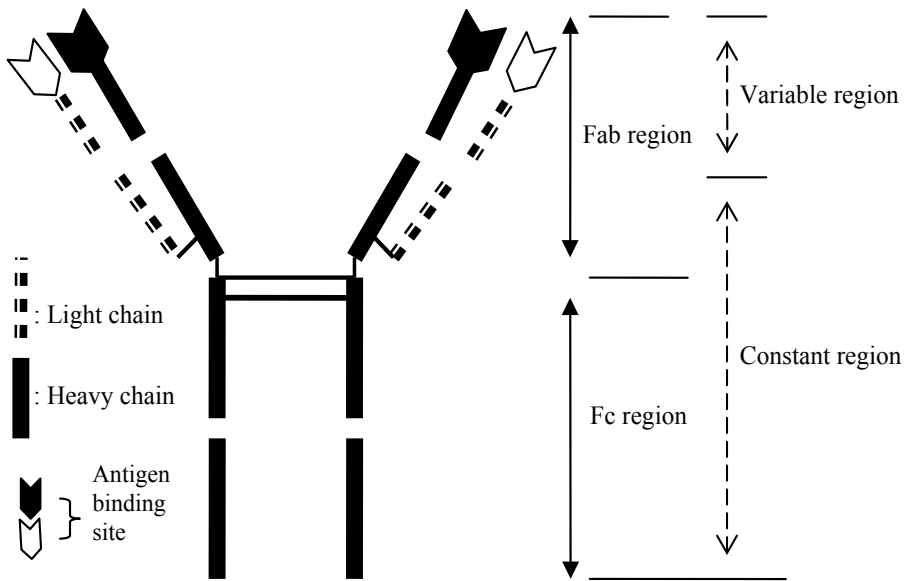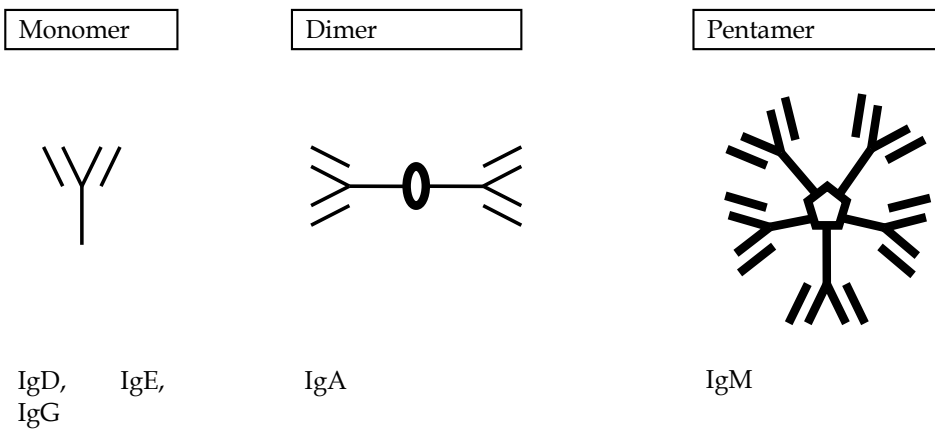
Fig. 1. The structure of an antibody

Fig. 2. Structures of the five types of immunoglobulin

Immunoglobulin D (IgD) is on the surface of B cells, and this antibody comprises less than 1% of immunoglobulin. Its molecular weight is 180 kDa and its half-life is 2.8 days. The significance of this antibody is unknown, but IgD is often measured and increased in cases of myeloma and monoclonal gammopathy. This antibody is a monomer. As monomers, IgE and IgG have essentially the same structure (Figure 2). Immunoglobulin G (IgG) comprises about 75% of immunoglobulins, and has a molecular weight of 150 kDa (Figure 2). This antibody has four sub-classes. The half-life of this antibody is 25 days. Only this antibody has the ability to pass through the placenta. Mother's IgG goes to the baby via transitional milk (after colostrum, but before mature milk), and this antibody plays essential roles in the immunity of newborns. About 2-3 months after birth, the level of this antibody in mother's milk drops, and newborns begin to produce it themselves. IgA and IgG are essential for the immunity of newborns. Immunoglobulin M (IgM) has a pentamer structure (Figure 2) and a molecular weight of 900 kDa, making it the largest immunoglobulin. Its half-life is 5 days. The level of this globulin is higher in females than males. When people are exposed to allergens, IgM is the first antibody produced in the body. Only IgG and IgM have the function of activating complements; other antibodies—IgA, IgD and IgE—cannot do this.

IgE was discovered by Ishizaka (Ishizaka K & Ishizaka T. 1967). This antibody has a molecular weight is 200 kDa and a half-life of 2 days. As mentioned above, we will describe the role of the IgE antibody in type I "food" allergy last, at the end of the next section. Only IgG and IgM can activate complements, other antibody, IgA, IgD and IgE can not do this.

2.3 Types of allergic reaction
2.3.1 Type II allergy
Type II allergy is specific reactions when IgG and IgM are activated against allergens bound to the individual's own cells; thus, the lymphocytes attack the body's own cells. The diseases of type II allergies include idiopathic thrombocytopenia (ITP), malignant anemia, rheumatism, Good pasture's syndrome, myasthenia gravis, etc.

2.3.2 Type III allergy
The mechanism of type III allergy is that immune complexes are deposited on the tissue; then the tissue with these complexes is damaged by the activated complements. There are two mechanisms for type III allergies, arthus reactions and serum sickness.   Arthus reactions are caused in a local area whereas serum reactions result in systemic disorder. The diseases of this allergy are hypersensitivity pneumonitis, arthus reaction, systemic lupus erythematosus and poststreptococcal acute glomerulonephritis.

2.3.3 Type IV allergy
Type IV allergy is a delayed-type hypersensitivity in response to foreign allergens. Important cells in this immune process are M cells in the intestinal mucosa and Langerhans cells (LCs) in the skin. In the case of digestive organs, exposure to allergens occurs via the mucous membrane. The mechanism of delayed-type hypersensitivity reactions has been proven: when people are exposed to foreign allergens, M cells in the Peyer's patches capture the antigens and deliver them to antigen-presenting cells (APCs), and these APCs present the antigen's information to T lymphocytes (T cells). T cells contact B lymphocytes (B cells), and B cells produce antibodies which react to specific allergens. Various kinds of antibodies and chemical mediators are produced which attack tissue, and inflammation cells (eosinophils, lymphocytes, monocytes, mast cells, plasma cells, etc.) cluster at the exposure

points due to signals from these chemical mediators. These mediators, antibodies and cells attack the antigens and organs, and the attacks appear as delayed hypersensitivity reactions. Peyer's patches, located at the lower ileum, comprise the immune system of the mucous membranes of the gastrointestinal tract.

The human skin is also exposed to allergens; one cutaneous APCs is Langerhans cells (LCs), which present in the epidermal layer. LCs capture antigens and present the antigen's information to the cell surface. LCs are capable of movement; they migrate from the skin to lymph nodes via lymph vessels with (Sugiura K, et al. 2003). Mature LCs, known as Birbeck granules, have an interesting and unique character. There are two kinds; tennis racket-shaped or coffee-bean shaped atypical granules (tennis racket-shaped granules can become coffee-bean shaped granules) (Shamoto M. 1970). Mature LCs migrate and present the antigens' information to T cells. T cells contact B cells, and B cells produce antibodies against the specific allergens. These antibodies cause delayed hypersensitivity reactions at the skin. Our study showed that LCs started migration one hour after exposure to allergens. Allergic contact dermatitis, sarcoidosis, multiple sclerosis, etc. are type IV allergy diseases.

2.3.4 Type V allergy

In type V allergy, antibodies against certain receptors attack (stimulate) the body's own cells having these receptors as ligands, and then these cells secrete mediators (ex. thyroid hormone). Self-antibodies acts like the allergen and combine ligand. Grave's disease is based on type V allergy.

2.3.5 Type I allergy

Food allergies comprise the type I allergy, which are signaled by (immediate IgE-mediated reactions such as urticaria. Type I allergy symptoms occur within 30 minutes of exposure and often threaten a patient's life (ex. anaphylactic shock). The mechanisms of IgE-mediated allergic reactions have been demonstrated: when people are exposed to foreign allergens, specific T cells against allergens produce IL-4 cytokines, which causes B cells to produce a specific IgE antibody against the particular allergen. When people are exposed to allergens again, mast cells and basophiles with IgE receptors (FcεRI), monocytes, lymphocytes and platelets with IgE receptors (FcεR II) bind with specific IgE antibodies and allergens, then these cells degranulate, liberating chemical mediators. Chemical mediators cause allergic reactions (permeability of blood vessels, twitching of smooth muscle, etc.), which appear within 10-30 minutes from exposure to allergens. There are many cases of IgE abnormalities: allergy, parasite infection, liver disorder, collagen disease, nephritic syndrome, chronic lymphocytic leukemia, etc. IgE antibodies attack antigens and the body's own cells, and immunological reactions increase with more and more exposure; these attacks often manifest as allergic reactions.

3. Food allergy

3.1 Introduction

Adverse reactions to foods are classified into two categories: food allergies and food intolerance (Cianferoni A & Spergel JM. 2009). These categories are divided into two and three subcategories, respectively. The frequency of food allergy has been reported, with varying results in different countries; the prevalence rate of food allergy is about 5% in

three-year-old children in Japan (Ebisawa M. 2006); the prevalence in children was reported to be 3-5% in France (Kanny G, et al. 2001) and 3.5-4 % in the USA (Sicherer SH, et al. 2004); 6 % of one-year-olds in Iceland were reported to have food allergy (Kristinsdóttir H, et al. 2011) and 11 % of Ghanaian school children were reported to develop adverse reactions to foods (Obeng BBet al. 2010). In the case of delayed-hypersensitivity foods allergy, because the frequency of this food allergy is lower than that of the immediate type. A frequent clinical symptom of food allergy is eruptions on the skin, which cause itching or irritation. Food allergies are classified as IgE-mediated allergies, non-IgE-mediated allergies, and mixed allergies. Food allergies are often caused by IgE-mediated and most food allergies are immediate allergy. The symptoms occur within 15-30 minutes of exposure to allergens. Most food allergens are glycoproteins, which have molecular weights ranging from about 10,000 to 60,000. The properties of these allergens are heat stability and resistance to proteolysis enzymes. If these allergens are cooked or digested, most of their antigenic qualities persist and cause allergic reactions. Japanese food allergy management guidelines is classified 5 categories; newborn infant's digestive symptoms, infantile atopic dermatitis associated with food allergy, an immediate-type reaction, food-dependent exercise-induced anaphylaxis (FDEIA)(Kidd JM 3rd, et al. 1983) and oral allergy syndrome (OAS) (Kondo Y & Urisu A. 2009). These allergies are related with IgE-mediated allergy. Generally, the antigenic quality of heated foods is lower than that of raw foods. Anyone who is concerned about having a food allergy or develops gastro-entero disturbances should eat heated foods.

When a food allergy is diagnosed, differential diagnoses should be considered, especially food intolerance. Food intolerance is characterized by the interaction between foods that contain chemical agents and a host who does not have the ability to resolve them. Food intolerance is not an immunological reaction, which is caused by food contents such as pharmacological active agents (ex. histamine, serotonin and caffeine), salicylic acid compounds (ex. tomato, potato and strawberry), or food additives (ex. paraben and benzoic acid) and by a characteristic of the host such as a metabolic or psychological abnormality (ex. lactose intolerance). Adverse effects due to soy sauce should be caused by soy sauce allergy and soy sauce intorelance. Histamine is related with both soy sauce allergy and soy sauce intorelance. For the diagnosis of a food allergy, the serum-specific IgE levels must be investigated, and skin test (prick test, scratch test or patch test) and oral challenge test must be performed. A diagnosis can be made based on the results of the examination, a clinical examination, the patient history (past history, present history and family history) and the patient's symptoms. Serum IgG is often not measured, because IgG is not always useful for the diagnosis of food allergy. Prick and scratch tests are used to diagnose immediate allergies, and these tests are performed on the forearm or back. After 20 minutes of prick or scratch test, physicians evaluate the results using their own criteria. However, the evaluation of prick and scratch tests is done differently depending on the physician, institute and country, these differences can cause confusion among physicians and patients, especially with respect to the prick test. If the prick test is performed using foods containing histamine or serotonin, these agents could modify some results of the prick test, and the prick test should be evaluated using plural criteria. We presented our criteria for evaluating the results of prick test, and then our criteria resulted in decreasing the number of false-positive reactions and reflecting clinical symptoms. The patch test is the main method for studying delayed-hypersensitivity reactions. The patch test is performed on the back or forearm. The evaluation of results read at 48 and 72 hours after removing allergens. The

International Contact Dermatitis Research Group (ICDRG) advocated that uniform criteria be adopted to evaluate the results of the patch test (Wilkinson JD & Shaw S. 2004). If international standards for the prick test were established, the confusion regarding prick test results would be eliminated. There are three kinds of oral challenge food tests: open, single-blind and double-blind tests. Basically, method of open test is that patients ingest small amounts of foods that may cause allergic reactions, and physicians then observe and evaluate the patient's reactions.

There are two interesting conditions resulting from food allergies: food-dependent exercise-induced anaphylaxis (FDEIA) (Kidd JM 3rd, et al. 1983) and oral allergy syndrome (OAS) (Kondo Y & Urisu A. 2009). These two conditions are IgE-dependent allergic reactions. Because the frequency of the shock symptoms of FDEIA is higher than that of OAS, people must know and it needs enlightenment about this disease. One common trigger of FDEIA is exercise, especially after allergens have been consumed. The kinds of exercise that can trigger FDEIA are different depending on the case, but they include running, tennis, basketball, baseball, golf, cycling, cleaning, etc. Some individuals can precipitate FDEIA just by walking after a meal. Other triggers of FDEIA are drugs (ex. aspirin) and the patient's status (physical condition). Present illness (episode of this disease) is important for the diagnosis. Most FDEIA occurs at 20-40 minutes after eating; patients should rest of 2-3 hours after meals. Some students develop allergic symptoms during physical education class after lunch. Teachers must be aware of this condition, and patients must obtain and know how to use an Epipen®. The Epipen®is an injection of epinephrine, which is a vasoconstrictor, and it is portable. An Epipen® injection causes vasoconstriction and results in a rise in blood pressure. This drug is a single use injection by own self. In the provocation test of FDEIA, an urgent treatment is often required.

OAS refers to contact urticaria of the mucosa (oral cavity, lip, throat, nose, esophagus, and trachea). This condition usually occurs within 20 minutes of the exposure to allergens. This allergy has become more prevalent in recent years, and the number of OAS has been increased. Most OAS allergens are fruits and vegetables. Some OAS patients have allergies to some kinds of pollen, which can cause cross-reactions (Table 1). The cross-reactions between pollen and vegetables or fruits are caused by PR-10 protein or profiling (Kondo Y & Urisu A. 2009). It is interesting that some cases of OAS are complicated with allergic conjunctivitis, allergic rhinitis, or latex allergy. The mechanism by which this occurs is likely a cross-reaction to these antigens.

Pollen	Foods
alder	melon, kiwi, soy, apple, peach mango, avocado, orange, potato, tomato, nut, carrot
white birch	melon, apple, peach, pear, almond, walnut. nut, peanut, potato, kiwi, celery, orange
orchard grass, timothy grass	tomato, flour, melon, rice, watermelon, potato, onion
hogweed	banana, watermelon, melon
Japanese cedar, cypress	tomato

Table 1. These pollens caused cross-reactions with foods.

3.2 Provision for foods allergy

The therapies for allergic reactions are different depending on the symptoms. The most important therapy for a food allergy is to avoid or eliminate the allergens. Individuals with food allergies and their family or caregivers must be attentive to the allergy at home or at school. At home, contamination during cooking is the most frequent cause of allergic reactions, there is a strong possibility of contamination because multiple people are involved in preparing and serving the school lunch at school. The mistaken distribution of foods at lunch time and in class is also an important cause. A child with a food allergy may suffer persecution if other children around him or her do not understand the food allergy. Children, teachers and the patient's family need to understand the psychological dimensions of food allergies. Therefore it is important that the patient's family, teacher and home doctor have a good relationship. Notices in the school are food restriction, bring lunch, and avoid allergens. Food allergy guidance and foods without allergens are essential for patients and their families. Depending on the allergen, a food allergy can be a significant burden for the patient and his or her family and can have a significant economic cost. Food restriction sometimes causes nutritional or eating disorders and can result in abnormal growth. If the patients have severe allergic reactions, and especially if the patients develop immediate allergy (shock), it is important for the patient to be able to inject epinephrine (Epipen®) by themselves. When people with severe allergic reactions are exposed to allergens, they usually inject this drug into their thigh by themselves before suffering shock. In Japan, processed foods using 7 materials (milk, egg, peanut, flour, buckwheat, shrimp and crab) must be labeled by law. And then, eighteen other foods (abalone, apple, banana, beef, chicken, gelatin, kiwi fruit, mackerel, matsutake mushroom, orange, peach, pork, salmon, salmon roe, soy, squid, walnut and yam) are not restricted but labeling is recommended. Because many foods today are widely distributed, common methods of labeling processed foods are needed worldwide. There are many kinds of foods allergy, in the case of flour allergy, it is difficult to avoid the allergen completely, because flour is often used not only in foods but also other items (ex. soap, shampoo, cosmetics). Processed foods and seasonings sometimes contain flour. The ingredients of these foods must be confirmed before they are consumed. International food labeling standards are useful for people with food allergies.

4. Soy products allergy

4.1 Introduction

Soybean allergy is 2% of food allergy. Leading cause of food allergy is eggs, the 2nd cause is milk, the 3rd cause is flour and the 10th cause is soybean (Rodrigo MJ, et al. 1990). There are many kinds of soy products, soy milk, tofu, natto. When we grind soybean and filter it, soybean changes soy milk. Coagulated soy milk is tofu. Soy oil is extracted from soybean. Fermented soybean is natto, soy sauce and soybean paste (miso). Roasting soybean is soy powder (Figure.3).

4.2 Classification according to types of soy allergy

Recently, we distinguish food allergy as a mechanism of pathogenesis. There are 6 parts of food allergy, class 1 allergy, class 2 allergy, inhalant allergy, contact urticaria, protein contact dermatitis and others.

soybean flour

Roasting

Fermentation

Natto

Soy sauce   Soy   Grinding and filtration   Soy milk   Coagulation   Tofu

Miso   Extraction

Oil
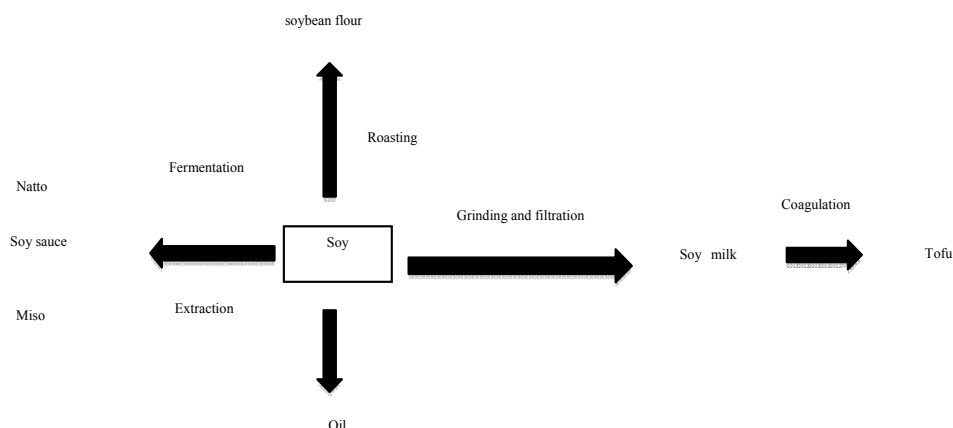
Fig. 3. Soy is processed into a wide variety of products

4.2.1 Class 1 allergy

Class 1 allergy is quote-unquote archaic food allergy. In this type, sensitized antigens equal to pathogenic antigens. Symptoms are urticaria, diarrhea, vomitting and anaphylactic shock. Basement of pathogenesis is an antigen permeability of mesentery and incomplete immune tolerance in infants. Class 1 allergy almost heal naturally expect some antigens as crabs, shrimp, buckwheat noodles. Main causative antigens are soybean, eggs, milk and flour. Causative antigens have digestive tolerance and fever tolerance. The structure is small molecular weight and contain s-s band.

4.2.2 Class 2 allergy

Transmucosaland percutaneous sensitization of pollen and latex tap, then similar molecules of food from vegetable sources trigger food allergy. Usually, adults with a pollen allergy and / or latex allergy cause this type of allergy, and natural healing is a rare occurrence. Symptoms are itching of the mouth and pharynx, frog and anaphylactic symptoms like facial edema, airway constriction dyspnea. Main causative antigens are vegetable, fruits, soybean and walnuts. The character of causative antigens is food protein from vegetable sources and water-soluble low molecular protein. Digestive resistibility of digestive enzyme isn't involved in this allergy because the allergens are assimilated by the mouth not the digestive canal (Table 2).

4.2.3 Inhalant allergy

Someone may sensitize soy protein when they inhale it, then they inhale soy protein again and soy protein evokes asthma attack. Over 20 patients passed away because they worked unloading the equipment from the ship in Barcelona, Spain, from 1981 to 1987 (Ikeda I & Ogawa T. 2000).

4.2.4 Contact urticaria

Some cases induce contact urticaria when they contact soy product such as tofu with hands or some body parts. They suffer from local parts urticaria that is only contact area with soy products (Amin S & Maibach HI. 1997). There is a staging of contact urticaria syndrome

(Iijima S. 2008). Stage 1 is local urticaria and dermatitis, stage 2 is generalized urticaria, stage 3 is bronchial asthma, rhinitis, conjunctivitis, orolaryngeal symptoms and gastrointestinal symptoms. Stage 4 is anaphylactic reactions.

	Class 1 allergy	Class 2 allergy
Causative food	Tofu, soy sauce, boiled soybean, soy sauce, bean paste (miso)	soymilk
Age	young children	adults
IgE RAST of soy (blood test)	high score	low score or negative
Source of sensitizing antigens	food	pollen
Pathway of sensibilization	oral sensitization	inhalation sensitization contact sensitization
Induction allergens	as same as induction allergens	cross-reaction between pollen and food
Symptoms	urticaria, diarrhea, vomitus anaphylactic shock.	oral allergy syndrome

Table 2. Class allergy and class 2 allegry

4.2.5 Protein contact dermatitis (PCD)
PCD is an allergic skin reaction induced by proteins of ether animal or plant origin. When they contact the causative protein, they have a chronic eczema and an acute urticaria occurring within 15-30 minutes. Prick or scratch test usually show positive, and patch test results are often negative.

4.2.6 Others
Some cases have both class 1 and class 2 allergies. When they eat freeze-dried bean curd or snacks including soybean, they suffer from orolaryngeal symptoms (class 2 allergy) at first, then urticaria, gastrointestinal symptoms and anaphylactic reactions (class 1 allergy).

4.3 Allergens
Table 3 shows main allergens of soy bean. Ogawa (Ogawa T, et al. 1993) reported Gly mBd was a main allergen of class 1. Soy 7s globulin is also a main allergen of class 1 (Ogawa T, et al. 1995). In Japan, we have an eating habit of soy. Therefore, there are a lot of studies of class 1 allergy. In the United States and Europe, they don't have an eating habit of soy, so they study about class 2 allergy and inhalant allergy. Main allergens of Barcelona asthma are Gly m1 and Gly m2, and main allergens of class 2 allergy are oleosin, SAM 22 (Gly m4), soy profilin (Gly m3). Soy milk contains a lot of Gly m4 and tofu dose not contain Gly m4 so much.

classification of soy allergy	molecular size	classification
Soy 7s globulin	68kDa 66kDa 50kDa	class 1
glycin A3 subunit	43kDa	class 1
Gly m Bd30K	30kDa	class 1
Gly m Bd28K	28kDa	class 1
oleosin	23-24kDa	class 2
Kunitz-type soybean trypsin inhibitor	18-20kDa	class 1
SAM 22 (Gly m 4)	17kDa	class 2
soy profilin (Gly m 3)	13kDa	class 2
Gly m 1	7.5kDa	inhalant antigen
Gly m 2	8kDa	inhalant antigen
2S albumin	9kDa 5kDa	class 1?

Table 3. Main allergens of soy bean

4.4 Tests
4.4.1 Blood tests
When we suspect our patients suffer from soybean allergy, we study IgE RAST of soy. We also study IgE RAST of pollen such as Japanese white birch, Alnus japonica, Japanese cedar and Japanese cypress, IgE RAST of fruits and vegetables such as apples, strawberries, carrots and celeries.

4.4.2 Skin tests
We conduct prick tests using tofu, soy milk, some fruits and vegetables. Histamine dihydrochloride is a positive control and saline is a negative control. We also conduct a 48-hour closed patch testing. Prick test results are usually positive, and patch test results often negative as PCD.

4.5 Treatments
Soy-free diet is important for class 1 allergy, soy milk, fruits and vegetables-free diet is important for class 2 allergy. They should protect their hands with gloves when they contact soybean product in PCD patients(Table 4). They suffer from class 1 allergy and class 2 allergy, they should take medicines such as anti-histamine internal medicine. If they have eczematous symptoms, they need to put on steroid ointment.

Tipe of food	processed food
grain	fried noodles, popcorn, macaroni, spaghett, instant noodle
potato and amyloid processed products	mashed potatoes, crisps, french fries
nuts and seeds processed products	fried almond, peanuts
pulse	red bean paste, kidney beans, peas, green peas, broad beans, bean sprout, soy beans, Tofu, fried tofu, a fried bean curd cake with vegetables and other ingredients in it, freeze-dried tofu, fermented soy beans, soy pulp, tofu skin, soybean flour, soymilk, gelatin
fish and shellfishseafood	tsukudani that preserved small fish, shellfish, konbu, etc. boiled down in soy sauce and sugar, fish sliced open that seasoned with mirin, soy sauce, etc. and dried in the sun, smoked fish, fish preserved in miso
meat processing products	meat with miso, soy sauce and oils
oils and fats	margarine, soybean oil, sesame oil and safflower oil contain with soy, bean- jam- filled wafers, oiled beans, agar- agar cubes, other delicacies in sugar syrup
sweets	a bar of sweet jellied adzuki- bean paste, a bun with a bean- jam filling, a rice ball coated with sweetened red beans, soybean flour, or sesame and salt, cubic rice crackers, fried dough cookies, Japanese sponge cake, chocolates
preference beverage	cocoa, coffee
seasoning	curry roux, white sauce, mayonnaise, dressings, a seasoned powder for sprinkling over rice, miso, soy sauce

Table 4. Soy products

4.6 Countermeasures

Moriyama reported some soy products improve hypoallergenic products (Moriyama T & Ogawa T. 2010). They attempt using hypoallergenic soybean paste (miso), hypoallergenic soy milk, hypoallergenic boiling soy and hypoallergenic soy cookies.

5. Soy sauce allergy

5.1 The history of soy sauce

Soy sauce originated from preserved foods in ancient China about 3000 years ago. The introduction of preserved foods from China into Japan occurred in about 500 A.D. Grains, especially soy, are easer to preserve than foods such as fish, duck, deer, and other meat and are suitable for Japanese-style cuisine. Preserved grain foods had developed in Japan because Japanese have been agricultural people and cultivated some kinds of grain. A Japanese Buddhist monk introduced the process of making miso (soybean paste), miso was developed in Japan. Some Japanese people in the Kamakura or Muromachi period (about 1200 - 1500 A.D.) found that the fermented solution obtained from preserved soy or miso was delicious. Both preserved soy and the fermented solution were then used for cooking, and soy sauce, fermented solution would be soy sauce. First, only noblemen (Buddhist monks, the samurai classes and court nobles) ate soy sauce, because soy sauce was a expensive food. The first soy sauce shop opened in about the 1580s in Japan, and then soy sauce began to be used for cooking by the public. The term 'soy sauce' started being used during the same period. The soy sauce trade started in about 1700 with the growth of the economy and commerce. In the Edo period (about 1700 A.D.), industrial production of soy sauce began, and mass production began in about 1900 A.D. (the Taisho period). Since the beginning of the 19th century, soy sauce began being exported abroad, and soy sauce has now become a global product due to the popularity of Japanese food worldwide. Soy sauce is now exported to over 100 countries.

5.2 Soy sauce

Soy sauce ' 醤油 ' is a traditional Japanese sauce made from fermented soybeans. Soy sauce is called 'shoyu' in Japan, and is made from soy, flour and salt (Kobayashi M. 2005). Soy sauce has 5flavors: tasty, salty, sweet, acidic and bitter, and the delicious of soy sauce are composed by striking a balance among these 5 flavors. Glutamic acid is a key to the taste. The concentration of salt in the soy sauce is about 16-18%, and the pH (potential of hydrogen) is about 5. This concentration of salt (about 16-18%) is high. Organic acids cause acidity and soften the saltiness. Three microbes, malt bacteria, lactic acid bacteria and yeast, play important roles in producing soy sauce. Japanese foods (washoku, 和食) are now being consumed worldwide, and soy sauce is thus widely consumed. The reason why Japanese foods are popular worldwide is because Japanese cuisine is known to be healthy. Soy sauce has been considered a healthy food which is anti-allergic, inhibits allergic reactions (Kobayashi M, et al. 2004 a), promotes the secretion of gastric juice (Kojima T. 1954), and improves anemia (Kobayashi M, et al. 2006) and hypolipidemia (Kobayashi M, et al. 2008). There are five kinds of soy sauce: koikuchi, usukuchi, tamari, siro and saisikomi shoyu. There are uses for each of these soy sauces. Koikuchi shoyu is the most consumed soy sauce in Japan; about 75 % of this shoyu is produced by honjouzo (production process). There are three production processes, honjouzo, kongoujouzo and kongou. The ingredients and additives used in this process are salt, alcohol, amino acid, glucose and sugar. The source, quality and volume of these substances are different depending on the kind of soy sauce being made or the companies that make them. Shoyu is made by brewing soybeans. Some of the production stages are shown in Figure 4.
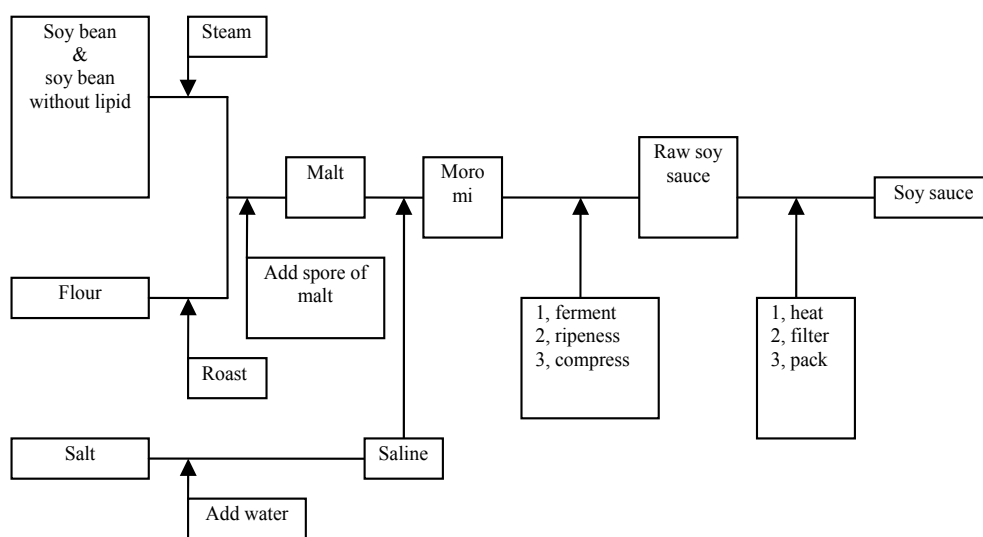
Fig. 4. This figure shows the process of honjouzou.

These processes take about 6-8 months. The same amount of soybeans (including soybeans without lipids) and flour are used. The soybeans (including soybeans without lipids) are steamed, and the flour is roasted. The kinds of soy and flour used depend on what type of soy sauce is being made or the soy sauce companies. The soybeans(including soybeans without lipids) and flour are mixed, and mold is added to this mixture. The mixture is then

mixed for a few days, and turns into malt. Saline is put into the malt, which changes it to moromi. The moromi is then turned into raw soy sauce by three steps (fermentation, ripening and compression). Raw soy sauce change soy sauce products throgh three process. The other 4 sauces (usukuchi, tamari, siro and saisikomi shoyu) are produced by different methods. The features of the other four sauces are as follows. About 15% of the soy sauce produced is uskuchi soy sauce. This sauce originates from the Kansai area. There is a greater amount of salt in usukuchi sauce, at about 10%, than koikuchi. Tamari, manufactured in the Chubu area of Japan, is often used for sashimi or sushi. Saisikomi is used in the Kyushu area, in western Japan, and is made from soy, flour and soy sauce. Siro is light in color and tastes sweet. This sauce is produced in the Hekinan area of Aichi, Japan. In Japan, except for soy, there are some other kinds of primary materials of shoyu sauce; fish, kelp, millet, rice, barnyard grass, etc. In these sauces, fish sauce smelled particular and unique. You can see or eat fish sauce when you go to East Asia. In the future, because of this increased consumption, the number of adverse effects, such as allergic reactions, may increase.

5.2.1 Introduction of soy sauce allergy

In Japan, some people develop irritation, cellulites or dermatitis around the lips after using soy sauce. The source of these symptoms is a possible soy sauce allergy, histamine poisoning, soy allergy, flour allergy, etc. Allergies to soy products and flour products are often caused by soy and flour, respectively. In particular, a major allergen of soybean is Gly mBD 30k (Tsuji H, et al. 1995) and Gly mBD 28k (Bando N, et al. 1998), but it is not detected in fermented soy products because of the heating used for production. However, allergens of fermented soy and flour foods were not detected, and fermented soy products are hypoallergenic foods (Kataoka S. 2005) because allergens of soy and flour should be degrade during fermentation (Kataoka S. 2005, Kobayashi M, et al. 2004 a,b). Why does irritation, cellulites, or dermatitis around the lips occur after using soy sauce? What allergens cause these symptoms? When cellulites and dermatitis around the lips occur, the person should be tested prick test and determine the volume of histamine. These symptoms are likely caused by a soy sauce allergy or histamine poisoning.

5.2.2 Cases of soy sauce allergy

We present eight cases of persons who developed allergic reactions after using their soy sauces. Case 1: A 35-year-old female who had been suffering from cellulites, dermatitis around the lips and coughing since 2004. Case 2: A 51-year-old female who has suffered swelling of the lips since November, 2008 (Figure 5). Case 3: A 10-year-old female who, since infancy, sometimes developed cellulites and scales after meals. Case 4: A 46-year-old female who had developed dermatitis around the lips with itching after meals since April, 2009. Case 5: A 10-year-old female exhibiting cellulites, scales with itching and dermatitis around the lips (Figure 6), and whose mother was the case 4 patient; both used the same soy sauce. Case 6: A 41-year-old female who suffered scales and swelling on her lips. Case 7: A 39-year-old female cook who developed swelling of the lips while working. Case 8: A 34-year-old female who sometimes suffered scales and swelling on her lips after meals. The average age of the 8 patients was 33.25 years. Their symptoms improved after they changed the brand of soy sauce they used. The eight patients did not develop any symptoms when they used salt, alcohol, amino acid, glucose and sugar.

Fig. 5. This figure showed Case 2; swelling of her lips.
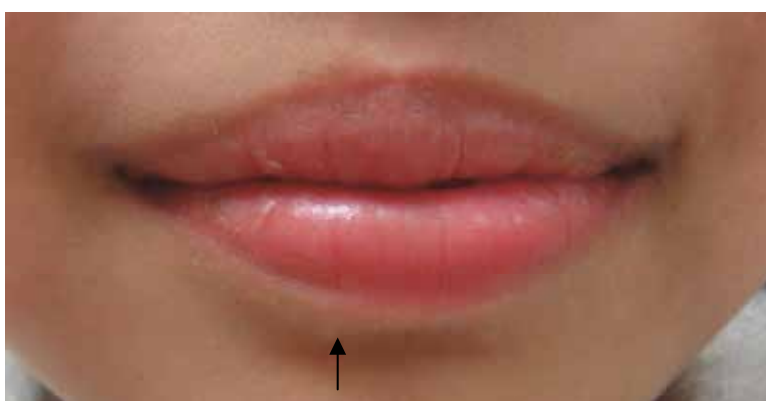
Fig. 6. This figure showed case 5; Allow indicated cellulites, scales with itching and dermatitis around lips.

5.2.3 Materials and methods for studying soy sauce allergy

5.2.3.1 Materials and methods for the prick test

Using prick tests and laboratory tests are essential for investigating allergies. The prick test is often used to study immediate allergy. We use Lancet needles (Leti, Madrid, Spain)for these tests. To conduct a prick test, we prick the substances suspected of causing patient's allergic reaction, and then we prick the patient's skin using the same needles. To read the results correctly, control substances and control people are needed. Generally, control substances are negative and positive controls, and control people are healthy without allergy. 15-20 minutes after the prick test, the results are evaluated using established criteria. Typical (conventional) criteria are 5-mm wheals with erythema (Roll A, et al. 2006), wheals 5 mm larger than in the negative control (Kivity S, et al. 2005), a mean diameter of two wheals that was 3 mm larger or more than the mean diameter of the negative control (Douglass JA and O'Hehir RE. 2006), half or more of wheals induced by histamine (Lee S, et al. 2001), 3-mm-diameter or larger wheals (Abi Berger. 2002, Brockow K & Romano A. 2008, S Kirschner, et al. 2009) and wheals with diameters 3 mm larger than the wheals of the negative controls (Lopes MI, et al 2006, Fereidouni M, et al. 2009, Christopher W Calabria & Larry Hagan. 2008, Baral VR & Hourihane JO. 2005). In our method, a wheal with a

diameter 3 mm smaller than the average wheal diameter of the controls with 21 mm or larger flare or wheals with diameters 3 mm larger than the average wheal ((maximum + minimum wheal diameter) / 2) diameter of the controls is considered to be a positive reaction. The A/H ratio (Sang-Ha Kim, et al. 2006)is the ratio of allergen-induced wheal size (A) to histamine-induced wheal size (H). A score of 1 indicates an A/H ratio of 0.1 to 1 with a flare of less than 21 mm. A score of 2 is an A/H ratio of 0.1 to 1 with a flare of 21 mm or more. A score of 3 is an A/H ratio of 1 to 2, a score of 4 is an A/H ratio of 2 to 3. And a score of 5 is an A/H ratio of 3 or more. A score of 2 or more was determined to be a positive reaction. The criteria varies among physicians, countries and institutes, and some criteria have often shown pseudo-positive reactions when substances containing histamine are tested. Because our method was not sufficient for evaluating results, we used both our method and A/H ratio as our new criteria. Therefore, we read the results of a prick test using soy sauce, and then positive reactions in both our method and A/H ratio are considered to be positive reactions in our criterion (Sugiura K & Sugiura M. 2011). Our criterion could decrease the number of pseudo-positive reactions.

Our volunteers without food allergies were four females as negative controls. The 4 controls 24, 27, 35 and 40 years old; the average age was 31 years. We obtained written consent for study participation from 12 persons (8 patients and 4 volunteers) before prick testing. We performed the prick test on their back or forearms. We used 15 sauces to investigate soy sauce allergy, including one barnyard grass sauce, one flour sauce, one garlic sauce, one kelp sauce, one millet sauce, one nam pla (Thai fish sauce), one rice sauce, one sardine sauce and 7 soy sauces. We used saline as the negative control and histamine chloride 1% aq as the positive control. These fifteen sauces were the commercial products from different companies, and there were one tamari sauce, one usukuchi sauce and 5 koikuchi sauces among the 7 soy sauces.

5.2.3.2 Laboratory test

We measured the percentage of eosinophils, IgE RIST and specific IgE against soy and flour by the capsulated hydrophilic carrier polymer-radioallergosorbent test (CAP-RAST) (ImmunoCAP®, Phadia KK, Tokyo). The concentration of IgE was divided into 6 classes; class 0 is less than 0.34 IU/ml, class 1 is 0.35-0.69 U/ml, class 2 is 0.7-3.49 IU/ml, class 3 is 3.5-17.49 IU/ml, class 4 is 17.5-49 IU/ml, class 5 is 50.0-99.99 IU/ml and class 6 is 100 IU /ml and more. The results evaluated as positive are class 2 and more.

5.3 Results of the laboratory test and prick test
5.3.1 The results of the laboratory test

The average percentage of eosinophils in patients was 3.35% (1%～5%), and that of serum IgE was 190 IU/ml. The values for specific IgE against soy in all cases were class 0; one patient was classified in class 2 for specific IgE against flour. The average percentage of eosinophils in the control volunteers was 0.45%, and the average of serum IgE was 91.75 IU/ml. The specific IgE against soy and flour in all volunteers received a score of 0.

5.3.2 The results of the prick test

We compared the results using our criteria with those using the 6 conventional criteria and A/H ratio described above. We observed positive reactions in 9 colors and various patterns (Table 5, Sugiura K & Sugiura M. 2011). It was interesting that some positive reactions by the conventional criteria described negative reactions by our method and A/H ratio, and

cases with negative reactions by conventional criteria did not show any positive reactions by our criteria (that is, there were no cases with false-negative reactions). Figure 7 showed positive reactions by the conventional criteria, but by our new criteria judged negative reactions. The positive ratio of the controls according to our new criteria was lower than that of the controls according to the conventional criteria (Table 6). Both the conventional and

Table 5. The results of prick test using 15 sauces. Each table indicated as follows.

Fig. 7. This reaction show negative or positive reaction.

new criteria described that some soy sauces that contained 10 mg/100 g or more histamine showed higher positive ratios in patients; however, the positive ratio by our new criteria was lower than that by the conventional criteria in the controls (Table 6). The positive ratios of the prick test using soy sauce in this study had different results between the conventional and new criteria (Table 6). We speculated that the concentration of histamine would be closely related to the type of shoyu (for example, koikuchi, usukuchi), but we did not see any significant relations. Some soy sauces containing extremely high concentrations of histamine may cause positive reactions, but there was no relation between the positive ratio and the concentration of histamine in our study.

Conventinal Criteria	Our new Criteria		Conventinal Criteria	Our new Criteria	
Positive ratio of patients (%)	Positive ratio of patients (%)	Sauces	Positive ratio of controls (%)	Positive ratio of controls (%)	Histamine (mg/100g)
46.87	25	Flour sauce	15.62	0	4.6
57.81	37.5	Soy sauce 6 (koikuchi)	12.5	0	5.1
65.62	37.5	Nanplar	21.87	0	7.1
71.87	62.5	Soy sauce 7 (tamari)	46.87	25	13.5
68.75	62.5	Soy sauce 3 (koikuchi)	56.25	25	27.6
85.93	62.5	Garlic sauce	84.37	25	29.4
85.9	62.5	Kelp sauce	78.12	25	30.2
79.68	62.5	Soy sauce 5 (koikuchi)	75	0	41.3
78.12	62.5	Soy sauce 1 (koikuchi)	78.12	0	50.5
87.5	75	Soy sauce 2 (usukuchi)	78.12	0	54
85.93	50	Soy sauce 4 (koikuchi)	59.37	0	75.2
21.87	0	Sardine sauce	0	0	not detected (Minimum limit of determination; 0.5mg/100g)
25	0	Millet sauce	0	0	not detected (Minimum limit of determination; 0.5mg/100g)
23.43	0	Rice sauce	0	0	not detected (Minimum limit of determination; 0.5mg/100g)
3.12	0	Barnyard grass sauce	3.12	0	not detected (Minimum limit of determination; 0.5mg/100g)

*Request for this to Japan Food Research Laboratories authorrized by Japaneze government
*Certificate of analyisis: No. 10024724001-10024724015
*We made this table based on the results provided by the Japane Food Research Laboratory.
*Each of there soy sauces were different brand

Table 6. The contrecation of histamine and positive ratio of prick test

5.3.3 Determination of histamine in each sauce

Histamine is an important factor for studying soy sauce allergy. Because the concentration of histamine in soy sauce is high and a false-positive skin reaction could be present, the results of a skin prick test must be evaluated strictly.

There are a variety of histamine-containing foods (Wantke F, et al 1993, BJ Vlieg-Boerstra, et al. 2005) change to (Wantke F, et al. 1993, BJ Vlieg-Boerstra, et al. 2005) in the world. The histamine-containing foods are cheese, sausages, fish, wine and soy products, and the histamine-releasing foods are strawberries, chocolate, tomatoes, peanuts and additives. Histamine-releasing foods act on mast cells for degranulating. Because histamine sometimes causes adverse reactions such as histamine poisoning, the concentration of histamine in the foods is provided on food packaging. Food Standards Australia and New Zealand (FSANZ) show that the concentration of histamine must be 20 mg/100 g or less, and codex standards

for hygiene and handling demonstrate that any fish used for human consumption must not contain a concentration of histamine of 20 mg /100 g or more. There is currently no rule regarding the acceptable concentration of histamine in soy sauces. The histamine in soy sauce comes during the brewing of soy (BJ Vlieg-Boerstra, et al. 2005, KW Chin, et al. 1989). The concentration is various depending on the kind of sauce, the manufacturing company and the preservation conditions.

We asked the Japan Food Research Laboratories (Tokyo) to determine the volume of histamine in 15 sauces. They used high-performance liquid chromatography (HPLC) for their testing, as shown in Table 7. The minimum limit of determination by this method was 0.5 mg/100 g.

5.3.4 The volume of histamine in the sauces

We tested various colors of soy sauce; those containing histamine expressed a deep color (Figure 8). No histamine was detected in the millet *(kibi)*, barnyard grass, sardine and rice sauces, all of which are lighter-colored than the sauces containing histamine. The concentrations of detected histamine in the tested sauces are described in Table 5. In those results, three soy sauces contained 50 mg/100 g and more, which is a high concentration of histamine. These soy sauces were soy sauce 4 at 75.2 mg/100 g, soy sauce 2 at 54.0 mg/100 g and soy sauce 1 at 50.5 mg/100 g. The minimum concentration of histamine was detected in the flour sauce, 4.6 mg/100 g. Seven sauces contained histamine at concentrations of 20 mg/100 g or more. In the case of soy sauce, the positive ratio of the prick test with concentrations of 50 mg/100 g and more of histamine was high. In fact, the level of toxicity or poisoning by histamine is low; the concentration of histamine that will cause food poisoning is 50 mg/100 g or more (Gilbert RJ, et al. 1980, Lehane L & Olley J. 2000, Brink B, et al. 1990). Because soy sauces containing high concentrations of histamine could cause adverse effects and affect medical tests, we need a new world standard for the concentration of histamine in soy sauces.

Fig. 8. The color of the tested soy sauces. The bottle at the far left contains distilled water. 4th, 5th, 6th and 8th from left side are barnyard grass, rice sauces, millet *(kibi)* and sardine.

Sample 1.5 g

← − − − 0.2 mol/l Perchloric acid 20 ml

← − − − 0.01 W/V% Octamethylenediamine 5 ml (internal standard)

← − − − Hexane 12 ml

Homogenize

Centrifuge at 2000 rpm for 5 min

Water layer

Filter through a filter paper (No. 5, Advantec MFS, Inc.)

Transfer 4 ml of filtrate to a brown test tube

← − − − 30W/V% Sodium carbonate 0.7 ml

← − − − 1W/V% Dancyl chloride in acetone 5 ml

After mixing and sealing, stand in a water bath at 37℃ for over night

← − − − Hexane 4 ml

Hexane layer

Evaporate to dryness

← − − − Ethanol 2 ml

HPLC

<Condition for HPLC>

Pump: LC-10ADvp [Shimadzu Co., Ltd.], Detector: UV Spectrophotometric Detector SPD-10Avp [Shimadzu Co., Ltd.]
Column: Mightysil RP-18GP, Φ4.6 mm×150 mm [Kanto Chemical Co., Inc.]
Column temperature: 45℃,Mobile phase: Acetonitrile-Methanol – 0.01 mol/l Acetic acid (2:3:2 V/V/V)
Flow rate: 1.0 ml/min,Wave length: 254 nm
(This table is based on the information provided by the Japan Food Research Laboratory)

Table 7. Analytical Method for Histamine

6. Conclusion

Irritation of the lips and the skin can occur around the mouth after a meal containing soy sauce, but physicians do not always have any idea what the origin of these symptoms is. We hope our study contributes to awareness about these symptoms and soy sauce allergy. Our challenges were that the allergen of soy sauce allergy without soy and flour allergy is unknown, and there is no established method for evaluating the results of prick tests.

First, we suspected that unknown substances made during fermentation were the source of allergic symptoms. When cellulites or dermatitis around the lips occurs, a soy sauce allergy is suspected, and clinicians investigate patient's symptoms. What materials cause soy sauce allergy? Previous reports described that no allergens were detected in fermented soy products and that some allergens were lost in the process of fermentation (Kataoka S. 2005, Kobayashi M, et al. 2004 a,b). We found that soy sauce allergy was caused by unknown substances generated during fermentation, as our cases did not have positive reactions on skin tests or show a negative class of specific IgE to soy or flour (score 0). One problem with soy sauce is that it is often of poor quality and contains unknown substances. There often are sediments of soy sauce that are affecting its quality. These sediments are proteins produced by the molding of malt that are not always obvious. We speculated that these sediments might cause soy sauce allergy, and thus when examining soy sauce allergy, one must investigate not only soy allergy but also allergies to other materials (flour, salt, additives) as well as histamine poisoning. Making a differential diagnosis of soy sauce allergy and histamine poisoning requires knowledge about a patient's present illness and past history as well as performance of a skin test that is strictly evaluated. If histamine poisoning is the origin of symptoms, other foods containing histamine may have caused the symptoms. In fact, when patients eat some foods contained histamine, they do not appear allergic symptoms. It is difficult to diagnose correctly using the conventional prick test criteria, because soy sauces contain histamine that affects the results. Ideally, the volume of histamine should be determined, and the prick test should be given to healthy volunteers at the same time it is given to the persons suspected of having allergies. If the patients show positive reactions and control volunteers show negative reactions, it can diagnose that test materials must be origin of allergy. The results must be read by the strictly evaluation.

The consumption of soy sauce is increasing, and hence the number of cases with soy sauce allergy could increase. Companies that manufacture soy sauce should educate people about the advantages of healthy eating, the acceptable concentration of histamine, histamine poisoning and allergic reactions. Histamine poisoning presents like an allergic reaction, with

the subject experiencing vomiting, diarrhea, urticaria, hypotension, headache, flushing, itching, etc (Steve L Taylor. 1986). Histamine is produced during the fermentation of soy, and we studied the concentration of histamine in soy sauce, which is different depending on the sauce. The concentration is related to the soy sauce color, with most soy sauces with deep color containing a high concentration of histamine and soy sauces with light color containing low concentrations of histamine (Figue. 8, Table 6). It is important to study the concentration of histamine when a soy sauce allergy is suspected. Consideration of how the histamine level will affect the results of a prick test is also important. The conventional criteria for evaluating prick test results are not suitable for soy sauce allergy, because the variety of results by these criteria can confuse both patients and physicians. We note that the conventional criteria have the possibility of not reflecting clinical symptoms and of including false-positive reactions. Our criteria are suitable for the evaluation of prick tests using soy sauce, because the number of false-positive reactions can be decreased (Sugiura K & Sugiura M. 2011). It is important for patients to know whether what they are experiences is a food allergy, and thus whether they can eat a given food. The International Contact Dermatitis Research Group (ICDRG) (Wilkinson JD & Shaw S. 2004) provided criteria for patch testing (used for testing delayed-hypersensitivity allergies), but there are no appropriate criteria for the prick test using foods containing histamine; if people have false-positive reactions according to the conventional criteria, they must eat a soy-sauce-free diet. This is unfortunate, as they may not actually have to eliminate soy sauce from their diet. Not only the results of the prick test but also clinical findings are essential for diagnosing an allergy, and thus globally correct criteria for diagnosis are needed. Our new criteria are useful and superior for prick tests using soy sauce.

In this chapter, patients with soy sauce allergy who do not have allergies to soy, flour or additives are described, including their symptoms with figures, laboratory data, the results of skin tests, treatment and their condition at present. We recommend a new criterion for prick tests using soy sauce for the accurate diagnosis of soy sauce allergy.

7. References

Abi Berger. 2002. Skin prick testing. BMJ, 325, 414

Amin S & Maibach HI. 1997. Introduction, 1-3, Contact urticaria syndrome, CRC Press LLC (USA)

Bando N, Tsuji H, Hiemori M, Yoshizumi K, Yamanishi R, Kimoto M & Ogawa T. 1998. Quantitative analysis of Gly mBd 28K in soybean products by a sandwich enzyme-linked immunosorbent assay. J Nutr Sci Vitaminol, 44, 655-664

Baral VR & Hourihane JO. 2005. Food allergy in children. Postgrad Med J., 81, 693-701

BJ Vlieg-Boerstra, S van der Heide, Oude Elberink JN, Kluin-Nelemans JC & Dubois AE. 2005. Mastcytosis and adverse reactions to biogenic amines and histamine-releasing foods: what is the evidence?. Netherlands J Med, 63, 244-249

Brink B, Damink C, Joosten HM & Huis in 't Veld JH. 1990. Occurrence and formation of biologically active amines in foods. Int J Food Microbiology, 11, 73-84

Brockow K & Romano A. 2008. Skin tests in the diagnosis of drug hypersensitivity reactions. Curr Pharm Des., 14, 2778-91

Christopher W Calabria & Larry Hagan. 2008. The role of intradermal skin testing in inhalant allergy. Ann Allergy Asthma Immunol.; 101: 337-347

Cianferoni A & Spergel JM. 2009. Food allergy: review, classification and diagnosis. Allergol Int. Dec,58(4), 457-66

Douglass JA, O'Hehir RE. 2006. Diagnosis, treatment and prevention of allergic disease: the basics. Med J Aust; 185: 228-233

Dreborg S, Backman A & Basomba A. 1989. Skin tests used in type I allergy testing Position paper.. Sub-Committee on Skin Tests of the European Academy of Allergology and Clinical Immunology. .Methods for skin testing. Allergy., 44 Suppl 10,1-59

Ebisawa M. 2006. Management of food allergy (food allergy management 2005 by national food allergy research group) Arerugi, 55, 107-114 (in Japanese).

Fereidouni M, Hossini RF, Azad FJ, Assarehzadegan MA, Varasteh AAzad FJ. 2009. Allergol Skin prick test reactivity to common aeroallergens among allergic rhinitis patients in Iran. Immunopathol, 37, 73-79

Gilbert RJ, Hobbs G, Murray CK, Cruickshank JG & Young SE. 1980. Scombrotoxic fish poisoning: features of the first 50 incidents to be reported in Britain (1976-9). Br Med J, 281, 71-72

Iijima S. 2008. Haveing something on our chest especially allergic diseases. Q & A of allergy 4, 589-591

Ikeda I & Ogawa T. 2000. Tofu-induced urticarial contact dermatitis, Arch Dermatol 136, 127-128

Ishizaka K & Ishizaka T. 1967. Identification of E-antibodies as a carrier of reaginic activity. J Immunol, 99, 1178

Kanny G, Moneret-Vautrin DA, Flabbee J, Beaudouin E, Morisset M & Thevenin F. 2001. Population study of food allergy in France. J Allergy Clin Immunol., 108(1), 133-40

Kataoka S. 2005. Functional effects of Japanese style fermented soy sauce (shoyu) and its components. J bioscience and bioengineering, 100, 227-234

Kidd JM 3rd, Cohen SH, Sosman AJ, Fink JN. 1983. Food-dependent exercise-induced anaphylaxis. J allergy Clin Immunol, 71, 1407-1411

Kirschner S, Belloni B, Kugler C, Ring J & Brockow K. 2009. Allergenicity of Wine Containing Processing Aids: A Double-Blind, Placebo-Controlled Food Challenge. J Investig Allergol Clin Immunol, 19, 210-217

Kivity S, Fireman E & Sade K. 2005. Bronchial hyperactivity, sputum analysis and skin prick test to inhalant allergens in patients with symptomatic food hypersensitivity. Isr Med Asso J, 7, 781-784

Kobayashi M. 2005. Immunological functions of soy sauce: hypoallergenicity and antiallergic activity of soy sauce. J bioscience and bioengineering, 100, 144-151

Kobayashi M, Nagatani Y, Magishi N, Tokuriki N, Nakata Y, Tsukiyama R, Imai H, Suzuki M, Saito M & Tsuji K. 2006. Promotive effect of shoyu polysaccharides from soy sauce on iron absorption in animal and humans. Int Mol Med, 18, 1159-1163

Kobayashi M, Magishi N, Matsushita H, Hashimoto T, Fujimoto M, Suzuki M, Tsuji K, Saito M, Inoue E, Yoshikawa Y & Matsuura T. 2008. Hypolipidemic effect of shoyu polysaccharides from soy sauce in animal and humans. Int Mol Med, 22, 565-570

Kobayashi M, Matsushita H, Yoshida K, Tsukiyama R, Sugimura T & Yamamoto K. 2004a. In vitro and in vivo anti-allergic activity of soy sauce. Int J Mol Med., 14, 879-84

Kobayashi M, Hashimoto Y, Taniuchi S & Tanabe S. 2004b. Degradation of wheat allergen in Japanese soy sauce. Int J Mol Med, 13, 821-827

Kojima T. 1954. Effect of soy sauce on gastric juice secretion. Rinsho Shoukakibyougaku Zassi, 2, 1107-1110 (in Japanese)

Kondo Y & Urisu A. 2009. Oral allergy syndrome. Allergol Int, 58(4), 485-91

Kristinsdóttir H, Clausen M, Ragnarsdóttir HS, Halldórsdóttir IH, McBride D, Beyer K & Sigurdardóttir ST. 2011. Prevalence of food allergy in Icelandic infants during first year of life. Laeknabladid, 97(1), 11-8

KW Chin, MM Garriga & DD Metcalfe. 1989. The histamine content of oriental foods. Fd Chem Toxic, 27, 283-287

Lehane L & Olley J. 2000. Histamine fish poisoning revisited. Int J Food Microbiology, 58, 1-37

Lee S, Noh GW & Lee KY. 2001. Clinical application of histamine prick test for food challenge in atopic dermatitis. J Korean Med Sci, 16, 276-282

Lopes MI, Miranda PJ & Sarinho E. 2006. Use of the skin prick test and specific immunoglobulin E for the diagnosis of cockroach allergy. J Pediatr, 82, 204-209

Mikihiro S. 1970. Langerhans cell granulein Letterer-Siwe disease. An electron microscopic study. Cancer, 26, 1102-1108

Moriyama T & Ogawa T. 2010. Variety of soy allergy and a strategy of lowering risk of soy allergy, Food Engineering 53, 75-87

Obeng BB, Amoah AS, Larbi IA, Yazdanbakhsh M, van Ree R, Boakye DA & Hartgers FC. 2010. Food Allergy in Ghanaian Schoolchildren: Data on Sensitization and Reported Food Allergy. I nt Arch Allergy Immunol. 26, 155(1), 63-73

Ogawa T, Tsuji H, Bando N, Kitamura K, Zhu YL, Hirano H & Nishikawa K. 1993a. Identification of the soybean allergic protein, Gly m Bd 30k, with the soybean seed 34-kDa oil-body-associated protein, Biosci Biotech Biochem 57, 1030-1033

Ogawa T, Bando N, Tsuji H, Nishikawa K & Kitamura K. 1995b. α-subnit of β-conglycinin, an allergic protein recognized by IgE antibodies of soybean-sensitive patients with atopic dermatitis, Biosci Biotech Biochem 59, 831-833

Proverbio D, Perego R, Spada E & Ferro E. 2010. Prevalence of adverse food reactions in 130 dogs in Italy with dermatological signs: a retrospective study. J Small Anim Pract, 51(7), 370-4

Rodrigo MJ, Morell F, Helm M, Swanson M, Greife A, Anto JM, Sunyer J & Reed CE. 1990. Identification and partial characterization of the soybean-dust allergens involved in the Balcelona asthma epidemic. J Allergy Clin Immunol, 85, 778-784

Roll A, Hofbauer G, Ballmer-Weber BK & Schmid-Grendelmeier P. 2006. Safety of specific immunotherapy using a four-hour ultra-rush induction scheme in bee and wasp allergy. J Investig Allergol Clin Immunol, 16, 79-85

Sang-Ha Kim, Hyun-Mi Kim, Young-Min Ye, Kim SH, Nahm DH, Park HS, Ryu SR & Lee BO. 2006. Evaluating the Allergic Risk of Genetically Modified Soybean. Yonsei Med J., 47, 505–512

Shamoto M. 1970. Langerhans cell granulein Letterer-Siwe disease. An electron microscopic study. Cancer, 26, 1102-1108

Sicherer SH, Munoz-Furlong A & Sampson HA. 2004. Prevalence of seafood allergy in the United States determined by a random telephone survey. J Allergy Clin Immunol, 114: 159-165

Steve L Taylor. 1986. Histamine food poisoning: toxicology and clinical aspects. Critical Reviews in Toxicology; 17, 91-128

Sugiura K & Sugiura M. 2011. Criteria for prick test using soy sauce. Int J Dermatology, in print.

Sugiura K & Sugiura M. 2010a. The study of occupational skin disease. Occup Environ Allergy, 17, 11-16

Sugiura K & Sugiura M. 2010b. Soy sauce allergy. JEADV, 24, 852-855

SugiuraK, Shamoto M, Sakamoto N, Shinzato M, Osada A, Sugiura M, Hayakawa R & Kato Y 2003. It is true that, when Langerhans cells migrate from the skin to the lymph node, they are transported via lymph vessels. Dermatology, 206(3), 222-224

Tsuji H, Okada N, Yamanishi R, Bando N, Kimoto M & Ogawa T. 1995. Measuremen of Gly m Bd 30K, a major soybean allergen, in soybean products by a sandwich enzymelinked immunosorbent assay. Biosci Biotechnol Biochem, 59, 150-151

von Pirquet, C. 1906 Allergie. Munch. Med, Wochenschr, 53. 1457

Wantke F, Gtz M & Jarisch R. 1993. Histamine-free diet: treatment of choice for histamine-induced food intolerance and supporting treatment for chronical headaches. Clin Exp Allergy, 23, 982-985

Wilkinson JD & Shaw S. 2004. Contact Dermatitis: Allergic. In: Rook's Textbook of dermatology. (Burns Tony, Breathnach Stephen, Cox Neil, Griffiths Christopher, eds), 7th edn. Oxford: Blackwell Science Ltd, 20

Soybean: Food or Remedy?

Barbalho, Sandra Maria[1,2] and Farinazzi-Machado, Flávia, M. V.[2]
[1]Faculty of Food Technology – FATEC Marília
[2]University of Marilia – UNIMAR
Brazil

1. Introduction

The soybean plant (*Glycine max* L) belongs to the family Leguminosae (Order Rosacea) and probably originated from China, 4000 to 5000 years ago. It was introduced in Europe circa 1700 and today is one of the world's most important crops. It is an important plant for human and animal nutrition as well as for industrial purposes because about 60% of the world's processed food products contain ingredients derived from soy (Liu, 1999; Friedman & Brandom, 2001; Soares et al., 2005; Priolli et al., 2002).

Soybean is an almost unique food because of its protein, mineral and fiber content. In the plant kingdom it has the highest content of phytochemicals (such as flavones), which are compounds whose structure closely resembles that of estrogen, suggesting that it could exert similar activities (Lissin & Cooke, 2000; Mateos-Aparicio et al., 2008).

The processing cost of soybean oil is very low. In addition to oil, soybeans are processed into many foods and food additives such as lecithin (Gesteira et al., 2003; Mateos-Aparicio et al., 2008). Lecithin is widely used as a functional food and for industrial applications due to its emulsifying properties (Awazuhara et al., 1998; Taylor & Kabourek, 2003).

The soybean plant has oblong pods with 2 to 4 seeds containing approximately 38% protein, 18% oil, 30% carbohydrates, 14% moisture, ash, and secondary components and it is an important source of vitamins (A, thiamin, riboflavin, pyridoxine and folic acid) and minerals (Fe, Zn, Mg, K, Ca, Mn, and Se), phytoestrogens and fibers (Liu; 1997b; Anderson et al., 1995; Soares et al., 2005; Reynolds et al., 2006).

Soy concentrates and isolates provide highly concentrated protein sources, high lysine content, a bland flavor, and reduction in flatulence factors and reducing sugars, and they may improve the overall quality of products (Singh et al, 2008). Soy protein is low in viscosity; therefore, it can be used in beverage applications (Xie & Hettiarachchy, 1997; Jasentuliyana et al., 1998), helping to achieve the desired mouth-feel. The viscosity promoted by soy isolate makes it ideal for other nutritious liquid products such as infant formulas, creamers, milk replacers, and spray-dried products (Yao et al., 1988; Riaz, 1999). Textured soy flour is also known as TPS (textured soy protein) or TVP (textured vegetable protein) (Endres, 2001). Textured protein products are prepared commercially by thermoplastic extrusion of flours, grits, and protein concentrates under heat and pressure to form chips, chunks, flakes, and a variety of other shapes (Singh et al, 2008; Mesa et al., 2009). TPSs are frequently made to resemble beef, pork, seafood, or poultry in structure and appearance when hydrated (Endres, 2001; Macedo-Silva et al., 2001).

Despite its advantages, the soybean possesses antinutritional factors such as protease inhibitors, which inhibit proteolytic enzymes and decrease food digestion. These factors can reduce the bioavailability of the proteins ingested by the organism (Miura et al., 2001; Moughan, 2003; Monteiro et al., 2003).

Soybeans and soy foods have been recognized for their low fats and good quality protein. The soybean proteins, fibers and phytoestrogens are known to decrease cholesterol levels and to produce positive effects in reducing chronic disease risk such as diabetes, obesity and vascular diseases (Allison et al., 2003; Martin et al., 2008; Jimenez-Escrig et al., 2008; Messina, 2010; Chen et al., 2010; Kang et al, 2010). Since the 1960s, soybean protein products have been used as nutritional or functional food ingredients in every food group available to the consumer (Endres, 2001). In Asia, soy proteins have been used for human consumption for centuries, during which various unique soy protein foods have been established. Onuegbu et al. (2011) studied the effect of soymilk on the lipid and lipoprotein profile of normocholesterolemic men and found that the consumption of 500 mL of this drink can decrease cholesterol and triglycerides levels and increase HDL-c levels.

Bansai & Parle (2010) observed that, due to its proestrogenic, antioxidant and neuroprotective properties, soybeans can be used as a remedy to improve memory and to manage cognitive deficits. Moreover, soy and soy products provide an alternative source of protein for people with cow milk protein intolerance (Riaz, 1999; Dupont et al., 2010) and an alternative to the use of probiotics. Supplementation with prebiotics enhances the potential of soymilk as a carrier for probiotics (Yeo & Liong, 2010).

2. Protein composition

Proteins are essential for the growth and maintenance of corporal structures. Vegetable proteins usually affect protein nutrition because they are not complete in essential amino acids, thus reducing the quality of the protein. Animal proteins are considered complete because they contain all the amino acids required to produce the human body proteins (Martínez Augustin & Martínez de Victoria, 2006).

The protein content of soybean is about 40% and it is equivalent to animal proteins. Soybean has a protein content of about 40%, which is equivalent to animal proteins. The most significant limiting amino acids are methionine (10mg/g protein) and cystine (25.00), which are sulfur-containing amino acids, but soy is rich in lysine (68.37g/mg protein) (Chung et al., 1996; Sacks et al., 2006; Mateos-Aparicio et al., 2008). Liu (1997b) reported that soybean has the following amino acids content in milligrams per gram of protein: arginine (77.16), alanine (40.23), aspartic acid (68.86), glutamic acid (190.16), glycine (36.72), histidine (34.38), isoleucine (51.58), leucine (81.69), lysine (68.37), phenylalanine (56.29), proline (52.92), serine (54.05), threonine (41.94), tryptophan (12.73), tyrosine (41.55) and valine (41.55).

The presence of antinutritional factors such as proteolytic enzymes, trypsin inhibitors, phenolic compounds and phytic acid can reduce the quality of proteins and their use in the organism (Liu, 1999; Monteiro et al., 2003).

Animal proteins are almost always related to high levels of saturated fats. This is not the case of soy proteins, which are rich in essential amino acids, making them suitable to substitute animal protein sources. Soy proteins can also partly replace other sources of calories such as fats and carbohydrates (Sacks et al., 2006; Mateos-Aparicio et al., 2008).

Soy protein ingredients are used in compound foods for their functional properties, including water and fat absorption, emulsification, aeration (whipping) and heat setting and

for increasing total protein content and improving the essential amino acids profile (Jasentuliyana et al., 1998).

2.1 Soy protein and health

The high content of fats in the proteins of several animal species can be implicated in the increase of blood cholesterol, triglycerides and LDL-c (low density lipoprotein). The widespread concern about vascular disease (associated with the daily ingestion of fats and sugars) has motivated numerous studies on soybean protein (Allison et al., 2003; Sacks et al., 2006; Martin et al., 2008; Messina, 2010; Bermudez et al., 2010). Maki et al. (2010) studied the effects of the consumption of soy protein on subjects with hypercholesterolemia and concluded that it can reduce total blood cholesterol, LDL-c and triglycerides levels. Lerman et al. (2010) studied the effects of a diet containing soy protein and sterols in adults with metabolic syndrome and observed improvements in their total levels of cholesterol, LDL-c and triglycerides, as well as HDL-c. Metabolic syndrome can be characterized by low HDL-c and high total cholesterol and triglycerides and is associated with increased risk of cardiovascular disease and death, which is significantly increased when accompanied by elevated low-density lipoprotein cholesterol (LDL-c). Their study led these authors to conclude that individuals at high cardiovascular risk benefit from a soy/phytosterol-containing medical food. In addition, Torre-Villalvazo et al. (2009) showed that dietary soy protein can reduce the ceramide concentration in the heart and may be considered a dietary therapeutic approach for the prevention of lipotoxic cardiomyopathy.

Because of its protein content, soy has gained attention for its potential role in improving risk factors for cardiovascular disease, which is one of the leading causes of death worldwide. In 1999, the FDA (US Food and Drug Administration) approved labeling for foods containing soy protein as a protection against coronary heart disease. Soybean's potential to reduce total cholesterol and LDL-c and increase HDL-c (high density lipoprotein) was observed in epidemiological studies on diet and vascular disease in Japan and other Asian countries that consume large amounts of soybeans. These studies revealed that these countries had lower heart disease rates (Reinolds et al., 2006; Lukaczer et al., 2006; Hirayama et al., 2010).

Soy proteins can also produce beneficial effects on the activity of the angiotensin converting enzyme (ACE). They act as ACE inhibitor peptides that can be released enzymatically from precursor proteins *in vitro* during food processing and *in vivo* during gastrointestinal digestion. These peptides can reduce blood pressure by limiting the vasoconstrictive effects of angiotensin II and potentiating the vasodilatory effects of bradykinin (De Leo et al., 2009).

Results of epidemiological and animal studies suggest that consuming soy-containing diets reduces the incidence of certain types of cancer. Some authors have shown that soy protein isolate may protect against cancer via multiple mechanisms, including: 1) increased mammary gland differentiation, 2) decreased activation of procarcinogens to carcinogens, and 3) regulation of genes in signal transduction pathways underlying tumor initiation, promotion and/or progression (Badger et al, 2005).

Proteins have a higher satiety power than carbohydrates and fats. Studies suggest that this effect can be attributed to a higher thermogenic response of protein. Soy protein may have an important effect on postprandial energy expenditure compared to animal protein preparations and this can reduce ingestion and obesity, which is a risk factor for heart disease (Jequier, 2002; Paddon-Jones et al., 2008; Alfenas et al., 2010).

Trevisan et al. (2010) showed that the ingestion of soy protein can help increase the resting energy expenditure of post-menopausal women who practice exercises.

3. Carbohydrate composition

Soybeans are also rich in carbohydrates, which represent approximately 35% of their compounds. They contain about 1% of starch and non-starch polysaccharides such as cellulose, hemicelluloses and pectic polysaccharides. Their main sugars are arabinose, galactose, uronic acids and glucose (cellulose) (Sosulsk et al., 1982; Wilcox & Shibles, 2001; Espinosa-Martos & Ruperes, 2006; Redondo-Cuenca et al., 2007).

3.1 Soy carbohydrates and health

Soybeans are a rich source of raffinose, a family of oligosaccharides such as α-galactosides or galactooligosaccharides, which are non-digestible carbohydrates. Human beings do not have α-galactosidase, which is needed to hydrolyze these molecules, so they cannot be digested. These molecules are digested by beneficial bifidogenic microorganisms which produce gases and short chain fatty acids that have prebiotic activity (functional effects).

Soybeans also contain sucrose in the form of low amounts of the monosaccharides fructose, rhamnose and arabinose. Significant levels of glucose occur only in immature seeds. Non-digestible oligosaccharides, one of the most popular components of functional food, are related to many health benefits (Tomomatsu; 1994; Crittenden & Playne, 1996; Torres y Torres et al., 2006). Monosaccharides such as glucose and arabinose, and measurable amounts of di- and oligosaccharides with sucrose, raffinose, and stachyose can be found in soybean soluble carbohydrates (Liu, 1999; Sosulski et al., 1982; Huisman, 2000; Mateos-Aparicio et al., 2008).

The amount of fibers in soybean can reduce blood cholesterol levels and insulin resistance in diabetics. These effects can reduce the incidence of cardiovascular disease and death (Chandalia et al., 2000; Redondo-Cuenca et al., 2007; Reynolds et al., 2006; Chen et al., 2010). Fibers in diet can also produce anti-inflammatory and anti-carcinogenic effects (Scheppach et al., 2004).

4. Lipids composition

Liu (1999) found that soybean contains about 22% of oil. The fractions contain triglycerides (about 99%) and other components such as phospholipids, unsaponifiable matter (tocopherols, phytosterols and carbohydrates) and free fatty acids (Mateo-Aparicio et al., 2008).

Soybean contains high percentages of functional polyunsaturated fatty acids, as well as 54% linoleic acid and 4-5% linolenic acid, considerable percentages of oleic acid (22-25%) and moderate percentages of saturated fatty acids such as palmitic (11%) and stearic acids (4%). The predominant fatty acid in soybeans is linoleic acid, which accounts for approximately 54% of their total fatty acid content (Martins et al., 2003). There are few good sources of -3 fatty acids such as linolenic acid. Available data suggest that many people have a diet poor in this acid; hence, soybeans could be a good source to increase the intake of linolenic acid (Messina, 1997; Martin et al., 2008).

Refined soybean oil is the most widely consumed cooking oil in many countries. Its refining process includes degumming, neutralization, bleaching and deodorization steps. The

isomerization reactions carried out during these steps may reduce alpha-linolenic acid levels significantly. The high temperatures employed in processing increase the rate of isomerization reactions, giving rise to increased *trans* fatty acid levels that may vary from 0.8 to 2.6% of the product's total fatty acids and comprise 18:1, 18:2, and 18:3 isomers (Martin et al., 2008).

Trans isomers of monounsaturated and polyunsaturated fat acids are implicated in negative effects on human health, such as reduction of cell membrane fluidity, increase in LDL-c and decrease in HDL-c levels, modifications in prostaglandin metabolism, and changes in platelet aggregation and vascular function. Many authors have demonstrated that the consumption of *trans* fats from partially hydrogenated fats may be associated with the development of coronary heart disease (Valenzuela & Morgado, 1999; Oomen et al., 2001; Fernandez-San, 2009).

4.1 Soy lipids and health

The functional and nutritional value of vegetable oils is based in their fatty acid composition. Linolenic acid, with three double bonds, is extremely susceptible to oxidation (Gesteira et al., 2003). Omega polyunsaturated fatty acids are well known for their role in many physiological functions and seem to reduce risks of cardiovascular diseases. Several mechanisms have been proposed to explain the cardioprotective effect of W-3 PUF, including antiarrhythmic, hypolipidemic, and antithrombotic roles (Harper & Jacobson, 2001; Holguin et al., 2004). These fatty acids may also have beneficial effects on pancreatitis, arthritis, allergy and inflammatory diseases (Seki et al., 2010; Lund et al., 2010; Persson & Uller, 2010). They can modulate the anti-inflammatory process inside the blood vessels, changing the composition of the atherosclerotic plaque and reducing its rupture, which precedes infarction (Tull et al., 2009).

Burghardt et al. (2010) demonstrated the effects of dietary ratios of omega-3 (n-3) to omega-6 (n-6) polyunsaturated fatty acids (PUFAs) in controlling the markers of metabolic syndrome, including insulin sensitivity, inflammation, lipid profiles and adiposity. N-3 polyunsaturated fatty acids (n-3 PUFAs) reduce plasma triacylglycerols and improve the lipoprotein profile by decreasing the fraction of atherogenic small dense LDL (Carpentier et al., 2006) and serum cholesterol levels (Chandrashekar et al., 2010). High stearic acid soybean oil is a trans-free, oxidatively stable, non-LDL-cholesterol-raising oil that can be used to replace trans fatty acids in solid fat applications. In comparison with other saturated fatty acids, stearic acid lowers LDL cholesterol and is neutral with respect to HDL cholesterol (Hunter et al., 2010). Lemke et al. (2010) showed that stearidonic acid-enriched soybean oil increases the omega-3 index by raising erythrocyte eicosapentaenoic acid concentrations, suggesting that it may contribute to cardiovascular protective effects.

Studies correlate the role of n-3 PUFA in the beneficial effects on obesity, insulin resistance and secretion of bioactive adipokines including leptin, adiponectin and visfatin (Moreno-Aliaga et al., 2010).

Omega-3 fatty acids seem to have a small, dose-dependent hypotensive effect, the extent of which seems to be dependent on the degree of hypertension, with a greater effect in hypertensive patients and those with high-normal blood pressure (Kris-Etherton et al., 2003; Mori, 2010).

Supplementation of human diets with omega-3 fatty acids reduced several aspects of neutrophil, monocyte, and lymphocyte functions, including the production of inflammatory mediators (Kelley, 2001). N-3 PUFAs inhibit inflammatory signaling pathways (nuclear

factor-kappa B activity), down-regulate fatty acid (FA) synthesis and gene expression (sterol regulatory element binding protein-1c) and up-regulate gene expression involved in FA oxidation (peroxisome proliferator-activated receptor alpha) (Adkins & Kellen, 2010).

5. Isoflavones

Isoflavones, which are phenolic compounds that belong to the phytochemicals, are called phytoestrogens in soybeans because they exhibit estrogenic effects. They belong to a group called flavonoids, whose structure consists of a flavone nucleus with two benzene rings linked to heterocyclic pyrane. In the plant kingdom, soy contains the largest amount of isoflavones (Setchell & Cassidy, 1999; Carrão-Panizzi et al., 2002; Cederroth & Nef, 2009).

The chemical structure of these phenolic compounds is similar to that of cholesterol except for the presence of an extra methyl or ethyl group. However, plant sterols are much less absorbed in humans than cholesterol (Abumweis & Jone, 2008). Isoflavones are found mostly in soybeans in the form of ß-glucoside conjugates, which include daidzin, genistin, glycitin and their malonyl and acetyl derivatives. Genistein and daidzein are the primary isoflavones in soybean. Isoflavone glucosides, which are hydrolyzed by intestinal microflora glycosidases, are released in the form of daidzein, genistein and glycitein aglucones: the b-glycosides daidzin, genistin, and glycitin; the acetyl-b-glycosides 6"-O-acetyl-b-daidzin, 6"-O-acetyl-b-genistin, 6"-O-acetyl-b-glycitin; and the malonyl-b-glycosides 6"-O-malonyl-b-daidzin, 6"-O-malonyl-b-genistin, and 6"-O-malonyl-b-glycitin (Genoveze et al., 2006; Torrezan et al., 2008). Isoflavones are non-nutritive substances that may be related to beneficial health effects (Sacks et al., 2006; Carrara et al., 2009).

Soybeans are known to be rich in isoflavones, making them important components of human diet. The content of these compounds in different cultivars and different soy products may be variable. However, studies have shown that soybean processing causes a loss of isoflavones, e.g., in tofu: a 38% loss in normal soybean tofu, 31% in bleached soybean tofu, and 56% in lipoxygenase-free tofu (Ciabotti et al., 2006; Mateos-Aparicio et al., 2008).

5.1 Soy isoflavones and health
The use of soybeans in human diet can be associated with a reduction in health problems involving the risk of several chronic diseases such as diabetes, obesity, coronary heart disease, osteoporosis, and breast and prostate cancer (Brouns, 2002; Jenkins et al., 2003; Sacks et al., 2006; Mateos-Aparicio et al., 2008; Rajasree et al., 2009; Dong & Qin, 2011).

5.1.1 Soy isoflavones and menopause
Soy is widely employed to treat menopausal symptoms and its use has been studied in cross-cultural comparisons. These studies have shown that women living in countries where large amounts of soy are normally consumed (e.g., Japan) have fewer menopausal symptoms and lower rates of coronary heart disease, fractures, and uterine or breast cancer than women living in countries where soy is not part of the normal diet (e.g., the United States and Western Europe) (Adlercreutz et al., 1992; Oddens, 1994; Vitolins et al., 2010; Cho et al., 2010).

Soybean isoflavones are believed to be beneficial for menopausal women because this phase is characterized by suppression of ovarian function and decreased estrogen secretion, with consequent morphological and metabolic disorders resulting from hormonal decline (Setchell & Cassidy, 1999; Kang et al., 2010).

The daily ingestion of soybean and its isoflavones reduces the frequency of hot flashes. It is possible that these isoflavones can bind to free estrogen receptors and produce a weak estrogenic effect. This could be useful as a dietary alternative or supplement in postmenopausal hormone replacement therapy (Cancelo-Hidalgo & Castelo-Branco, 2010).

The consumption of soybean isoflavones can also improve lipid profiles in postmenopausal women and contribute to reduce the risk of coronary heart disease (Merz-Demlow et al., 2000; Wangen et al., 2001; Nikander et al., 2004).   Carmignani et al. (2010) evaluated the effects of dietary soy supplementation and concluded that it may be an effective alternative therapy for somatic and urogenital symptoms of menopause.

Soy isoflavones can also improve mood, vasomotor and other symptoms of menopause (Chedraui et al., 2010; Vitolins et al., 2010). Some authors have demonstrated the effects of soy isoflavones on of immune and oxidative stress markers in postmenopausal women (Ryan-Borchers et al, 2006).

The use of isoflavones also can be beneficial for postmenopausal women suffering from insomnia. In a study on postmenopausal women who took isoflavones for four months, Hachul et al. (2010) found a decrease in the intensity and number of hot flashes and reduced symptoms of insomnia (data confirmed by increased sleep efficiency, which was observed in a polysomnographic analysis).

5.1.2 Soy isoflavones and heart disease

Current guidelines recommend diet as the most effective way to prevent dyslipidemias, and some studies indicate that a plant-based diet is more effective in increasing blood lipid levels. The American Diabetes Association considers that a plant-based diet is healthful, nutritionally adequate, and may provide health benefits for the prevention and treatment of certain diseases (Ferdowsian & Barnard, 2009; Craig & Mangels, 2009).

Numerous epidemiological studies suggest that the regular ingestion of flavonoids can reduce the risk of many pathological conditions such as hypertension, diabetes, coronary heart disease, stroke and dementia (Ghosh & Schhepens, 2009). Sagara et al. (2004) observed significant reductions in systolic and diastolic blood pressure, total cholesterol and non-high density lipoprotein cholesterol (non-HDL-C) in a group treated with a daily diet of 20 g of soy protein and 80 mg of isoflavones. In their review, Taku et al. (2010) reported that a daily ingestion of 25-375 mg of soy isoflavones (aglycone equivalents) for 2-24 weeks significantly decreased systolic blood pressure by 1.92 mmHg compared with placebo in adults with normal blood pressure and prehypertension.

The main objective in the treatment of heart diseases with isoflavones is to decrease LDL-c because it is the most common cardiac risk factor (Nagarajan, 2010).

Soybean-based diets have been reported to prevent cardiac events such as atherosclerosis. This condition is traditionally associated with obesity, insulin resistance, diabetes, dyslipidemia and inflammatory processes. The latter condition is considered the main factor for the initiation and progression of atherosclerosis. Dyslipidemia by itself is a primary risk factor for cardiovascular disease, peripheral vascular disease, and stroke. Soy-based diets can decrease all these risk factors because of their protein and flavonoids content (Jenkins et al., 2002; Kreijkamp-Kaspers et al., 2004; Sacks et al., 2006; Abumweis & Jone, 2008; Wong et al., 2010; Nagarajan, 2010; Shukla et al., 2010).

Many studies have shown that isoflavones only affect the lipid profile when consumed in the presence of soy protein. Greaves et al. (1999) showed that monkeys responded to

isoflavonoids only if they also received soy protein. Probably these proteins facilitate the transport of isoflavonoids in the blood or their entry into target organs such as liver or muscle cells (Mikkola et al., 2003; Nikander et al., 2004).

Hyperglycemia is implicated in many vascular complications because it is related to oxidative stress and endothelial dysfunction. Soy isoflavones (genistein and daidzein) can protect the cells against hydrogen peroxide-induced apoptosis and promote inhibition of cell proliferation due to oxidative stress (Xu et al., 2009).

Although isoflavone activity has been linked to atheroprotective effects, it has been increasingly accepted that isoflavones may activate other nuclear receptors regulating lipid metabolism, such as liver x receptor, farnesoid x receptor and peroxisome proliferator-activated receptors (PPARs). PPARs, which are members of the nuclear hormone receptor superfamily of ligand-activated transcription factors, regulate the expression of target genes involved in several physiological processes such as lipid catabolism. This explains their hypolipidemic effects and glucose because they increase insulin sensitivity. This would be useful in the treatment of type 2 diabetes (Ricketts et al., 2005; Carrara et al., 2009).

5.1.3 Soy Isoflavones and bones

Osteoporosis involves the reduction of bone mass, leading to enhanced bone fragility and increased risk of fractures. This condition can affect postmenopausal women because the ovaries stop producing estrogens. The treatment for osteoporosis is long and therapeutics may also causeside effects (Bitto et al., 2010).

Osteoporosis is a skeletal disorder that predisposes to fractures. Postmenopausal women are susceptible to this condition because they go through a rapid phase of bone loss. This is preceded by declining concentrations of circulating estrogen, which plays an important role in maintaining the integrity of bone density by regulating bone formation and resorption. The intake of soybean isoflavones can help prevent the symptoms caused by loss of estrogen. Asian women are at lower risk of osteoporosis than Western women, which can be explained by the soybean-rich diet in Asian countries (Setchell & Cassidy, 1999; Nilsson & Gustafsson, 2002; Bawa, 2010).

The use of soybeans or isoflavone supplement foods may increase bone mass. The intake of genistein aglycone stimulates osteoblast and inhibits osteoclast function, preventing bone loss, and daidzein has been shown to increase bone mass in postmenopausal women. Soy isoflavone extract supplements increase lumbar spine bone mineral density in menopausal women (Kreijkamp-Kaspers et al., 2004; Bitto et al., 2010; Taku et al., 2010; Hooshmand et al., 2010; Bawa, 2010). Yamori et al. (2002) studied forty healthy female postmenopausal Japanese immigrants living in Brazil and found that urinary excretion of bone resorption markers was reduced in the isoflavone-treated group (37.3 mg per day for 10 weeks), while the placebo group showed no significant reduction. Differences in levels of urinary isoflavones and bone resorption markers between the two groups were significant.

In addition, studies have shown that soy can also be applied clinically to substitute calcium phosphate cements on bone regeneration. Perut et al. (2010) describe the osteoblastic response to injectable bone cement based on a composite formulation including bioactive soybean and gelatin foaming agents. They took advantage of the foam-forming capacity of defatted soybean and gelatin gels to establish porosity and aid in osteoblast adhesion and growth, suggesting that the use of this bioactive compound can help bone regeneration through less invasive surgery.

5.1.4 Soy isoflavones and cancer

Many studies have suggested that the use of soybeans in the regular diet can reduce levels of different cancers (Mateos-Aparicio et al., 2008; Liss et al., 2010). The consumption of soybeans can prevent prostate cancer through the anti-androgenic effects of isoflavones (Liss et al., 2010).

Oncology research has focused much attention on genistein (isoflavones found in soy and soy products) because it exerts a wide range of biological effects. Several epidemiological studies suggest that genistein is able to inhibit growth of primary tumors, which is the critical first step in the progression of metastatic disease. It can inhibit the development of metastatic disease in a variety of cancer types and inhibit cellular proliferation. It also increases the rate of cancer cell death, called apoptosis, and decreases the rate of cancer cell migration, which is important in the metastatic cascade (Pavese et al., 2010).

The use of soy isoflavones and its benefits for women with breast cancer has become a subject of public consensus. Dong & Qin (2011) observed that the consumption of soy isoflavones is associated with a significantly reduced risk of breast cancer incidence in Asian populations and is inversely associated with risk of breast cancer recurrence. Kang et al. (2010) reported that the intake of soy isoflavones is related to a lower risk of recurrence among postmenopausal breast cancer patients.

Soybean intake may be related to lower risk of developing breast cancer due to the presence of genistein (Cho et al., 2010). This isoflavone is a tyrosine kinase inhibitor and agonist of estrogen receptor-β, which is known to have antitumor properties. Lattrich et al. (2010) showed that genistein can enhance the antitumor activity of trastuzumab (a common therapy for women with breast cancer whose tumors have excessive HER2 protein) in breast cancer cells *in vitro*.

Genistein also may exert cytotoxic activity against breast cancer cells. This mechanism possibly involves mobilization of endogenous copper, whose levels are considerably increased in many types of cancers. Ullah et al. (2010) showed that this isoflavone can target endogenous copper, leading to prooxidant signaling and consequent cell death. The authors of this study attribute the anticancer effect of genistein to its preferential cytotoxicity towards cancer cells.

In addition to the above, the antioxidant and anti-inflammatory effects of genistein can protect normal tissues from the adverse effects of chemotherapy and radiation and can be helpful in ameliorating the side effects of chemotherapy (Tacyildiz et al., 2010).

5.1.5 Soy isoflavones and blood pressure

Arterial hypertension is related to vascular disease and death. Nevertheless, some factors are associated with the improvement of this condition. Soy products can reduce blood pressure. Longitudinal and clinical studies have shown that soy protein and isoflavones play an important role in diastolic blood pressure. The following mechanisms may explain the effects of soy protein and isoflavones on blood pressure: 1) improvement of systemic arterial compliance, 2) a natriuretic effect similar to the commonly used drug furosemide, 3) amino acid composition, and 4) lower salt retention. Soy protein and isoflavones also increase nitric oxide levels. This substance plays an effective role in regulating blood pressure and its increase is associated with the reduction of blood pressure via vasodilatory effects (Gimenez et al., 1998; Woodman et al., 2002; Nyby et al., 2004; Yang et al., 2005; Hermann et al., 2006; Taku et al., 2007; Azadbakht et al., 2007; Simão et al., 2010; Galleano et al., 2010).

6. Soy-derived phosphatidylserine and memory function

A gradual decline in cognitive function is very common with aging. Many neurochemical substances are associated with functional neuronal membrane and memory. Alterations in these substances and in their mechanisms of action are related to a decline in cognitive functions. Many studies have demonstrated the important role of phosphatidylserine in reducing and reversing age-related neurochemical damage in non-demented people with memory impairment and in some diseases (Ritcher et al., 2010).

Phosphatidylserine, an abundant phospholipid in the brain, can be extracted from bovine cortex to help improve the cognitive function of the elderly, including Alzheimer's patients. However, the use of bovine cortex may be unfeasible due to the minuscule quantity obtained from each animal and the risk of bovine spongiform encephalopathy. This fact emphasizes the importance of soy-derived phosphatidylserine (soybeans are used to produce soy-derived phosphatidylserine, which is produced from lecithin by an enzymatic reaction with L-serine). Kato-Kataoka et al. (2010) reported improvements in neuropsychological test scores and in memory ability in their test subjects treated with phosphatidylserine. These subjects were 50 to 69-year-old Japanese men and women suffering from subjective memory impairment. After six months of treatment, the authors found that soy-derived phosphatidylserine is a safety food ingredient that can improve memory functions in the elderly with impaired memory and can be useful as a supplement to prevent the development of dementia in men and women suffering from memory impairment (Amaducci, 1988; Zanotti et al., 1989; Engel et al., 1992; Vakhapova et al., 2010; Kato-Kataoka et al., 2010).

7. Conclusions

Soybeans represent an alternative food that is easy to consume while simultaneously possessing functional properties. They can be used as additives in new food products such as soy protein concentrates and soy protein isolates, soy milk, yogurt and tofu, grist, soybean flour, breads, crackers, cookies, breakfast cereals, diet supplements and textured soy protein used in meat products.

Furthermore, soy can be used as a functional food. It has an important protective effect against many chronic diseases because of its content of bioactive components, whose characteristics are beneficial for nutritional physiology and metabolism. Soy protein, isoflavones (phytoestrogens), non-digestible carbohydrates, and omega polyunsaturated fatty acids have gained considerable attention for their potential role in reducing the risk factors for cardiovascular disease, the incidence of certain cancers, inflammatory diseases, diabetes, osteoporosis and other disorders attributed to modern life styles and food habits, which have been confirmed by epidemiological and clinical studies.

In view of the above, it can be concluded that soybeans have multiple effects on human health and well-being, both as food and as remedy.

8. References

AbuMweis, S. S. & Jones, P. J. (2008). Cholesterol-lowering effect of plant sterols. *Current Atherosclerosis Reports.* Vol.10, No.6, (December 2008), pp. 467-72, ISSN 1534-6242

Adkins, Y. & Kelley, D. S. (2010). Mechanisms underlying the cardioprotective effects of omega-3 polyunsaturated fatty acids. *The Journal of Nutritional Biochemistry*, Vol.21, No.9, (May 2010), pp.781-92, ISSN 1873-4847

Adlercreutz, H.; Hamalainen, E.; Gorbach, S. & Goldin, B. (1992). Dietary phytooestrogens and the menopause in Japan. *Lancet.*Vol.339, No.1233, (May 1992), pp. 56-78, ISSN 1474-547X

Alfenas, R. C. G.; Bressan, J. & Paiva, A. C. (2010). Effects of protein quality on appetite and energy metabolism in normal weight subjects. *Arquivos Brasileiros de Endocrinologia e Metabologia*, Vol.54, No.1, (February, 2010), pp. 45-51, ISSN 1677-9487

Allison, D. B.; Gadbury, G.; Schwartz, L. G.; Murugesan, R.; Kraker, J. L.; Henshka, S.; Fontaine, K. R. & Heymsfield, S. B. (2003). A novel soy-based meal replacement formula for weight loss among obese individuals: a randomized controlled clinical trial. *European Journal of Clinical Nutrition*, Vol.57, No.4, (April 2003), pp. 514-522, ISSN 1476-5640.

Amaducci, L. (1988). The SMID group Phosphatidylserine in the treatment of Alzheimer's disease: results of a multicenter study. *Psychopharmacology Bulletin,* Vol.24, No.1, (1988), pp. 130–134, ISSN 0048-5764

Anderson, J. W.; Johnstone, B. M. & Cook-Newell, M. E. Meta-analysis of the effects of soy protein intake on serum lipids. *The New England Journal of Medicine*, Vol.333, No.5, (August 1995), pp. 276-82, ISSN 1533-4406

Awazuhara, H.; Kawai, H.; Baba, M.; Matsui, T. & Kamiyama, A. (1998). Antigenicity of the proteins in soy lecithin and soy oil in soybean allergy. *Clinical and Experimental Allergy*. Vol.28, No.12, (December 1998), pp.1559-1564, ISSN 1365-2222

Azadbakht, L.; Kimiagar, M. & Mehrabi Y. (2007). Soy inclusion in the diet improves features of the metabolic syndrome: a randomized crossover study in postmenopausal women. *American Journal of Clinical Nutrition*, Vol.85, No.3, (March 2007), pp. 735-41, ISSN 1938-3207

Badger, T. M.; Ronis, M. J. J.; Simmen, R. C. M. & Simmen, F. A. (2005). Soy Protein Isolate and Protection Against Cancer. *Journal of the American College of Nutrition*, Vol.24, No.2, (April 2005), pp.146S-149S, ISSN 1541-1087

Bansal, N. & Parle, M. (2010). Soybean supplementation helps reverse age- and scopolamine-induced memory deficits in mice. *Journal of Medicinal Food*, Vol.13, No.6, (December 2010), pp. 1293-300, ISSN 1096-620X

Bawa, S. (2010). The significance of soy protein and soy bioactive compounds in the prophylaxis and treatment of osteoporosis. *Journal of Osteoporosis*, Vol.8, (March 2010), in press. ISSN 891058.

Bermúdez, V.; Arráiz, N.; Aparicio, D.; Rojas, E.; Gotera, D.; Guerra, X.; Canelón, R.; Faría, J.; Sorell, L.; Amell, A.; Reyna, N.; Cabrera, M.; Mengual, E.; Cano, R.; Cano, C. & Velasco, M. (2010). Lipoprotein(a): from molecules to therapeutics. *American Journal of Therapeutics*, Vol.17, No.3, (May-June, 2010), pp. 263-73. ISSN 1536-3686

Bitto, A.; Polito, F.; Squadrito, F.; Marini, H.; D'Anna, R.; Irrera, N.; Minutoli, L.; Granese, R. & Altavilla, D. (2010). Genistein aglycone: a dual mode of action anti-osteoporotic soy isoflavone rebalancing bone turnover towards bone formation. *Current Medicinal Chemistry*. Vol.17, No.27, (2010), pp. 3007-18, ISSN 1875-533X

Brouns, F. (2002). Soybean isoflavones: a new and promising ingredient for the health foods sector. *Food Research International,* Vol. 35, No.2-3, (2002), pp.187-193, ISSN 0963-9969.

Burghardt, P. R.; Kemmerer, E. S.; Buck, B. J.; Osetek, A. J., Yan, C.; Koch, L. G., Britton, S. L. & Evans, S. J. (2010). Dietary n-3:n-6 fatty acid ratios differentially influence hormonal signature in a rodent model of metabolic syndrome relative to healthy controls. *Nutrition & Metabolism*, Vol.7, No.53, (June 2010), 8 pages, ISSN 1743-7075.

Cancelo-Hidalgo, M. J. & Castelo-Branco, C. (2010). Optimizing soy isoflavones effect in postmenopausal women: the impact of timing on climacteric symptoms. *Gynecological Endocrinology*, (October 2010), in press, ISSN 0951-3590

Carmignani, L. O.; Pedro, A. O.; Costa-Paiva, L. H. & Pinto-Neto, A. M. (2010). The effect of dietary soy supplementation compared to estrogen and placebo on menopausal symptoms: a randomized controlled trial. *Maturitas*, Vol.67, No.3, (November 2010), pp. 262-9, ISSN 1873-4111

Carpentier, Y. A.; Portois, L. & Malaisse, W. J. (2006). n-3 fatty acids and the metabolic syndrome. *The American Journal of Clinical Nutrition*, Vol. 83, S.6, (May 2006), pp. 1499S-1504S, ISSN 1938-3207

Carrara, V. S.; Amato, A. A.; Neves, F. A. R.; Bazotte, R. B.; Mandarino, J. M. G.; Nakamura, C. V.; Filho, B. P. D. & Cortez, D. A. G. (2009). Effects of a methanolic fraction of soybean seeds on the transcriptional activity of peroxisome proliferator-activated receptors (PPAR). *Brazilian Journal of Medical and Biological Research*, Vol.42, No.6, (June 2009), pp. 545-550, ISSN 0100879X

Carrão-Panizzi, M. C.; Favoni, S. P. G. & Kikuchi, A. (2002). Extraction time for soybean isoflavone determination. *Brazilian Archives of Biology and Technology*, Vol.45, No.4, (2002), pp.515-518, ISSN 15168913

Cederroth, C. R. & Nef, S. (2009). Soy, phytoestrogens and metabolism: A review. *Molecular and Cellular Endocrinology,* Vol.304, No.1-2, (May 2009), pp. 30-42, ISSN 1872-8057

Chandalia, M.; Garg, A.; Lutjohann, D.; Von Bergmann, K.; Grundy, S. M. & Brinkley, L. J. (2000). Beneficial effects of high dietary fiber intake in patients with type 2 Diabetes Mellitus. *The New England of journal of medicine*, Vol.342, No.19, (May 2000), pp. 342:1392-1398, ISSN 0028 4793

Chandrashekar, P.; Lokesh, B. R. & Gopala Krishna, A. G. (2010). Hypolipidemic effect of blends of coconut oil with soybean oil or sunflower oil in experimental rats. *Food Chemistry*, Vol.123, No.3, (December 2010), pp. 728-733, ISSN 0308-8146

Chedraui, P.; San Miguel, G. & Schwager, G. (2010). The effect of soy-derived isoflavones over hot flushes, menopausal symptoms and mood in climacteric women with increased body mass index. *Gynecological Endocrinology*, (June 2010), in press, ISSN 1473-0766

Chen, W.; Duizer, L.; Corredig, M. & Goff, H. D. (2010). Addition of soluble soybean polysaccharides to dairy products as a source of dietary fiber. *Journal of Food Science,* Vol.75, No.6, (August 2010), pp. 478-84, ISSN *0022-1147*

Cho, Y.A.; Kim, J.; Park, K.S.; Lim, S.Y.; Shin, A.; Sung, M.K. & Ro, J. (2010). Effect of dietary soy intake on breast cancer risk according to menopause and hormone receptor status. *European journal of clinical nutrition,*Vol.64, No.9, (September 2010), pp. 924-32, ISSN 1476-5640

Chung, T. K. H.; Yip, S. K.; Lam, P.; Chang, A. M. Z. & Haines, C. J. (1996). A randomized, double-blind, placebo-controlled, crossover study on the effect of oral estradiol on acute menopausal symptoms. *Maturitas*, Vol 25, No.2, (October 1996), pp. 115-123, ISSN 1873-4111

Ciabotti, S.; Barcellos, M. F. P.; Mandarino, J. M. G. & Tarone, A. G. (2006). Chemical and biochemical evaluation of grains, soymilk and tofus of normal soybean and Lipoxygenase-free soybeans. *Ciência e Agrotecnologia,* Vol.30, No.5, (October 2006), pp. 920-929, ISSN 1413-7054

Craig, W. J. & Mangels, A. R. (2009). American Dietetic Association. Position of the American Dietetic Association: vegetarian diets. *Journal of the American Dietetic Association*, Vol.109, No.7, (July 2009), pp. 1266-82, ISSN 00028223

Crittenden, R. G. & Playne, M. J. (1996). Production, properties and applications of food-grade oligosaccharides. *Trends in Food Science & Technology*, Vol.7, No.11, (November 1996), pp. 353-361, ISSN *0924-2244*

De Leo, F.; Panarese, S.; Gallerani, R. & Ceci, L. R. (2009). Angiotensin converting enzyme (ACE) inhibitory peptides: production and implementation of functional food. *Current pharmaceutical design,* Vol.15, No.31, (December 2009), pp. 3622-43, ISSN 1381-6128

Dong, J. Y. & Qin, L. Q. (2011). Soy isoflavones consumption and risk of breast cancer incidence or recurrence: a meta-analysis of prospective studies. *Breast Cancer Research and Treatment,* Vol.125, No.2, (January 2011), pp. 315-23, ISSN 0167-6806

Dupont, C.; Chouraqui, J. P.; de Boissieu, D.; Bocquet, A.; Bresson, J. L.; Briend, A.; Darmaun, D.; Frelut, M. L.; Ghisolfi, J.; Girardet, J. P.; Goulet, O.; Hankard, R.; Rieu, D.; Rigo, J.; Vidailhet, M. & Turck, D. (2010). Comité de nutrition de la Société française de pédiatrie. Dietetic treatment of cow's milk protein allergy. *Archives de Pediatrie*, (November 2010), in press, ISSN 0096-6630

Endres, J. G. (Month 2001). Soy protein products: characteristics, nutritional aspects and utilization (edition). Libray of Congress, AOCS Press ISBN 1-893997-27-8. United States of America (http://books.google.com.br/books?hl=ptBR&lr=&id=3RNa1vS0sZYC&oi=fnd&pg=PR5&dq=Soy+protein+products+characteristics,+nutritional+aspects+and+utilization&ots=r5evi_iCW7&sig=-mkmRHmHwnh1aD1wmHslxcfYzEA#v=onepage&q&f=false).

Engel, R. R.; Satzger, W.; Günther, W.; Kathmann, N.; Bove, D.; Gerke, S.; Münch, U. & Hippius, H. Double-blind cross-over study of phosphatidyl-serine vs. placebo in patients with early dementia of Alzheimer type. European Neuropsychopharmacology, Vol.2 , No.2 (June 1992), pp. 149–155, ISSN 1873-7862

Espinosa-Martos, I. & Rupérez, P. (2006). Soybean oligosaccharides. Potencial as new ingredients in functional food. *Nutricion Hospitalaria,*Vol.21, No.1, (January 2006), pp. 92-6, ISSN 0212-1611

Ferdowsian, H. R. & Barnard, N. D. (2009). Effects of plant-based diets on plasma lipids. *The American Journal of Cardiology,*Vol.104, No.7, (October 2009), pp. 947-56, ISSN 00029149

Fernández-San, J. P. M. (2009). Trans fatty acids (tFA): sources and intake levels, biological effects and content in commercial Spanish food/ Ácidos grasos trans (AGt): fuentes y niveles de ingesta, efectos biológicos y contenido en los alimentos comerciales

españoles. *Nutricion Hospitalaria*, Vol.24, No.5, (September 2009), pp. 515-520, ISSN 0212-1611

Friedman, M. & Brandon, D. L. (2001). Nutritional and health benefits of soy proteins. *Journal of Agricultural and Food Chemistry*, Vol.49, No.3, (March 2001), pp. 1069-1086, ISSN 1520-5118

Food and Drug Administration (FDA). FDA approves new health claim for soybean protein and coronary heart disease. FDA Talk Paper 1999, in www.fda.gov.

Galleano, M.; Pechanova, O. & Fraga, C. G. (2010). Hypertension, nitric oxide, oxidants, and dietary plant polyphenols. *Current Pharmaceutical Biotechnology*, Vol.11, No.8, (December 2010), pp. 837-48, ISSN 1389-2010

Genovese, M. I.; Davila, J. & Lajolo, F. M. (2006). Isoflavones in processed soybean products from Ecuador. *Brazilian Archives of Biology and Technology*, Vol.49, No.5, (September 2006), pp. 853-859, ISSN 1516-8913

Gesteira, A. S.; Schuster, I.; José, I. C.; Piovesan, N. D.; Viana, J. M. S.; Barros, E. G. & Moreira M. A . (2003). Biometrical analyses of linolenic acid content of soybean seeds. *Genetics and Molecular Biology.* Vol.26, No.1 (September 2003), pp. 65-68, ISSN 1415-4757

Ghosh, D. & Scheepens, A. (2009). Vascular action of polyphenols. *Molecular Nutrition and food research*, Vol.53, No.3, (March 2009), pp. 322-31, ISSN 1613-4133

Gimenez, I.; Martinez, R. M.; Lou, M.; Mayoral, J. A.; Garay, R. P. & Alda, J. O. (1998). Saliuretic action by genistein in the isolated, perfused rat kidney. Hypertension, Vol.31, No.2, (February 1998), pp. 706-11, ISSN 0194911X

Greaves, K. A.; Parks, J. S.; Williams, J. K. & Wagner, J. D. (1999). Intact dietary soy protein, but not adding an isoflavone-rich soy extract to casein, improves plasma lipids in ovariectomized cynomolgus monkeys. *Journal of Nutrition*, Vol.129, No.8, (August 1999), pp. 1585–1592, ISSN 0022-3166

Hachul, H.; Brandão, L. C.; D'Almeida, V.; Bittencourt, L. R.; Baracat, E. C. & Tufik, S. (2010) Isoflavones decrease insomnia in postmenopause. *Menopause*, (August 2010), in press, ISSN 1530-0374

Harper, C. R. & Jacobson, T. A. (2001). The Fats of Life - The Role of Omega-3 Fatty Acids in the Prevention of Coronary Heart Disease. *Archives of Internal Medicine,* Vol.8, No.61, (October 2001), pp. 2185-2192, ISSN 1538-3679

Hermann, M.; Flammer, A. & Lüscher, T. F. (2006). Nitric oxide in hypertension. Journal of Clinical Hypertension, Vol.8, No.12, (May 2006), pp. 17-29, ISSN 1938-3207

Hirayama, F.; Lee, A. H.; Binns, C. W.; Hiramatsu, N.; Mori, M. & Nishimura, K. (2010).Dietary intake of isoflavones and polyunsaturated fatty acids associated with lung function, breathlessness and the prevalence of chronic obstructive pulmonary disease: Possible protective effect of traditional Japanese diet. *Molecular Nutrition and food research,*Vol.54, No.7, (July 2010), pp. 909-17, ISSN 1613-4133

Holguin, F.; Téllez-Rojo, M. M.; Lazo, M.; Mannino, D.; Schwartz, J.; Hernández, M. & Romieu, I. (2004). Cardiac Autonomic Changes Associated With Fish Oil vs Soy Oil Supplementation in the Elderly. *Chest Journal*, Vol.127, No.4, (April 2004), pp. 1102-1107, ISSN 1931-3543

Hooshmand, S.; Juma, S. & Arjmandi, B. H. (2010). Combination of genistin and fructooligosaccharides prevents bone loss in ovarian hormone deficiency. *Journal of medicinal food*, Vol.13, No.2, (April 2010), pp. 320-5, ISSN 1557-7600

Huisman, M. M. H. (2000). Elucidation of the chemical fine structure of polysaccharides from soybean and maize kernel cell walls. PhDthesis, Wageningen University, (March, 2000), ISBN 9058081877, Wageningen, Netherlands

Hunter, J. E.; Zhang, J. & Kris-Etherton, P. M. (2010). Cardiovascular disease risk of dietary stearic acid compared with trans, other saturated, and unsaturated fatty acids: a systematic review. *American Journal of Clinical Nutrition,* Vol.91, No.1, (January 2010), pp. 46-63, ISSN 1938-3207

Jasentuliyana, N.; Toma, R. B.; Klavons, J. A. & Medora, N. (1998). Beverage cloud stability with isolated soy protein. *Journal of the Science of Food and Agriculture,* Vol.78, No.3, (November 1998), pp. 389–394, ISSN 1097-0010

Jequier, E. (2002). Pathways to obesity. *International journal of obesity and related metabolic disorders,*Vol.26, No.6, (September 2002), pp. 12-7, ISSN 0307-0565

Jenkins, D. J. A.; Kendall, C. W. C.; Marchie, A Alexandra L Jenkins, A. L.; Augustin, L. S. A.; Ludwig, D. S.; Barnard, N. D. & Anderson, J. W. (2003). Type 2 diabetes and vegetarian diet. *American journal of clinical nutrition*, Vol.78 No.3, (September 2003), pp. 610-616, ISSN 1938-3207

Jenkins, D. J.; Kendall, C. W.; Jackson, C. J.; Connelly, P. W.; Parker, T.; Faulkner, D.; Vidgen, E.; Cunnane, S. C.; Leiter, L. A. & Josse, R. G. (2002). Effects of high- and low-isoflavone soyfoods on blood lipids, oxidized LDL, homocysteine, and blood pressure in hyperlipidemic men and women. *American journal of clinical nutrition*, Vol.76, No2., (August 2002), pp. 365–372, ISSN 1938-3207

Jiménez-Escrig, A.; Tenorio, M. D.; Espinosa-Martos, I. & Rupérez, P. (2008). Health-promoting effects of a dietary fiber concentrate from the soybean byproduct okara in rats. *Journal of agricultural of food chemistry*, Vol.56, No.16, (August 2008), pp. 7495-501, ISSN 1520-5118

Kang, J.; Badger, T. M.; Ronis, M. J. & Wu, X. (2010). Non-isoflavone phytochemicals in soy and their health effects. *Journal of agricultural of food chemistry*, Vol.58, No.14, (July 2010), pp. 8119-33, ISSN 1520-5118

Kang, X.; Zhang, Q.; Wang, S.; Huang, X. & Jin, S. (2010). Effect of soy isoflavones on breast cancer recurrence and death for patients receiving adjuvant endocrine therapy. *Canadian Medical Association Journal,* Vol.182, No.17, (November 2010), pp. 1857-62, ISSN 0820-3946

Kato-Kataoka, A.; Sakai, M.; Ebina, R.; Nonaka, C.; Asano, T. & Miyamori T. (2010). Soybean-derived phosphatidylserine improves memory function of the elderly Japanese subjects with memory complaints. *Journal of Clinical Biochemistry and Nutrition*. Vol.47, No.3, (November 2010), pp. 246-55, ISSN 1880-5086

Kelley, D. S. (2001). Modulation of human immune and inflammatory responses by dietary fatty acids. *Nutrition*, Vol.17, No.7, (July 2001), pp. 669-673, ISSN 1873-1244Kreijkamp-Kaspers, S.; Kok, L.; Grobbee, D. E.; de Haan, E. H.; Aleman, A.; Lampe, J. W. & van der Schouw, Y. T. (2004). Effect of soy protein containing isoflavones on cognitive function, bone mineral density, and plasma lipids in postmenopausal women: a randomized controlled trial. *JAMA: the journal of the American Medical Association*, Vol.292, No.1, (July 2004), pp. 65–74, ISSN 1538-3598

Kris-Etherton, P. M.; Harris, W. S.; Lawrence J. Appel, L. J. (2003). Fish Consumption, Fish Oil, Omega-3 Fatty Acids, and Cardiovascular Disease. *Arteriosclerosis, Thrombosis, and Vascular Biology,* Vol.23, No.2, (February 2003), pp. e-20-e-30, ISSN 10795642

Lattrich, C.; Lubig, J.; Springwald, A.; Goerse, R.; Ortmann, O. & Treeck, O. (2010). Additive effects of trastuzumab and genistein on human breast cancer cells. *Anticancer Drugs*, (December 2010), in press, ISSN 1473-5741

Lemke S. L.; Vicini, J. L.; Su, H.; Goldstein, D. A.; Nemeth, M. A.; Krul, E. S. & Harris, W. S. (2010). Dietary intake of stearidonic acid-enriched soybean oil increases the omega-3 index: randomized, double-blind clinical study of efficacy and safety. *American journal of clinical nutrition,* Vol.92, No.4, (October 2010), pp. 766-75, ISSN 1938-3207

Lerman, R. H.; Minich, D. M.; Darland, G.; Lamb, J. J.; Chang, J. L.; Hsi, A.; Bland, J. S. & Tripp, M. L. (2010). Subjects with elevated LDL cholesterol and metabolic syndrome benefit from supplementation with soy protein, phytosterols, hops rho iso-alpha acids, and Acacia nilotica proanthocyanidins. *Journal of Clinical Lipidology*, Vol.4, No.1, (January-February 2010), pp. 59-68, ISSN 1933-2874

Liss, M. A.; Schlicht, M.; Kahler, A.; Fitzgerald, R.; Thomassi, T.; Degueme, A.; Hessner, M. & Datta, M. W. (2010). Characterization of soy-based changes in Wnt-frizzled signaling in prostate cancer. *Cancer Genomics Proteomics,* Vol.7, No.5, (September-October 2010), pp. 245-52, ISSN 1790-6295

Lissin, L. W. & Cooke, J. P. (2000). Phytoestrogens and cardiovascular health. *Journal of the American College of Cardiology*, Vol.35, No.6, (May 2000), pp.1403-1410, ISSN 1558-3597

Liu, K. (1997). Soybeans chemistry technology and utilization. 2.ed. New York: Chapman & Hall. 532p. http://www.scielo.br/scieloOrg/php/reflinks.php?refpid=S0100-204X200700120001600010&pid=S0100-204X2007001200016&lng=en.

Liu, K. S. (1999). Chemistry and nutritional value of soybean components. In Soybeans: Chemistry, Technology and Utilization. *Aspen Publ. Inc,* pp. 25-113, ISBN 0834212994

Lukaczer, D.; Liska, D. J.; Lerman, R. H., Darland, G.; Schiltz, B.; Tripp, M. & Bland, J. S. (2006). Effect of a low glycemic index diet with soybean protein and phytosterols on CVD risk factors in postmenopausal women. *Nutrition,* Vol.22, No.2, (February 2006), pp. 104-113, ISSN 1873-1244

Lund, T.; Mangsbo, S.M.; Scholz, H.; Gjorstrup, P.; Tötterman, T.H.; Korsgren, O.; Foss, A. (2010). Resolvin E1 reduces proinflammatory markers in human pancreatic islets in vitro. *Experimental and Clinical Endocrinology & Diabetes*, Vol.118, No.4, (April 2010), PP.237-44, ISSN 1439-3646

Macedo-Silva, A.; Shimokomaki, M.; Vaz, A. J.; Yamamoto, Y. Y. & Tenuta-Filho, A. (2001). Textured Soy Protein Quantification in Commercial Hamburger . *Journal of Food Composition and Analysis*, Vol.14, No.5, (October 2001), pp. 469-478, ISSN *0889-1575*

Maki, K. C.; Butteiger, D. N.; Rains, T. M.; Lawless, A.; Reeves, M. S.; Schasteen, C. & Krul, E. S. (2010). Effects of soy protein on lipoprotein lipids and fecal bile acid excretion in men and women with moderate hypercholesterolemia. *Journal of Clinical Lipidology*, Vol.4, No.6, (November-December 2010), pp. 531-42, ISSN 1933-2874

Martínez Augustin, O. & Martínez de Victoria, E. (2006). Proteins and peptides in enteral nutrition. *Nutricion Hospitalaria,*Vol.21, Suppl 2:, (November 2006), pp. 01-14, ISSN 0212-1611

Martin, C. A.; Visentainer, J. V.; Oliveira, A. N.; Oliveira, C. C.; Matsushita, M. & Souza, N. E. (2008). Fatty acid contents of Brazilian soybean oils with emphasis on trans fatty

acids. *Journal of the Brazilian Chemical Society*, Vol.19, No.1, (2008), pp.117-122, ISSN: 0103-5053

Martin, D.; Song, J.; Mark, C. & Eyster, K. (2008). Understanding the cardiovascular actions of soy isoflavones: potential novel targets for antihypertensive drug development. *Cardiovascular & hematological disorders drug targets,* Vol.8, No.4, (December 2008), pp. 297-312, ISSN 1871-529X

Martins, R. T.; Cascabulho, A. R.; Baião, N. C. & Afonso, R. J. C. F. (2003). Effect of different soybean oils on fatty acids composition of broiler carcass. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia,* Vol.55, No.1, (February 2003), pp. 92-98, ISSN 0102-0935

Mateos-Aparicio, I.; Cuenca, A. R.; Villanueva-Soares, M. J. & Zapata-Revilla, M. A. (2008). Soybean, a promising health source. *Nutricion Hospitalaria*, Vol.23, No.4, (August, 2008), pp. 305-312, ISSN 0212-1611

Merz-Demlow, B. E.; Duncan, A. M.; Wangen, K. E.; Xu, X.; Carr, T. P.; Phipps, W. R. & Kurzer, M. S. (2000). Soy isoflavones improve plasma lipids in normocholesterolemic, premenopausal women. *American journal of clinical nutrition*, Vol.71, No.6, (June 2000), pp. 1462–1469., ISSN 1938-3207

Mesa, N. J. E.; Alavi, S.; Singh, N.; Shi, Y. C.; Dogan, H. & Sang, Y. (2009). Soy protein-fortified expanded extrudates: Baseline study using normal corn starch. *Journal of Food Engineering*, Vol.90, No.2, (January 2009), pp. 262-270, ISSN 0260-8774

Messina, M. J. (1997). Soybeanfoods: their role in disease prevention and treatment. In Soybeans: Chemistry, Technology and Utilization. Aspen Publicashion, p. 442-447, ISBN 0834212994

Messina, M. (2010). Insights Gained from 20 Years of Soy Research. *The journal of nutrition*, Vol.140, No.12, (December 2010), pp. 2289S-2295S, ISSN 1541-6100

Mikkola,T. S.; Anthony, M. S.; Clarkson, T. B. & St. Clair, R. W. (2003). Serum cholesterol efflux potential is an independent predictor of coronary artery atherosclerosis. *Atherosclerosis*, Vol.170, No.1, (September 2003), pp.31–38, ISSN 1879-1484

Miura, E. M. Y.; Binotti, M. A. R.; Camargo, D. S.; Mizubutti, I. Y. & Ida, E. I. (2001). Avaliação biológica da soja com baixas atividades de inibidores de tripsina e ausência do inibidor Kunitz. *Archivos Latinoamericanos de Nutrición*, Vol.51, No.2, (June 2001), pp.195-198, ISSN 0004-0622

Monteiro, M. R. P.; Moreira, M. A.; Costa, N. M. B.; Oliveira, M. G. & Pires, C. V. (2003). Avaliação da digestibilidade protéica de genótipos de soja com ausência e presença do inibidor de tripsina Kunitz e lipoxigenases. *Brazilian Journal of Food Technology*, Vol.32, No.4, (July-August, 2003), pp. 99-107, ISSN 0004-0622

Moreno-Aliaga, M. J.; Lorente-Cebrián, S. & Martínez, J. A. (2010). Regulation of adipokine secretion by n-3 fatty acids. *The Proceedings of the Nutrition Society*. Vol.69, No.3, pp. 324-34, ISSN 0029-6651

Mori, T. A. (2010). Omega-3 fatty acids and blood pressure. *Cellular and Molecular Biology*, Vol.56, No.1, pp. 83-92, ISSN 1165-158X

Moughan, P. J. (2003). Amino acid availability: aspects of chemical analysis and bioassay methodology. *Nutrition Research Reviews*, Vol.16, No.2, (December 2003), pp.127-141, ISSN 1475-2700

Nagarajan, S. (2010). Mechanisms of anti-atherosclerotic functions of soy-based diets. *The journal of nutritional biochemistry*, Vol.21, No.4, (April 2010), pp. 255-60, ISSN 1541-6100

Nikander, E.; Tiitinen, A.; Laitinen, K.; Tikkanen, M. & Ylikorkala, O. (2004). Effects of isolated isoflavonoids on lipids, lipoproteins, insulin sensitivity, and ghrelin in postmenopausal women. *The Journal of clinical endocrinology and metabolism*. Vol.89, No.7, (July 2004), pp. 3567–3572, ISSN 1945-7197

Nilsson, S. & Gustafsson, J. A. (2002). Biological role of estrogen and estrogen receptors. Critical Reviews in Biochemistry and Molecular Biology, Vol.37, No.1, (2002), pp. 1–28, ISSN 1040-9238

Nyby, M. D.; Abedi, K.; Eslami, P.; Hernandez, G.; Smutko, V.; Berger, M. E. & Tuck, M. L. (2004). Dietary fish oil prevents hypertension, oxidative stress and suppression of endothelial nitric oxide synthase expression in fructose-fed rats. American Journal of Hypertensive, Vol.17, S.1, (May 2004), pp. 216A. ISSN 1879-1905

Oddens, B. M. (1994). The climacteric cross-culturally: The International Health Foundation South-east Asia Study. *Maturitas*, Vol.19, No.3, (October 1994), pp. 155–156, ISSN 1873-4111

Oomen, C.; Ocké, M. C.; Feskens, E. J.; Van Erp-Baart, M. A. J.; Kok, F. J. & Kromhout, D. (2001). Association between trans fatty acids intake and 10-year risk of coronary heart disease in the Zutphen Elderly Study: a prospective population-based study. *Lancet,* Vol.357, No.9258, (March 2001), pp.746-751, ISSN 1474-547X

Onuegbu, A. J.; Olisekodiaka, J. M.; Onibon, M. O.; Adesiyan, A. A. & Igbeneghu, C. A. (2011). Consumption of Soymilk Lowers Atherogenic Lipid Fraction in Healthy Individuals. *Journal of Medicinal Food,* (January 2011), pp. 1-4, in press, ISSN 1557-7600.

Paddon-Jones, D.; Westman, E.; Mattes, R. D.; Wolfe, R. R.; Astrup, A. & Westerterp-Plantenga, M. (2008). Protein, weight management, and satiety. *American journal of clinical nutrition*, Vol.87, No.5, (May 2008), pp. 1558-1561, ISSN 1938-3207

Pavese, J. M.; Farmer, R. L. & Bergan, R. C. (2010). Inhibition of cancer cell invasion and metastasis by genistein. *Cancer Metastasis Reviews.*, Vol.19, No.3, (September 2010), pp. 465-82, ISSN 1573-7233

Perut, F.; Montufar, E. B.; Ciapetti, G.; Santin, M.; Salvage, J.; Traykova, T.; Planell, J. A.; Ginebra, M. P. & Baldini, N. (2010). Novel soybean/gelatine-based bioactive and injectable hydroxyapatite foam: material properties and cell response. *Acta Biomaterialia*, (December 2010), in press, ISSN 1878-7568

Persson, C.G.; Uller, L. (2010). Resolution of cell-mediated airways diseases. *Respiratory Research,* Vol.11, No.75 (June 2011), pp.1-12. ISSN 1465-993X

Priolii, R. H. G.; Mendes Junior, C. T.; Arantes, N. E. & Contel, E. P. B. (2002). Characterization of brazilian soybean cultivars using microsatellite markers. *Genetics and molecular biology*, Vol.25, No.2, (June 2002), ISSN 1415-4757

Rajasree, C. R.; Rajmohan, T. & Augusti, K. T. (2009). Antiatherogenic and antiperoxidative effects of garlic and soy proteins in alcohol fed rats. *Indian Journal of Experimental Biology*, Vol.47, No.3, (March 2009), pp. 169-75, ISSN 0975-1009

Reynolds, K.; Chin, A.; Lees, K. A.; Nguyen, A.; Bujnowski, D. & He, J. (2006). A Meta-Analysis of the Effect of Soybean Protein Supplementation on Serum Lipids. *The*

American journal of cardiology, Vol.98, No.5, (September 2006), pp. 633-640, ISSN 00029149

Redondo-Cuenca, A.; Villanueva-Suárez, M. J.; Rodríguez-Sevilla, M. D. & Mateos-Aparicio, I. (2007). Chemical composition and dietary fibre of yellow and green commercial soybeans (Glycine max). *Food Chemistry*, Vol.101, No.3, (March 2007), pp. 1216-1222, ISSN 0308-8146

Riaz, M. N. (1999). Soybeans as Functional Foods. *Cereal Foods World*, Vol.44, No.2, (February 1999), pp. 89-92, ISSN 0146-6283

Ricketts, M. L.; Moore, D. D.; Banz, W. J.; Mezei, O. & Shay, N. F. (2005). Molecular mechanisms of action of the soy isoflavones includes activation of promiscuous nuclear receptors. A review. *The Journal of nutritional biochemistry,* Vol.16, No.6, (June 2005), pp. 321-330, ISSN 1873-4847

Richter, Y.; Herzog, Y.; Cohen, T. & Steinhart, Y. (2010). The effect of phosphatidylserine-containing omega-3 fatty acids on memory abilities in subjects with subjective memory complaints: a pilot study. *Clinical Interventions in aging*, Vol.2, No.5, (November 2010), pp. 313-6, ISSN 1176-9092

Ryan-Borchers, T.A.; Park, J.S.; Chew, B.P.; McGuire, M.K.; Fournier, L.R.; Beerman, K.A. (2006). Soy isoflavones modulate immune function in healthy postmenopausal women. American Journal of Clinical Nutrition, Vol. 83, No.5, (May 2006), pp.1118-25, ISSN 1938-3207

Sacks, M.; Sacks, M. D.; Lichtenstein, A. D. S.; Van Horn,L.; Harris, W.; Kris-Etherton, P. & Winston, M. (2006). Soy Protein, Isoflavones, and Cardiovascular Health. *Circulation,* Vol.113, No.7, (February 2006), pp. 1034-1044, ISSN 1524-4539

Sagara, M.; Kanda, T.; NJelekera, M.; Teramoto, Y.; Armitage, L.; Birt, N.; Birt, C. & Yamori, Y. (2004). Effects of Dietary Intake of Soy Protein and Isoflavones on Cardiovascular Disease Risk Factors in High Risk, Middle-Aged Men in Scotland. *Journal of the American College of Nutrition*, Vol.23, No.1, (February 2004), pp. 85-91, ISSN 1541-1087

Scheppach, W.; Luethrs, H.; Melcher, R.; Gostner, A.; Schauber, J.; Kudlich, T.; Weiler, F. & Menzel , T. (2004). Antiinflammatory and anticarcinogenic effects of dietary fibre. *Clinical nutrition supplements*, Vol.1, No.2, (February 2004), pp. 51-58, ISSN 1744-1161

Seki, H.; Fukunaga, K.; Arita, M.; Arai, H.; Nakanishi, H.; Taguchi, R.; Miyasho, T.; Takamiya, R.; Asano, K.; Ishizaka, A.; Takeda, J.; Levy, B.D. (2010). The anti-inflammatory and proresolving mediator resolvin E1 protects mice from bacterial pneumonia and acute lung injury. *The Journal of Immunology,* Vol.184, No.2, (June 2010), pp.836-43, ISSN 1550-6606.

Setchell, K. D. R. & Cassidy, A. (1999). Dietary isoflavones: Biological effects and relevance to human health. *The journal of nutrition,* Vol.129, No.3, (March 1999), pp.758-767, ISSN 1541-6100

Simão, A. N.; Lozovoy, M. A.; Simão, T. N.; Dichi, J. B.; Matsuo, T. & Dichi, I. (2010). Nitric oxide enhancement and blood pressure decrease in patients with metabolic syndrome using soy protein or fish oil. *Arquivos Brasileiros de Endocrinologia e Metabologia*, Vol.54, No.6, (August 2010), pp. 540-5, ISSN 1677-9487

Soares L. L.; Lucas, A. M. M. & Boaventura, G. T. (2005). Can organic and transgenic soy be used as a substitute for animal protein by rats? *Brazilian journal of medical and biological research,* Vol.38, No.4, (April 2005), pp.583-586, ISSN 0100879X

Shukla, S. K.; Gupta, S.; Ojha, S. K. & Sharma, S. B. (2010). Cardiovascular friendly natural products: a promising approach in the management of CVD. *Natural Product Research,* Vol.27, No.9, (May 2010), pp. 873-98, ISSN 1478-6427

Singh, P.; Kumar, R.; Sabapathy, S. N. & Bawa, A. S. (2008). Functional and Edible Uses of Soy Protein Products. *Comprehensive Reviews in Food Science and Food Safety,* Vol.7, No.1, (January 2008), pp. 14–28, ISSN 1541-4337

Sosulski, F. W.; Elkowicz, L. & Reichert, R. D. (1982). Oligosaccharides in eleven legumes and their air-classified protein and starch fractions. *Journal of Food Science*, Vol.47, No.2, (March 1982), pp. 498-502, ISSN *0022-1147*

Tacyildiz, N.; Ozyoruk, D.; Yavuz, G.; Unal, E.; Dincaslan, H.;, Dogu, F.; Sahin, K. & Kucuk, O. (2010). Soy isoflavones ameliorate the adverse effects of chemotherapy in children. *Nutrition and Cancer*, Vol.62, No.7. (October 2010), pp.1001-5, ISSN 1532-7914

Taku, K.; Melby, M. K.; Takebayashi, J.; Mizuno, S.; Ishimi, Y.; Omori, T. & Watanabe, S. (2010). Effect of soy isoflavone extract supplements on bone mineral density in menopausal women: meta-analysis of randomized controlled trials. *Asia Pacific journal of Clinical Nutrition,* Vol.19, No.1, (March 2010), pp. 33-42, ISSN 1440-6047

Taku, K.; Umegaki, K.; Sato, Y.; Taki, Y.; Endoh, K. & Watanabe, S. (2007). Soy isoflavones lower serum total and LDL cholesterol in humans: a meta-analysis of 11 randomized controlled trials. *American Journal of Clinical Nutrition*, Vol.85, No.4, (April 2007), pp. 1148-56, ISSN 1938-3207

Taylor, S. L. & Kabourek, J. L.(2003). Soyfoods and allergies: separating fact from fiction. *The Soy Connection*, Vol.11, No.2.
        http://www.soyconnection.com/upload/SCNv11n2.pdf

Tomomatsu, H. (1994). Health effects of oligosaccharides. *Food Technology*, Vol.61, No.5, (October 1994), pp. 61-65, ISSN 0737-3937

Torrezan, R.; Gomes, R. M.; Ferrarese, M. L.; Melo, F. B.; Ramos, A. M. D.; Mathias, P. C. F. & Scomparin, D. X. (2008). Treatment with isoflavones replaces estradiol effect on the tissue fat accumulation from ovariectomized rats . *Arquivos Brasileiros de Endocrinologia e Metabologia*, Vol.52, No.9, (December 2008), pp. 1489-1496, ISSN 1677-9487

Torre-Villalvazo, I.; Gonzalez, F.; Aguilar-Salinas, C. A.; Tovar, A. R. & Torres, N. (2009). Dietary soy protein reduces cardiac lipid accumulation and the ceramide concentration in high-fat diet-fed rats and ob/ob mice. *The journal of Nutrition*, Vol.139, No.12, (December 2009), pp. 237-43, ISSN 1541-6100

Torres y Torres, N.; Palacios-González, B.; Noriega-López, L. & Tovar-Palacio, A. R. (2006). Índice glicémico, índice insulinémico y carga glicémica de bebidas de soya con un contenido bajo y alto en hidratos de carbono/ Glycemic, insulinemic index, glycemic load of soy beverage with low and high content of carbohydrates. *Revista de investigación clínica*, Vol.58, No.5, (October 2006), pp. 487-497, ISSN 0034-8376

Trevisan, M. C.; Souza, J. M. & Marucci, M. D. (2010). Influence of soy protein and exercises with weights on the resting energy expenditure of women in post-menopause.

Revista da Associação Medica Brasileira, Vol.56, No.5, (2010), pp. 572-578, ISSN 1518-7365

Tull, S.P.; Yates, C.M.; Maskrey, B.H.; O'Donnell, V.B.; Madden, J.; Grimble, R.F.; Calder, P.C.; Nash, G.B.; Rainger, G.E. (2009) Omega-3 Fatty acids and inflammation: novel interactions reveal a new step in neutrophil recruitment. *PLoS Biology*, Vol.7, No.8, (August 2009). ISSN1545-7885

Ullah, M. F.; Ahmad, A.; Zubair, H.; Khan, H. Y.; Wang, Z.; Sarkar, F. H. & Hadi, S. M. (2010). Soy isoflavone genistein induces cell death in breast cancer cells through mobilization of endogenous copper ions and generation of reactive oxygen species. *Molecular Nutrition & Food Research*, (December 2010), in press, ISSN 1613-4133

Vakhapova, V.; Cohen, T.; Richter, Y.; Herzog, Y. & Korczyn, A. D. (2010). Phosphatidylserine containing omega-3 fatty acids may improve memory abilities in non-demented elderly with memory complaints: a double-blind placebo-controlled trial. *Dementia and Geriatric Cognitive Disorders*, Vol.29, No.5, (June 2010), pp. 467-74, ISSN 1420-8008.

Valenzuela, A. & Morgado, N. (1999). Trans fatty acids isomersin human health and in the food industry. *Biological Research,* Vol.32, No.4, (1999), pp. 273-287, ISSN 0717-6287

Vitolins, M. Z.; Case, L. D.; Morgan, T. M.; Miller, M. A. & Burke, G. L. (2010). Soy use and vasomotor symptoms: Soy Estrogen Alternative follow-up study. *International Journal of Women´s Health*, Vol.2, No.2, (November 2010), pp. 381-6, ISSN 1179-1411

Wangen, K. E.; Duncan, A. M.; Xu, X. & Kurzer, M. S. (2001). Soy isoflavones improve plasma lipids in normocholesterolemic and mildly hypercholesterolemic postmenopausal women. *American journal of clinical nutrition,* Vol.73, No.2, (February 2001), pp. 225–231, ISSN 1938-3207

Wilcox, J. R.; & Shibles, R. M. (2001). Interrelationships among seed quality attributes in soybean. *Crop Science*, Vol.41, No.1, (January-February 2001), pp. 11-14, ISSN 1439-037X

Wong, J. M.; Kendall, C. W.; Souza, R.; Emam, A.; Marchie, A.; Vidgen, E.; Holmes, C. & Jenkins, D. J. (2010). The effect on the blood lipid profile of soy foods combined with a prebiotic: a randomized controlled trial. *Metabolism: clinical and experimental,* Vol.59, No.9, (September 2010), pp. 1331-40, ISSN *0026-0495*

Woodman, R. J.; Mori, T. A.; Burke, V.; Puddey, I. B.; Watts, G. F. & Beilin, L. J. (2002). Effects of purified eicosapentaenoic and docosahexaenoic acids on glycemic control, blood pressure, and serum lipids in type 2 diabetic patients with treated hypertension. *American Journal of Clinical Nutrition*, Vol.76, No.5, (November 2002), pp. 1007-15, ISSN 1938-3207

Xie, Y. R. & Hettiarachchy, N. S. (1997). Xanthan Gum Effects on Solubility and Emulsification Properties of Soy Protein Isolate. *Journal of Food Science*, Vol.62, No.6, (November 1997), pp. 1101–1104, ISSN *0022-1147*

Xu, S. Z.; Zhong, W.; Ghavideldarestani, M.; Saurabh, R.; Lindow, S. W. & Atkin, S. L. (2009). Multiple mechanisms of soy isoflavones against oxidative stress-induced endothelium injury. Free Radical Biology & Medicine, Vol.47, No.2, (July 2009), pp. 167-75, ISSN 1873-4596

Yang, G.; Shu X-O.; Jin, F.; Zhang, X.; Li, H-L.; Li, Q.; Gao, Y-T. & Zheng, W. (2005). Longitudinal study of soy intake and blood pressure among middle-aged and

elderly Chinese women. *American Journal of Clinical Nutrition*, Vol.81, No.5, (May 2005), pp. 1012-7, ISSN 1938-3207

Yao, J. J.; Wei, L. S. & Steinberg, M. P. (1988). Water-Imbibing Capacity and Rheological Properties of Isolated Soy Proteins. *Journal of Food Science*, Vol.53, No.2, (March 1988), pp. 464–467, ISSN *0022-1147*

Yeo, S. K. & Liong, M. T. (2010). Effect of prebiotics on viability and growth characteristics of probiotics in soymilk. *Journal of the Science of Food and Agriculture*, Vol.90, No.2, (January 2010), pp. 267-75, ISSN 1097-0010

Yamori, Y.; Moriguchi, E. H.; Teramoto, T.; Miura, A.; Fukui, Y.; Honda, K.; Fukui, M.; Nara, Y.; Taira, K. & Moriguchi, Y. (2002). Soybean Isoflavones Reduce Postmenopausal Bone Resorption in Female Japanese Immigrants in Brazil: A Ten-Week Study. *Journal of the American College of Nutrition*, Vol.21, No.6, (December 2002), pp. 560-563, ISSN 1541-1087

Zanotti, A.; Valzelli, L. & Toffano, G. Chronic phosphatidylserine treatment improves spatial memory and passive avoidance in aged rats. *Psychopharmacology*, Vol.99, No.3, *(*1989), pp. 316–321, ISSN 0033-315

Soybean the Main Nitrogen Source in Cultivation Substrates of Edible and Medicinal Mushrooms

Diego Cunha Zied[1], Jean-Michel Savoie[2] and Arturo Pardo-Giménez[3]
[1]Faculdade de Ciências Agronômicas, Universidade Estadual Paulista, FCA/UNESP.
Módulo de Cogumelos, Departamento de Produção Vegetal (Defesa Fitossanitária),
[2]INRA, UR1264, Mycologie et Sécurité des Aliments,F-33883, Villenave d'Ornon,
[3]Centro de Investigación, Experimentación y Servicios del Champiñón (CIES),
Quintanar del Rey, Cuenca,
[1]Brazil
[2]France
[3]Spain

1. Introduction

The "MUSHROOM" word is used in all part of world to describe the fruiting bodies of saprophytic, mycorrhizal and parasites fungi, belonging to the order of Basidiomycetes or Ascomycetes. They can be found in soils rich in organic matter and humus, moist wood, animal waste, etc., after heavy rain (with thunderstorm or not) or a sudden change of temperature and soon after a few hours or days they disappear, leaving no sign, but vegetative mycelium.

The terminology "MUSHROOMING", or mushroom cultivation refers to the intentional and directed production of mushrooms, substituting wild collection in the fields and forests with a harvest in defined conditions of growing, resulting in strict quality control, food safety without risk of consumption of poisonous or toxic species, and with guarantees on the benefits generated by these fungi.

The cultivation of edible mushrooms is actually an alternative biotech which is fast, environmentally friendly and feasible to recycle organic byproducts from agribusiness into high nutritional and medicinal quality food both with respect to the amount of protein or minerals and selected substances with medicinal and pharmacological properties, for example the presence of β-glucans like lentinan, and thus it can contribute significantly in feeding human.

Currently the most cultivated mushroom in the world are *Agaricus bisporus* (Lange) Imbach "Champignon" or botton mushroom, *Lentinula edodes* (Berk.) Pegler "Shiitake" and *Pleurotus ostreatus* (Jacq.) P. Kumm "Oyster Mushroom" and other *Pleurotus* species (Fig. 1). Recently, extreme attention is paid to *Agaricus subrufescens* (formely *Agaricus blazei* ss. Heinemann and *Agaricus brasiliensis*), the "Almond mushroom". The interest for this Basidiomycete fungus also called the Medicinal mushroom or Sun mushroom is due to its medicinal proprieties such as the presence of glucan protein which has tumor-inhibition activity. All of

them are saprophytic fungi but the *Agaricus* species are humicolous ones whereas *Pleurotus* spp. and *L. edodes* are lignicolous ones. Consequently, the former need a composted substrate for their cultivation and the later can be grown on raw lignocellulosic materials.
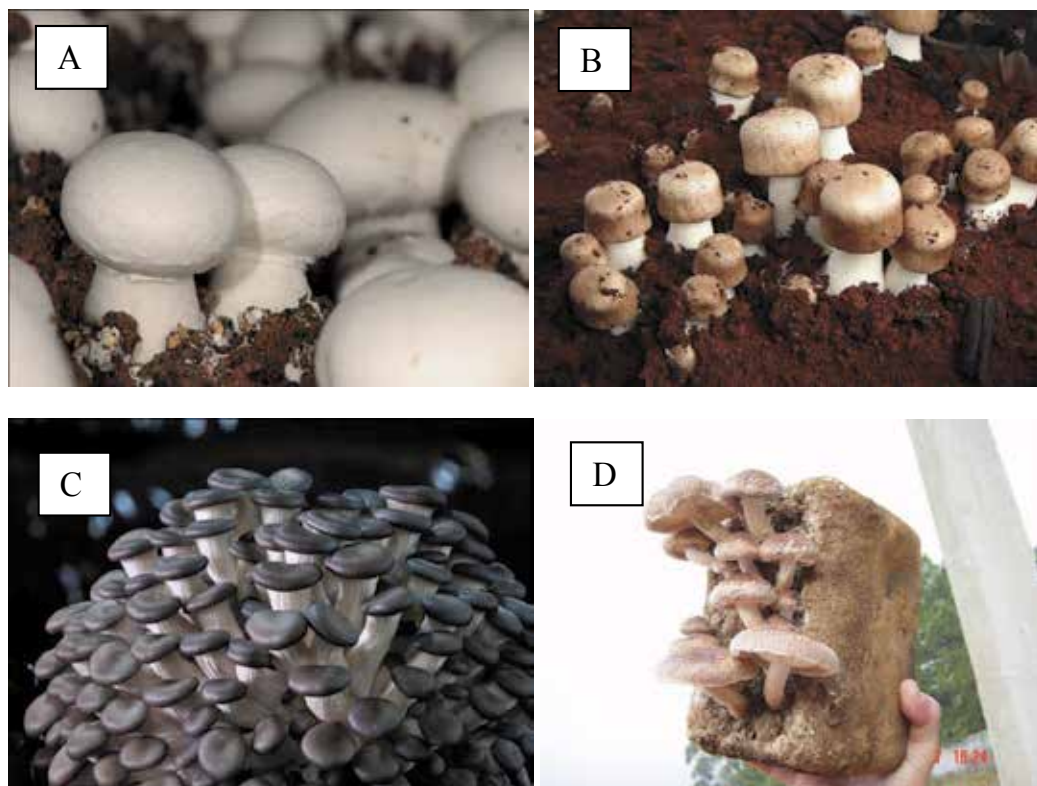
Fig. 1. A: Button mushroom, B: Almond mushroom, C: Oyster mushroom and D: Shiitake.

The production of the substrate to mushroom growth is recognized as the most critical stage of cultivation, having dramatic consequences on the yield and quality of the crop and consequently on the economic viability (Gerrits 1977, 1988; Cormican and Stauton, 1991; Dhar, 1994). The substrates are both a physical support and a source of nutrients for the mushrooms needed to complete their life cycle (vegetative and reproductive phases).

For the production of substrate, various materials (mostly agricultural residues and agro-industrial by-products) can be used, according to the location of the culture, the type, quality, distance and frequency of availability and finally the cost of these materials. According to many researchers the material used to produce the substrate may be classified as: bulky (make up about 60-85% of the total volume of the substrate, being formed with materials largely of cellulose, hemicellulose and lignin); concentrates (make up about 15-35% of the total volume of the substrate, and be materials with high contents of protein, nitrogen, fat and carbohydrates) and conditioners (make up about 5-10% of the total volume of the substrate, the base materials being a source of calcium). Table 1 shows the main types of materials used for production of mushroom cultivation substrates in Brazil and Europe.

Materials	Brazil	Europe
	Materials	
Bulky	Sugarcane bagasse, wheat, oats, rice, braquiária, coast cross and tifton straw, horse manure, etc.	Wheat straw, barley straw, horse manure, sawdust, grape stalks, kenaf fibre, etc.
Concentrated	Soybean meal, chicken manure, cereal meals, cottonseed meal, urea, ammonium, superphosphate, etc.	Chicken manure, urea, ammonium sulphate, grape pomace, cereal meals and brans, feather flour, etc.
Conditioners	Calcitic lime, calcium carbonate and gypsum.	Gypsum and calcium carbonate.

Table 1. Classification and types of materials used in mushroom cultivation.

Standards of mushroom cultivation substrates are: absence of pests and diseases, specific availability of nutrients for the cultivated fungi at the exclusion of other competing fungi, pH between 6 to 7.5, and a bulk density allowing gas exchanges and avoiding excess of moisture content. The use of concentrated materials in growing mushroom is intended to correct the C/N ratio (carbon / nitrogen). Each mushroom has an optimum of C/N ratio for growth, allowing getting the highest yield in a short period of production.

Mushroom	C/N ratio			Reference
	Minimum	Maximum	Optimum	
Agaricus subrufescens	16/1	33/1	27/1	Eira (2003); Kopytoswky-Filho (2004)
Agaricus bisporus	16/1	22/1	19/1	Sharma and Kilpatrick (2000)
Agaricus bitorquis	16/1	22/1	19/1	Oie (2003); Dhar (1994)
Agaricus brunescens	16/1	21/1	19/1	Cies (2009)
Pleurotus ostreatus	40/1	90/1	45-55/1	Heltay *et al.* (1960)
Pleurotus ostreatus f. florida	40/1	150/1	45-55/1	Heltay *et al.* (1960); Kaul *et al.* (1981)
Pleurotus sajor-caju	40/1	150/1	45-55/1	Gramss (1979); Poppe (1995)
Pleurotus flabellatus	40/1	100/1	45-60/1	Chang-Ho *et al.* (1979)
Pleurotus cornucopiae	40/1	97/1	45-55/1	Chang and Miles (1989)
Pleurotus eryngii	40/1	70/1	45-55/1	Chang and Miles (1989)
Lentinula edodes (sawdust)	25/1	55/1	30-35/1	Zied *et al.* (2009)

Table 2. Examples of C/N ratio being desirable to obtain highest yield in different species of mushrooms.

Due to its high protein content, soybean meal is a good source of nitrogen that reduces the levels of carbon in the substrate mainly because to the use of bulk materials. Several advantages are observed in the use of soybean with the production of substrates in Brazil and other American countries, such as: a protein source of high quality, without the presence of heavy metals, found during all seasons, which takes up little space (yet without the need to be stored) and a relatively low price. In Europe where soybean meal is less available as local production, nitrogen in raw ingredients is generally obtained from other sources.

In addition to the use of soybean meal as concentrated material in the formulation of the cultivation substrates it can be added as supplement at the time of inoculation of the substrate with the fungi to be cultivated or later during cultivation. Supplements used both in America and Europe are most of the time manufactured products containing denatured soybean meal and other organic protein sources enriched with minerals. These supplements are frequently used for cultivation of *Agaricus* species.

The Table 2 shows the minimum, maximum and optimum C/N ratio, that some mushrooms need at the time of inoculation. The differences in optimum C/N between *Agaricus* species and the others are consequences of their ecological differences: *Agaricus* species need nitrogen rich humus like substrates obtained by composting, the other species are adapted to lignocellulosic material degradation. The use of soybean meal in the cultivation of both groups of mushrooms as concentrated material or supplement is reviewed in the following parts.

2. In cultivation of *Agaricus* species

2.1 Use as concentrated ingredient for substrate preparation

Substrates for *Agaricus* species obtained after a composting process generally composed of two phases. After a pre-wetting period, ingredients are mixed and Phase I occurs for 1 to 2 weeks either outdoor in long rectangular stacks, or indoor with air flow and temperature controls. Phase I is a biological and chemical process with the breakdown of available organic materials for energy and its incorporation into microbial biomass being the crucial mechanism.

The use of soybean meal as the main source of organic nitrogen in the cultivation substrate of *Agaricus* species is illustrated with *A. subrufescens* for which it has been extensively tested by the scientific community (Eira, 2003). Soybean meal replaces partly chicken manure that is not used for *A. subrufescens* whereas it is commonly used for *A. bisporus.*

Table 3 shows a series of formulations using soybean meal and their respective levels of yield in the end of mushroom cycle and the C/N ratios at the end of the composting process. Some authors used values between 28-23/1 and nitrogen content between 1.15 to 1.45% (Kopytoswky-Filho and Minhoni, 2004) and other 22-17/1 and nitrogen content between 1.7-2.6% (Andrade et al., 2007). The consequences on the yields were not clear. The highest yield was obtained in a study where soybean meal was not used (formulation 4).

In a more detailed study, Kopytoswky-Filho and Minhoni (2004) tested different proportions of soybean meal and urea for obtaining C/N ratio of 37/1 at the beginning of Phase I composting. The highest yield (10.1%) was obtained with a ratio 1.5/1 (soybean meal/urea), while the ratio 4/1 and 1/1.5 resulted in a yield of 8.13 and 7.29%, respectively (Kopytoswky Filho and Minhoni, 2004) showing that a high quantity of soybean is not favorable to the yield.

However, the bulky material used in the production of substrate also influences the yield. Table 4 shows the chemical analysis and yield of mushrooms in an experiment conducted with three different composts with the same amount of soybean added during Phase I composting. It is noteworthy that addition of organic nitrogen source (soybean) and the relationship between organic and inorganic N, other factors also influence the quality of compost, such as: size of the particular material, moisture, density of compost, method of composting used, ammonia concentration in the final compost, etc. (Cormican and Stauton, 1991; Dhar, 1994)

Formulation	Materials	C/N (Phase II)	N (%)	Yield	Reference
1	Sugarcane bagasse, Coast-cross straw, soybean meal, urea, calcitic lime and gypsum.	23.7/1	-	12.9- 16.2%	Zied e*t al.* (2010)
2	Sparagus straw, cottonseed hull, soybean cake and gypsum.	-	1.47	9.8 kg of fresh mushroom per m²	Wang *et al.* (2010)
3	Sugarcane bagasse, brachiaria sp. grass, coast-cross grass, soybean bran, urea, ammonium sulfate and gypsum.	-	-	7.1 kg of fresh mushroom per m²	Colauto *et al.* (2010)
4	Sugarcane, coast-cross, wheat bran, limestone, gypsum, superphosphate and ammonia.	-	1.1	16.3% (mushroom fresh weight/compost fresh weight)	Siqueira *et al.* (2009)
5	Sugarcane bagasse, oat straw, soybean, urea, gypsum and calcitic lime.	25.6/1	-	8.7-12.8%	Zied *et al.* (2009)
6	Sugarcane, coast-cross, soybean meal, gypsum and limestone.	18/1	2.28	10.1% (mushroom fresh weight/compost fresh weight)	Andrade *et al.* (2007)
7	Sugarcane bagasse, braquiária straw, soybean meal, urea, gypsum and limestone.	27-33/1	1.15	7.29-10.01% (mushroom fresh weight/compost fresh weight)	Kopytoswky-Filho and Minhoni (2004)

Table 3. Example of materials used in production of the substrate, its C/N ratio, N content (%) and yield observed by the authors.

Compost	N	P_2O_5	K_2O	Ca	Mg	S	MO	C	Na	Cu	Fe	Mn	Zn	C/N	pH	Yield
				%					mg kg^{-1} dry matter							%
1	1.81	1	1.09	5.15	0.48	1.36	64	35.6	440	0	500	260	20	20/1	6.25	10.77
2	1.76	0.66	1.04	5.44	0.48	1.26	68	37.8	440	0	400	160	18	21/1	7.04	10.72
3	1.6	0.64	1.31	5.20	1.68	1.40	65	36.2	1160	172	400	306	212	23/1	6.94	9.13

Compost 1. Sugar cane bagasse, Massai straw, soybean meal, urea, ammonia, superphosphate, limestone and gypsum.
Compost 2. Sugar cane bagasse, oat straw, soybean meal, urea, ammonia, superphosphate, limestone and gypsum.
Compost 3. Sugar cane bagasse, Aruana straw, soybean meal, urea, ammonia, superphosphate, limestone and gypsum.

Table 4. Chemical analysis of three composts (Phase II) and yield of mushrooms (%).

Even if the different data presented here came from different works, they tend to show that the performance of soybean used with nitrogen source during Phase I of composting, for having a positive effect on mushroom yield with *A. subrufescens* is not completely defined.

2.2 Use as supplement in compost

At the end of composting, the nitrogen content and the value of C/N may be corrected by a supplementation performed either at spawning (inoculation with the mycelium) or at casing (addition of a layer of peat, clay and gypsum at the surface of the culture for inducing the fruiting). Supplementation of compost with soybean based products is common in the cultivation of *A. bisporus* with the aim to increase the nutritional value of mushrooms, which directly consume them, in order to enhance performance, but without affecting quality. Yields generally increase by 5-20%, and occasionally by more.

This technique emerged in the 1960s (Lemke, 1963; Schisler and Sinden, 1962; Sinden and Schisler, 1962). Outstanding aspects to be considered include, on the one hand, the types of nutrients required and the most suitable time for them to be applied without forgetting, on the other hand, economic costs and profits (Randle, 1985). In their study into the economic aspects of supplementing compost, Randle and Smith (1986) estimated that the cost of supplementation is covered by a 1.5 kg m^{-2} increase in yield.

Cereal grains and oilseeds, widely used as mushroom compost supplements, contain varying amounts of the three basic nutritional requirements: carbohydrates, proteins and fats (Randle, 1985). Oil-extracted seed meals and whole-seed meals rich in protein and lipids have proved most consistent in increasing mushroom yields, irrespectively of being added to compost at spawning or at casing (Randle, 1985). Thus, the majority of modern supplements are based on protein-rich vegetable-based raw materials. The products generally consist of soybeans or soybean by-products or other vegetable by-products. Soya meal, maize gluten, potato protein and feather meal are particularly suitable (Gerrits, 1985). In practice, soya meal is the most commonly used. Many products consist of finely textured grain by-products, while others are larger particles or cracked full-fat soybeans (Dahlberg, 1990). The use of a cracked soybean has the added advantage that the protein, fat and carbohydrate contents remain in a natural nutritional balance. The grain by-product supplements generally consist of defatted soybean meal or corn gluten meal (Dahlberg, 1993). However, the need for micronutrient technology that can continue to stimulate yields, but improve quality, shelf life and hopefully disease control, is beginning to be discussed (Wheeler and Wach, 2006; Peeters, 2008).

Supplement can be added at two times: at spawning or at casing after an incubation of the inoculated compost. The results of addition at casing as opposed to spawning are better but this one is only possible when incubation is performed in bulk before filling trays or other containers and casing. This type of incubation is called Phase 3 and is applied only in some countries in large facilities. Applying supplement at spawning appears to be the stage that offers the best operational advantage if added with the inoculum. In this case, however, compost selectivity may be affected by the increased risk of competing fungi appearing (Gerrits, 1985). The optimum quantity of supplement depends on a number of factors, first on the thickness of the compost layer. In general, the best results are obtained with least risk if 1 kg m^{-2} is used. This applies to products with a protein content of no more than 50%. If the protein content is much higher, it is better to limit the quantity to 0.5-0.7 kg m^{-2} (Gerrits, 1985).

The increase in temperature immediately after supplementing due to the high metabolic activity of the fungus and other microorganisms in compost should be controlled and supplements treated to delay the immediate availability of nutrients are needed for successful supplementation at spawning. The first references of treating mushroom compost supplements with formaldehyde, which had been previously applied in animal feed, correspond to Carrol and Schisler (1976), and led to the so-called delayed-release nutrients. These authors assumed that as formaldehyde reduced the solubility and denatured the proteins of supplements, it inhibited their utilization by weed moulds so that the mushroom mycelium, when it was dominant in the compost at 2 weeks post-spawning, could utilize the slowly-available lipo-protein supplement which was then effective through the whole cropping period. While preparing their product, cotton-seed meal was blended with peanut oil, spray-dried and then treated with 10% formaldehyde to denature the protein, making it less soluble and less readily available.

Gerrits (1986) evaluated the supplementation at spawning and at casing with soybean meal treated with solutions of formaldehyde in water (formalin) at different concentrations. It was proved that supplementation with soybean meal treated with 0.2% formalin at casing gives positive response; while at spawning it is advisable to apply soybean-meal treated with 0.6% formalin, in order to offer a better protection. The mushroom yield increase achieved by supplementation at spawning is just a half of that achieved by supplementation at casing. For *Agaricus bitorquis* (Quel.) Sacc, a mushroom replacing *A. bisporus* for cultivation at high temperature, Saharan and Guleria (2001b) treated different supplements with various concentrations (0.2, 0.3 and 0.5%) of formaldehyde and the maximum increase (37.4%) in mushroom yield was recorded in compost supplemented with 0.3% of soybean cake treated with formaldehyde.

Delayed release supplement gives up its nutrients right thought out the life of the crop. And today numerous studies support the positive effect of post-composting supplementation with soybean-based supplements in *Agaricus bisporus* (J.E. Lange) Imbach all over the world. For example, we can find references to its use in Germany (Lemke, 1963; Lelley, 1984), UK (Randle et al., 1983; Randle, 1985; Randle and Smith, 1986), Canada (Rinker, 1991), the Netherlands (Gerrits, 1983, 1985, 1986, 1989; Gerrits and Amsing, 1996; Resink and de Leeuw, 1993; Peeters, 2008), USA (Schisler and Sinden, 1962; Sinden and Schisler, 1962; Schisler, 1970, 1971, 1979; Schroeder and Schisler, 1981; Abell, 1988; Dahlberg, 1990, 1993; Wach and Wheeler, 1998), Ukraine (Petrenko and Bisko, 2004), India (Garcha et al., 1987; Gupta y Vijay, 1992), Italy (Lanzi, 1985), Belgium (Pitblado, 1993) and France (Vedie, 1990; Vedie and Retailleau, 1992; Desrumaux et al., 1999). However,due to the toxicity of formaldehyde other kinds of treatments resulting in protein tanning have to be investigated, specifically for organic production of mushrooms.

Various soybean products and commercial supplements based on treated soybeans may be used alone or in mixtures with other nitrogen rich components. Depending on the types of soybean supplements and mixtures added to the compost the consequences on the yield may vary. Petrenko and Bisko (2004) explored the influence of soybean extrudate, soybean meal and protein soybean concentrate on yield of *A. bisporus* under commercial conditions. Soybean extrudate produced a statistically significant increase (37%). Concentrations from 0.7 to 1% (compost wet wt. basis) produced the greatest response. Supplementation with 2% rate (compost wet wt. basis) of soybean cake provided a 21.9% increase in yield over control for *A. bitorquis* production (Saharan and Guleria, 1993).

Dogan et al. (2000) investigated the effect of corn flour, wheat flour, hen grain, soybean meal and sunflower seed hulls added to synthetic compost on mycelium growth, yields and early ripe of *A. bitorquis*. The growth period of mycelium was shortened and amount of yield also was increased. Saharan and Guleria (2001a) supplemented wheat straw compost with oil seed cakes viz; cotton seed, groundnut, mustard, soybean and till each at three different rates (1.0, 1.5 and 2.0%) on wet weight basis of compost at spawning. Supplementation with soybean cake (1.5%) resulted in maximum increase (38.7%) in yield over control.

In our currently experiments conducted at the Centro de Investigación, Experimentación y Servicios del Champiñón (Cuenca, Spain), supplementation of commercial compost at spawning with three different delayed-release nutrients has shown a significant increase in biological efficiency (4.0-7.1%) and protein content of mushrooms (13.8-16.6%). Also, earliness and unitary weight of mushrooms were positively affected (Table 5).

Supplement and dose	Biological efficiency (kg 100kg^{-1} compost)	Earliness (days from casing)	Mushroom unitary wt (g)	Protein (Nx4.38) (g kg^{-1})
Unsupplemented control	81.42 b	21.6	8.22	219.0 b
Promycel® Gold (10 g kg^{-1})	84.68 a	21.4	8.98	249.2 a
Champfood® S (10 g kg^{-1})	87.18 a	21.2	8.69	250.1 a
Calprozime® (5 g kg^{-1})	84.78 a	21.2	8.44	255.4 a

(*) Values followed by a different letter within a column are significantly different at 5% level according to Tukey's HSD test.

Table 5. Production parameters with respect to various supplementations applied to commercial compost in *Agaricus bisporus* cultivation (*)

Finally, studies adding soybean meal and other commercial supplement (Champfood) at spawning and before casing were also done by Kopytoswky-Filho et al. (2008) in cultivation of *A. subrufescens*. The authors began the process of composting with a mixture of sugarcane bagasse, braquiária straw, soybean meal, urea, limestone and gypsum, with C/N ratio of 37/1. According to the results, the supplementation at spawning and before casing did not differ statistically. The yield values at spawning were 9.1, 9.6 and 8.6 kg of fresh mushroom per m² and before casing were 5.6, 6.1 and 9.3 kg of fresh mushroom per m², respectively, for the control (no supplement), supplemented with soybean and use of a commercial supplement.

2.3 Use for new cultivation substrates

Soybean based supplement are also precious compounds for the development of new ways to prepare the cultivation substrates. Royse et al. (2008) evaluated the effect of adding a delayed release nutrient (SoyPlus®, 4% dry wt) to colonized mushroom compost for the

production of a second crop of mushrooms. Re-casing compost after re-supplementing represents a potential opportunity for growers to increase revenues and reduce costs associated with preparation and disposal of compost. According with the authors, the ability to double crop mushroom compost would provide growers a chance to increase yields by 40% or more. In mushroom production on non-composted substrates, Till (1962) developed what is commonly known as the Till substrate which consisted primarily of straw and several additives (ground straw, white peat, calcium carbonate, cottonseed meal and soybean meal). After autoclaving, the substrate was inoculated under sterile conditions. This process was never adopted because of high operating costs.

Gibbons et al. (1991) evaluated synthetic compost formulations containing varying amounts of soybean meal as nitrogen source, with mushroom yields between 155.8 and 182.7 g kg^{-1} (fresh wt.). Sanchez and Royse (2001) showed that a pasteurized, non-composted substrate consisting of oak sawdust, millet, rye, peat, alfalfa meal, soybean flour, wheat bran, and calcium carbonate was suitable for the production of brown Portobello, a variety of the common cultivated mushroom *A. bisporus*. Biological efficiency ranged from a low of 30.1% (when wheat straw was substituted for sawdust) to 77.1% for the basal mixture.

Findings of Bechara (2007), developing an alternative commercial *A. bisporus* mushroom production system using grain-based substrates, suggested a promising alternative to commercial compost-based system and its environmental problems. In this research, the highest yield was observed for a millet/5% soybean substrate with an additional amendment of 5% delayed-release supplement which produced 21.3 kg/m^2 with a BE of 273%. Mamiro et al. (2007) and Mamiro and Royse (2008) grown *A. bisporus* on non-composted substrate containing ground soybean (4%), spent mushroom compost, and mixtures of them, non-supplemented or supplemented with different nutrients, obtaining yields comparable to non-supplemented Phase II compost. Results confirm the possibility of producing mushrooms on non-composted substrates. Soybean meal has also been used in spawn production (Stoller, 1974).

3. In Pleurotus spp. and *Lentinula edodes* growth

Several kinds of lignocellulosic residues may be used for lignicolous mushroom cultivation, like wheat straw, corn, cotton, coconut, crushed sugar-cane and sawdust. In favorable environments (temperature, relative humidity, luminosity) they produce lignocellulase enzymes, mainly laccases, Mn-peroxidases, cellulases and hemicellulases which convert these lignocellulosic residues into food. However, the addition of supplements to these substrates, such as wheat bran, rice and soybean is usually recommended, in order to obtain a satisfactory development (Melo de Carvalho et al., 2010). Although available commercial supplements were initially developed specifically for use with humicolous *Agaricus* species, researchers found that many of these supplements were effective in stimulating yields of some specialty mushrooms such as *Pleurotus* spp and *L. edodes*. In this way, an opportunity exists for commercial development of nutrients specifically designed for lignicolous mushroom cultivation (Royse et al., 1991).

However, as in the case of *A. bisporus*, the practice of supplementation with materials rich in nitrogen and carbohydrates is not without risks. Among the most important is the ability to promote the development of competitor moulds and cause dangerous increases in the temperature of the substrate, not always easy to control if there is no adequate climatic control systems. These problems diminish in any case where the practice of

supplementation is carried out with the so-called delayed-release nutrients, available after the mycelium has fully colonized the substrate (Muez y Pardo, 2001).

Several trends are evident for *Pleurotus* spp.: decrease in time to harvest, decrease in disease incidence under proper conditions, increase in yield and quality and increase in number of production cycles per room per year (Betterley, 1989).

3.1 Soybean as supplement to substrates based on cereal straws and other crops by-products

Lot of attention was paid on the cultivation of *Pleurous ostreatus* (Jacq.) P. Kumm. In Italy, Ferri (1985) proposed, among other formulations, a mixture of wheat straw (90%) and soybean meal (10%). Royse and Schisler (1987a) cultivated *P. ostreatus* on a pasteurized mixture of chopped wheat straw (70%) and milled corncobs (30%) non supplemented and supplemented with two levels of delayed-release nutrient. Yields increased 2.3 and 3.2 fold on substrate containing 16% and 32% (dry weight basis) of delayed-release nutrients, respectively. Larger mushrooms were produced on substrate containing higher levels of delayed-release nutrient. Mushrooms were harvested 12 to 14 days earlier from supplemented substrates.

Gea et al. (2009) obtained significant increases of 21.6% in the value of the biological efficiency when supplemented a wheat straw based commercial substrate with an additive containing denatured soybean meal and other organic protein sources. Biological efficiency values of 70.6 kg 100 kg^{-1} substrate (dry wt.) were reached.

Recently, Jafarpour et al. (2010) evaluated combination usage of substrates including wood chips, boll, sugar beet pellet pulp and palm fiber along with wheat bran, rice bran, soya cake powder, soya cake powder + rice bran, and carrot pulp as supplements. The least growth period (30.3 d) belonged to sugar beet pulp enriched with soya cake powder. In addition, the highest biological efficiency (158.9%) was found on boll substrate enriched with a mixture of soya cake powder and rice bran supplements, with an increase of 80% compared to control.

Experiments to study the feasibility of reusing the spent oyster mushroom substrate in new production cycles had provided, as a result of supplementation, increases of biological efficiency between 51 and 70%, depending on the base substrate used and supplementation applied, as listed in Table 6 and Fig. 2. As shown in Fig. 3, supplementation also produced increases in dry matter content of fruit bodies (Picornell, 2010; Pardo-Giménez et al., 2011).

All the experiments related above prove the interest of supplement based on soybean or other nitrogen sources for the cultivation of *P. ostreatus* in substrates that are naturally poor in this compound. Numerous similar positive effects have been obtained for the cultivation of other *Pleurotus* species (Fig. 4).

Naraian et al. (2009) evaluated different supplements in *Pleurotus florida* cultivation. Corn cob was employed as basal substrate while eight different additives such as urea, ammonium sulphate, gram flour, soybean meal, ground nut cake and molasses were used as supplements. The biological efficiencies in every supplemented set were increased over un-supplemented control set. The cotton seed cake was found the best supplement producing 93.75% biological efficiency while soybean meal was the second best additive producing 93.00% yield.

Zadrazil (1980) measured the effects of supplementing the straw substrate of *Pleurotus sajor caju* (Fr.) Singer with ammonium nitrate, alfalfa and soybean meal on the decomposition speed of the substrate, the yield of fruiting bodies and their nitrogen content. The yield of

Substrate formulation	Supplementation (20 g kg^{-1})	Biological efficiency (kg/100 kg substrate)
Wheat straw CaSO$_4$ (50 g kg^{-1})	Nonsupplemented	43.4 bcd
	Promycel® 600	73.3 a
	Champfood®	65.6 a
	Calprozime®	72.3 a
Wheat straw + Spent oyster mushroom substrate (1:1, w/w) CaSO$_4$ (50 g kg^{-1}) CaCO$_3$ (10 g kg^{-1})	Nonsupplemented	28.7 d
	Promycel® 600	42.8 bcd
	Champfood®	45.8 bc
	Calprozime®	48.9 b
Wheat straw based commercial control	Nonsupplemented	48.5 b

(*) Values followed by a different letter are significantly different at 5% level according to Tukey's HSD test.

Table 6. Results obtained for the biological efficiency assessed in oyster mushrooms originating from the various supplementations applied to two substrate formulations (*)

fruiting bodies and their nitrogen content increased more with addition of alfalfa and soybean meal than with ammonium nitrate supplementation. The highest nitrogen content in fruiting bodies (8.90%) was found using wheat straw substrate after supplementation with 30% of soybean meal (8.90%). Moreover, the yield coefficient (Yield of fruiting bodies/% Loss of organic matter) was higher in supplemented substrates (maximum 0.25) than in the nonsupplemented control (wheat straw, 0.11).
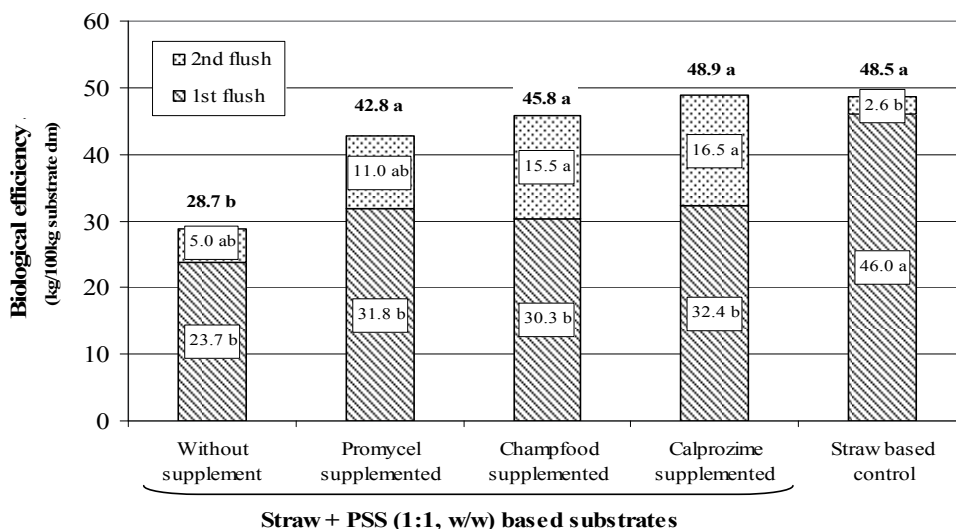

Fig. 2. Results obtained for the biological efficiency assessed in oyster mushrooms originating from the various supplementations applied to wheat straw + *Pleurotus* spent substrate (PSS) formulation
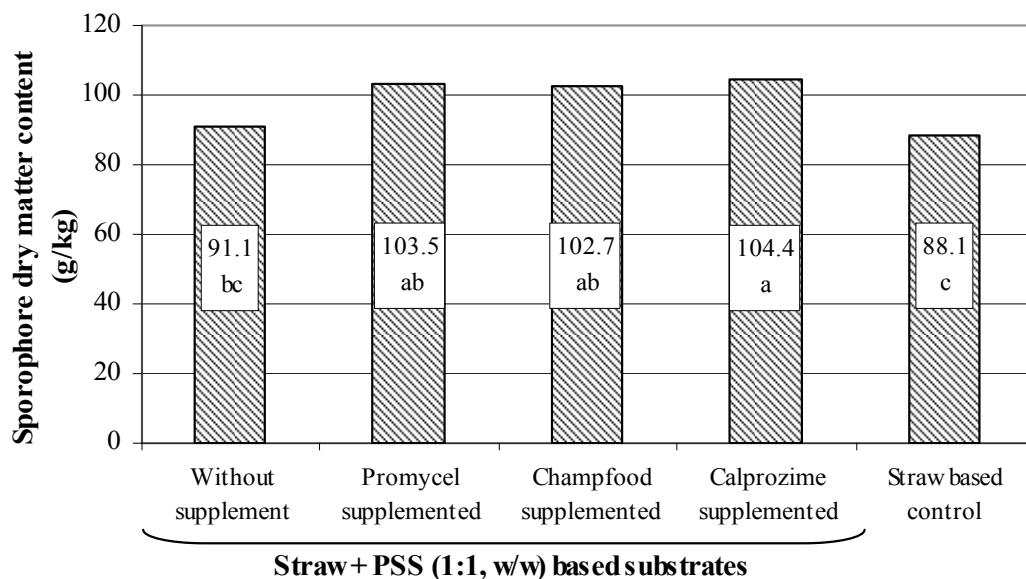
Fig. 3. Results obtained for the dry matter content of oyster mushrooms originating from the various supplementations applied to wheat straw + *Pleurotus* spent substrate (PSS) formulation

Royse and Schisler (1987a) cultivated *Pleurotus sajor-caju* on a pasteurized mixture of chopped wheat straw (70%) and milled corncobs (30%) nonsupplemented and supplemented with two levels of delayed-release nutrient. Yields increased 2.3 and 3.2 fold on substrate containing 16% and 32% (dry weight basis) delayed-release nutrient additions, respectively. For mushroom size, a differential response was observed for genotype and delayed-release nutrient. Smaller mushrooms were produced with higher levels of nutrient. Mushrooms were harvested 3 to 4 days earlier from supplemented substrate. The same authors (Royse and Schisler, 1987b) observed values of biological efficiency of 121% using high supplementation rates (63%, dry wt.) of a delayed release supplement (SpawnMate[®]) when Benomyl[®] (a fungicide) was applied during the substrate soaking process used for water absorption by wheat straw.

Royse and Bahler (1988) evaluated the combination of alfalfa hay with wheat straw and supplementation with delayed-release nutrient, with a significant increase of total yield and biological efficiency of *P. sajor-caju*. As substrate nitrogen content increased, biological efficiency increased. Highest yield (93.1% biological efficiency) was obtained from a mixture of straw and hay (80:20, w/w) supplemented at spawning with SpawnMate II[®] (3.5%, wet wt.). Later, Royse et al. (1991) cultivated *Pleurotus sajor-caju* grown on chopped, pasteurized wheat straw non-supplemented and supplemented with formaldehyde-treated soybean, commercial delayed-release nutrient (SpawnMate[®] II SE) or vegetable oil. Yield was 2.1-fold higher for substrate supplemented (12% dry wt.) with low-volume formaldehyde-treated soybean as compared to non-supplemented substrate. Mushroom yield from substrate supplemented with commercial nutrient was 1.7-fold higher than yield from non-supplemented substrate. As the supplement level increased, the mushroom yield response increased. The yield ranged from 3.56 kg/m² for non-supplemented substrate to 7.36 kg/m² for substrate supplemented (12% dry wt.) with formaldehyde-treated soybean.

Fig. 4. A: *P. ostreatus* var Florida; B: *P. pulmonarios*; C: *P. ostratus* and D: Overview of the cultivation of *Pleurotus* ssp.

The same author obtained high yields of *P. sajor-caju* (79.4% biological efficiency) by supplementing a spent shiitake basal medium with 12% of ground soybean and 1% $CaCO_3$ (Royse, 1992). Bano et al. (1993), supplementing the rice straw substrate colonized by the mushroom *Pleurotus sajor-caju* with powdered oil seed cakes (mustard, niger, sunflower, cotton, and soybean), observed increases of mushroom yields between 50 and 100%, compared to the unsupplemented substrate.

Royse y Zaki (1991) observed that supplementation of pasteurized wheat straw with two commercial nutrient supplements (Spawn Mate II[®] and Fast Break[®]) alone or in combination stimulated yield and biological efficiency (BE) of *Pleurotus flabellatus* (Berk & Broome) Sacc. A combination of both supplements at 84g each per kg dry wheat straw gave a yield of 6.7 kg/m² and a BE of 77.7%. Substrates supplemented only with Spawn Mate[®] (168 g/kg dry substrate) produced a yield of 5.8 kg/m² and a BE of 67.3%. Non supplemented substrate produced a yield of 1.7 kg/m² and a BE of 22.8%.

Royse (2002) evaluated the effect of spawning rate and supplementation level in the cultivation of *Pleurotus cornucopiae* (Paulet) Rolland. The substrate (a mixture of pasteurized cottonseed hulls, chopped wheat straw, and ground limestone) was spawned at various levels (1.25%, 2.5%, 3.75%, or 5% wet wt.) and not supplemented or supplemented with commercial delayed release nutrient (Campbell's S-41) at various levels (0%, 3%, 6%, 9%, or

12%). Maximum yield (weight of fresh mushrooms harvested at maturity) was obtained at 3.75–5% spawn level and 6% S-41 supplement. As supplement levels exceeded 6%, yields declined significantly.

In another series of experiments, Royse et al. (2004) found a cost effective alternative substrate. For this, *P. cornucopiae* was grown on: (1) chopped, pasteurized switch grass (99%) with 1% ground limestone and (2) a mixture of pasteurized cottonseed hulls (75% dry wt.), 24% chopped wheat straw, and 1% ground limestone (all ingredients wt./wt.). The substrates were spawned at various levels (2.5%, 3.75% or 5% wet wt., crop I) and non-supplemented or supplemented with commercial delayed release nutrient (Campbell's S-41) at various levels (0%, 1.5%, 3%, 4.5%, 6%, 7.5% and 9% dry wt., crop II). As in Royse (2002), maximum yield was obtained on cottonseed hull/wheat straw substrate at a 3.75-5% spawn level and 6% S-41 supplement. On switch grass substrate, increasing spawn levels and supplement levels stimulated yields in a linear fashion. However, maximum yields were only 46% or less for those of similar treatments on cottonseed hull/wheat straw substrate.

3.2 Soybean as supplement to substrates based on sawdust

With *Pleurotus eryngii* (DR.) Quél., Royse (1999) conducted several experiments to determine de effect of supplementing cottonseed hulls and oak sawdust with brewer's grain and Spawmate II-SE® (SM) a commercial delayed-release nutrient recommended for oyster mushroom production. In general, as percentage of SM increased, mushroom yield and biological efficiency increased. Cottonseed hulls based substrate supplemented with 9% of SM resulted in a 3-fold increase in yield over 3% supplementation.

Rodríguez Estrada and Royse (2007) performed experiments to determine effects of supplementation of cottonseed hull/sawdust substrate with Mn, Cu, and whole ground soybean (4%, 8% and 12%) on yield, mushroom size, and bacterial blotch resistance of two commercial strains of *Pleurotus eryngii*. Mushroom yields were significantly higher from substrates containing Mn at 50 μg/g and soybean at 8% and 12% supplementation compared to the basal substrate. As the level of soybean addition to substrate increased, yield also increased. Rodríguez Estrada et al. (2009) noticed that improved yield and biological efficiency (BE) of *P. eryngii* var. *eryngii* were achieved by supplementation of substrate with a commercial delayed-release nutrient and use of a casing overlay. Yield increases of 4% were achieved from cased substrates that were supplemented at time of casing with a corn and soybean delayed-release nutrient (4% dry wt.).

Species of *Eucalyptus* (*urophylla*, *grandis*, *camaldulensis* and *saligna*) and *Quercus* (*acutissima*, *dentata*, *serrata* and *mongolica*) are commonly used in the cultivation of Shiitake (*L. edodes*) for production of the substrate, which are additioned with meals (soybean, wheat, rice, cotton , corn, etc.) or bran (Table 7) in order to improve the properties of the substrate, mainly due to the increased content of nitrogen and carbohydrates available, resulting in fast spawn run and reduction the production phase, when compared with cultivation on logs (Rinker, 1991; Luo, 2004; Minhoni et al., 2007) (Fig. 5).

But high levels of nitrogen may result in a less dense mycelium growth and facilitate the presence of *Trichoderma*, the main competitor causing troubles in cultures of Shiitake. Thus the balance of nitrogen and carbon should be performed to establish a C/N ratio of 35-55/1 during mycelial growth, taking into account that in the time of fruiting it will be between 55-80/1. The recommended pH values for mycelial growth are 5.5 to 6.5, so in the time of fruiting it will be between 4.5 to 5.5 (Oei, 2003; Chen 2005; Zied et al., 2009a). Table 7 shows various formulations used in several countries.

Sawdust	Meal	Grain	Others	Author
80% Quercus	10% wheat	10% maize		Royse et al., 1985
80% Quercus	10% wheat	10% millet		Rinker, 1991
72%(1)	26% wheat		2% carbonate	Ghang, Miles, 1989
94%	3-4% rice 1% wheat or maize		1% carbonate	Oie, 2003
80% Eucalyptus	20% wheat		1 a 2% carbonate	Sant`anna, 1998
70-78%(2)	20% maaize		2% de carbonate	Kalberer, 2000

(1)Dalberia sisso, Acacia arabica and Populos alba; (2)70% Quercus, 20% Faia and 10% Bordo.

Table 7. Formulations examples of the substrates for the cultivation of *L. edodes.*

The yield achieved in the cultivation of *L. edodes* is between 15 to 30% (mushroom fresh weight/compost fresh weight) and the production time is around 90 to 180 days of cultivation. This technology by adding various types of meals in sawdust offers major advantages when compared to traditional cultivation on logs, such as use of various types of waste (sawdust, sugarcane bagasse, etc.), constant production throughout the year, reduction of production time and ease of management (since each block has about 2-3 kg). But some disadvantages are also observed, as higher investment in cultivation (production of the substrate and chambers of growing semi-controlled), high energy consumption and need for skilled labor (Badham, 1988; Pettipher, 1988).

Fig. 5. Cultivation of Shiitake in sawdust, supplemented with soybean (right); and traditional cultivation in logs of *Eucalyptus* (left)

4. Conclusions

The use of soybean meal other by-products from soybean transformation in cultivation of edible and medicinal mushrooms is possible, viable and represents a good source of organic N (to be used as concentrated material or supplement). For any application it is important to know exactly the physiological behavior and the nutrition of the mushroom. It is noteworthy that the numerous advantages of its use as: a protein source of high quality, without the presence of heavy metals, found during all seasons, which takes up little space (yet without the need to be stored), a relatively low price, increase the nutritional value of mushrooms and the yield values by 5-20% and occasionally by more.

5. References

Abell, S. (1988). What's new in supplementation? Mushroom News 36(10): 18-19.

Andrade, M.C.N., Kopytowski Filho, J., Minhoni, M.T.A., Coutinho, L.N., Figueiredo, M.B. (2007). Productivity, biological efficiency, and number of *Agaricus blazei* mushrooms grown in compost in the presence of *Trichoderma* sp. and *Chaetomium olivacearum* contaminants, Braz J Microbiol 38:243-247

Andrade, M.C.N., Zied, D.C., Minhoni, M.T.A., Kopytowski Filho, J. (2008). Yield of four *Agaricus bisporus* strains in three compost formulations and chemical composition analyses of the mushrooms. Braz J Microbiol 39:593–598.

Badham, E. R. (1988). Is autoclaving Shiitake substrate necessary? Mushroom Tropics 8: 129-36.

Bano, Z., Shashirekha, M.N., Rajarathnam, S. (1993). Improvement of the bioconversion and biotransformation efficiencies of the Oyster mushroom (*Pleurotus sajor-caju*) by supplementation of its rice straw substrate with oil seed cakes. Enzyme and Microbial Technology 15: 985-987.

Bechara, M.A. (2007). Alternative mushroom production systems using non-composted grain-based substrates. Ph. D. Thesis. The Pennsylvania State University, USA.

Betterley, D.A. (1989). Supplementation of *Agaricus* and *Pleurotus* substrates – an overview. Mushroom News 37(10), 10-16.

Carrol Jr, A.D., Schisler, L.C. (1976). Delayed release nutrient supplement for mushroom culture. Appl. Environ. Microbiol. 31(4): 499-503.

Chang, S.T., Milles, P.G. (1989). Edible mushroom and their cultivation, Florida: CRC press.

Chang-Ho, Y., Ho, T.M. (1979). Effect of nitrogen amendment on the growth of *Volvariella volvacea*. Mushroom Science 10(1): 619-625.

Chen, A. W. (2005). Shiitake bag cultivation. In: Mushroom Growers' Handbook 2, Mush World, Seoul, Korea, p. 73-87.

Cies (2009). Resultados de dos ciclos de cultivo de champiñón Portobello. El Champiñón en Castilla-La Mancha 29, 1:4.

Colauto, N.B., Silveira, A.R., Eira, A.F., Linde, G.A. (2010). Alternative to peat for *Agaricus brasiliensis* yield. Bioresource Technology 101: 712-716.

Cormican, T., Stauton, L. (1991). Factors in mushroom (*Agaricus bisporus*) compost producity. In: MAHER, M.J. (Ed.) Science and cultivation of edible fungi. Rotterdam: Balkema 1: 223-234.

Dahlberg, K.R. (1990). Particulate compost supplements. Mushroom News 38(10): 12-17.

Dahlberg, K.R. (1993). Selection of optimum spawn and supplement types and rates. Mushroom News 41(7): 4-7.

Desrumaux, B., Seydeyn, P., Werbrouck, A., Lannoy, P. (1999). Supplémenter dans la culture du champignon de couche: experience comparative avec quelques produits de supplementation du commerce. Bull. FNSACC 81: 789-802.

Dhar, B.L. (1994). Mushroom composting for *Agaricus bisporus/bitorquis*. In: NAIR, M.C. (Ed.) Advances in Mushroom biotechnology, Jophpur: Scientific Publishers, p. 84-90.

Dogan, H.H., Öztürk, C., Akköz, C. (2000). The effect of some nutrients supplementation to synthetic compost with wheat straw before mycel inoculation, on mycelium growth, yield and early ripe of *Agaricus bitorquis* (Quél.) Sacc. Turk. J. Biol. 24: 151-158.

Eira, A. F. (2003). Cultivo do cogumelo medicinal *Agaricus blazei* (Murrill) ss. Heinemann. Viçosa: Editora Aprenda Fácil, 308 p.

Ferri, F. (1985). I Funghi. Micologia, isolamento, coltivazione. Edagricole, Bolonia, Italia. 398 pp.

Garcha, H.S., Khanna, P.K., Sodhi, H.S., Dhanda, S., Sidhu, A., Phutela, R.P. (1987). Nutrient supplementation for *Agaricus bisporus* (Lange) Sing. cultivation. In: Developments in Crop Science 10: Cultivating Edible Fungi, P.J. Wuest,

D.J. Royse, R.B. Beelman (eds), 101-108. Elsevier Science Publishing Company Inc., New York, NY, USA.

Gea, F.J., Martínez-Carrasco, A., Navarro, M.J. (2009). Efecto de la suplementación del sustrato sobre la cosecha de setas. Horticultura Internacional 67: 32-40.

Gerrits, J.P.G. (1977). The significance of gypsum applied to mushroom compost. The Neth. J. Agric. Sci., 25: 288-302.

Gerrits, J.P.G. (1983). New products for compost supplementation. Mushroom Journal 126: 207-213.

Gerrits, J.P.G. (1985). Nutrition and compost. In: The Cultivation of Mushrooms, L.J.L.D. van Griensven (ed), 29-72. Interlingua T.T.I. Ltd., East Grinstead, Sussex, UK

Gerrits, J.P.G. (1986). Supplementation with formaldehyde treated soya bean meal. Mushroom Journal 161, 169-174.

Gerrits, J. P. G. (1988) Nutrition and compost. In: GRIENSVEN, L. J. L. D.(Ed.) The cultivation of mushrooms. Horst: Mushroom Experimental Station, p.29-72.

Gerrits, J.P.G. (1989). Supplementation of *Agaricus* compost with organic materials with special attention to the uptake of minerals and amino acids. Mushroom Science. 12(1): 361-370.

Gerrits, J.P.G., Amsing, J.G.M. (1996). Milli Champ 3000 en 6000. De Champignoncultuur 40(10): 397-400.

Gibbons, W.R., Maher, A.A., Todd, R.L. (1991). Button mushroom production in synthetic compost derived from agricultural wastes. Bioresource Technology 38: 65-77.

Gramss, G. (1979). Some difference in response to competitive microorganisms deciding on growing success and yield of wood destroying edible fungi.Mushroom Science4:265-285.

Gupta, Y., Vijay, B. (1992). Post-composting supplementation in *Agaricus bisporus* under seasonal growing conditions. Mushroom Research 1(2): 115-117.

Heltay, I., Zavodi, I. (1960). Rice straw compost. Mushroom Science 4: 393-399.

Jafarpour, M., Zand, A.J., Dehdashtizadeh, B., Eghbalsaied, S. (2010). Evaluation of agricultural wastes and food supplements usage on growth characteristics of *Pleurotus ostreatus*. African Journal of Agricultural Research 5(23): 3291-3296.

Kalberer, P. P. (2005). Influence of urea and ammonium chloride on crop yield and fruit body size of shiitake (*Lentinula edodes*). Mushroom Science 15: 361-366.

Kaul, T., Khurana, M., Kachroo, J. (1981). Chemical composition of cereal straw of the Kashmir valley. Mushroom Science 11 (2): 19-25.

Kopytowski Filho, J., Minhoni, M.T.A. (2004). C/N ratio on yield of *Agaricus blazei* Murrill ss. Heinemann. Mushroom Science 16: 213-220.

Kopytowski Filho, J., Minhoni, M.T.A., Andrade, M.C.N., Zied, D.C. (2008). Effect of compost supplementation (soybean meal and ChampFood) at different phases (spawning and before casing) on productivity of *Agaricus blazei* ss. Heinemann (*A. brasiliensis*). Mushroom Science 17: 260-271.

Lanzi, G. (1985), Banco di prova: Milki Champ 3000, Milki Champ 6000. Mushroom Information 6: 30-32.

Lelley, J. (1984). Effetto della supplementazione del substrato alla semina. Mushroom Information 4: 15.

Lemke, G. (1963). Champignonkultur auf nicht kompostiertem Strohsubstrat mit "Startddungung". Die Deutsche Gartenbauwirtschaft 11: 167-169.

Luo, X. C. (2004). Progress of xiang-gu (shiitake) cultivation in China. Mushroom Science 16: 317-322.

Mamiro, D.P., Royse, D.J., Beelman, R.B. (2007). Yield, size, and mushroom solids content of *Agaricus bisporus* produced on non-composted substrate and spent mushroom compost. World J. Microbiol. Biotechnol. 23: 1289-1296.

Mamiro, D.P., Royse, D.J. (2008). The influence of spawn type and strain on yield, size and mushroom solids content of *Agaricus bisporus* produced on non-composted and spent mushroom compost. Bioresource Technology 99: 3205-3212.

Melo De Carvalho, C.S., Sales-Campos, C., Nogueira De Andrade, M.C. (2010). Mushrooms of the *Pleurotus* genus: a review of cultivation techniques. Interciencia 35(3): 177-182.

Minhoni, M. T. A., Andrade, M. C. N., Zied, D. C., Kopytowski Filho, J. (2007). Cultivo de *Lentinula edodes*(Berk.) Pegler - (Shiitake). Botucatu: Fepaf.

Muez, M.A., Pardo, J. (2001). La preparación del sustrato. In: J.E. Sánchez and D. Royse (Eds.) La Biología y el Cultivo de *Pleurotus* spp., 157-186. Ecosur, Limusa, México.

Naraian, R., Sahu, R. K., Kumar, S., Garg, S.K., Singh, C.S., Kanaujia, R.S. (2009). Influence of different nitrogen rich supplements during cultivation of Pleurotus florida on corn cob substrate. The Environmentalist 29: 1-7.

Oei, P. (2003). Mushroom cultivation. 3 ed. The Netherlands: Backhuys Publishers Leiden, 429 p.

Pardo-Giménez, A., Picornell, M. R., De Juan, J.A., Pardo-González, J.E., Zied, D.C. (2011). Cultivation of Pleurotus ostreatus using supplemented spent oyster mushroom substrate. Acta Horticulturae (in press).

Pettipher, G. L. (1988). Cultivation of the Shiitake mushroom (*Lentinula edodes*) on lignocellulosic waste. J. Sci. Food Agric. 42: 195-198.

Peeters, J. (2008). The art of supplementing. Mushroom Business 30: 24-25.

Petrenko, B.F., Bisko, N.A. (2004). Influence of the addition of soybean supplements to the compost on the yield of Agaricus bisporus. Mushroom Science 16: 353-358.

Picornell, M.R. (2010). Reutilización de sustratos postcultivo de hongos comestibles en el cultivo de Pleurotus ostreatus (Jacq.) P. Kumm. Ph. D. Thesis. Universidad de Castilla-La Mancha, Albacete, Spain.

Pitblado, R.E. (1993). Supplementation – "Compost through to casing". Mushroom World 4(2): 45-46.

Poppe, J., Höfte, M. (1995). Twenty wastes for twenty cultivated mushroom. Mushroom Science 14(1): 171-179.

Randle, P.E. (1985). Supplementation of mushroom composts – a review. Mushroom Journal 151: 241-249.

Randle, P.E., Fermor, T.R., Flegg, P.B., Matcham, S.E., Smith, J.F., Wood, D.A. (1983). Mushroom compost supplementation. Trials with proprietary and others materials. Mushroom Journal 130: 345-349.

Randle, P.E., Smith, J.F. (1986). Economic aspects of compost supplementation. Mushroom Journal 165: 297-305.

Resink, J.W., De Leeuw, B. (1993). Mushroom supplementation in the Netherlands. Mushroom News 41(7): 20-25.

Rinker, D.L. (1991). Alternative additives, supplements, and casing amendments for *Agaricus bisporus*. Mushroom Science 13(2): 781-789.

Rinker, D. L. (1991). The influence of heat treatment, genotype and other cultural practices on the production of *Shiitake* mushrooms on sawdust. Mushroom Science 13: 497-502.

Rodríguez Estrada, A.E., Royse, D.J. (2007). Yield, Size and bacterial blotch resistance of *Pleurotus eryngii* grown on cottonseed hulls/oak sawdust supplemented with manganese, copper and whole ground soybean. Bioresource Technology 98: 1898-1906.

Rodríguez Estrada, A.E., Jiménez-Gasco, Mª.M., Royse, D.J. (2009). Improvement of yield of *Pleurotus eryngii* var. *eryngii* by substrate supplementation and use of a casing overlay. Bioresource Technology 100(21): 5270-5276.

Royse, D. J., Schisler, L. C., Diehle, A. (1985). Shiitake mushrooms: consumption, production and cultivation. Interdisciplinary Science Reviews, Bristol 10: 329-335.

Royse, D.J., Schisler, L.C. (1987a). Yield and size of *Pleurotus ostreatus* and *Pleurotus sajor-caju* as effected by delayed-release nutrient. Applied Microbiology and Biotechnology 26 (2): 191-194.

Royse, D.J., Schisler, L.C. (1987b). Influence of benomyl on yield response of *Pleurotus sajor-caju* to delayed-release nutrient supplement. Hortscience 22(1), 60-62.

Royse, D.J., Bahler, B.D. (1988). The effect of alfalfa hay and delayed release nutrient on biological efficiency of *Pleurotus sajor-caju*. Mushroom J. Tropics 8: 59:65.

Royse, D.J. (1992). Recycling of spent shiitake substrate for production of the oyster mushroom, *Pleurotus sajor-caju*. Applied Microbiology and Biotechnology 38: 179-182.

Royse, D.J. (1999). Yield stimulation of king oyster mushroom, *Pleurotus eryngii*, by brewer's grain and SpawnMate IISE® supplementation of cottonseed hull and wood chip substrates. Mush. News 47 (2): 4–8.

Royse, D.J. (2002). Influence of spawn rate and commercial delayed release nutrient levels on *Pleurotus cornucopiae* (oyster mushroom) yield, size, and time to production. Applied Microbiology and Biotechnology 58: 527-531.

Royse, D.J., Zaki, S.A. (1991). Yield stimulation of *Pleurotus flabellatus* by dual nutrient supplementation of pasteurized wheat straw. Mushroom Science 13 (2): 545-547.

Royse, D.J., Fales, S.L., Karunanandaa, K. (1991). Influence of formaldehyde-treated soybean and commercial nutrient supplementation on mushroom (*Pleurotus sajor-caju*) yield and in-vitro dry matter digestibility of spent substrate. Applied Microbiology and Biotechnology 36(3): 425-429.

Royse, D.J., Rhodes, T.W., Ohga, S., Sánchez, J.E. (2004). Yield, mushroom size and time to production of *Pleurotus cornucopiae* (oyster mushroom) grown on switch grass substrate spawned and supplemented at various rates. Bioresource Technology 91: 85-91.

Royse, D.J., Sanchez, J.E., Beelman, R.B., Davidson, J. (2008). Re-supplementing and re-casing mushroom (*Agaricus bisporus*) compost for a second crop. World J. Microbiol. Biotechnol. 24: 319-325.

Saharan, M.S., Guleria, D.S. (1993). Effect of oilseed cakes supplementation on yield parameters of mushroom (*Agaricus bitorquis* (Quel.) Sacc.). Crop Res. 6(1), 59-63.

Saharan, M.S., Guleria, D.S. (2001a). Effect of supplementation of compost parameters and yield of *Agaricus bitorquis* (Quel) Sacc. J. Mycol. Pl. Pathol. 31(1), 64-66.

Saharan, M.S., Guleria, D.S. (2001b). Effect of supplements treated with different concentrations of formaldehyde on yield of *Agaricus bitorquis* (Quel) Sacc. J. Mycol. Pl. Pathol. 31(1), 85-87.

Sanchez, J. E., Royse, D.J. (2001). Adapting substrate formulas used for shiitake for production of brown *Agaricus bisporus*. Biores. Technol. 77: 65-69.

Sant´Anna, A. (1998). Cultivo do cogumelo Shiitake Lentinula edodes (Berk.) Pegler em serragem e em toros de eucalípto. Rio Claro, 115p. Dissertação (Mestrado em Ciências Biológicas/ Microbiologia aplicada) – Instituto de Biociências, Universidade Estadual Paulista.

Shekhar Sharma, H.S., Kilpatrick, M. (2000). Mushroom (*Agaricus bisporus*) compost quality factors for predicting potential yield of fruiting bodies. Canadian Journal of Microbiology, 46, 515-519.

Schisler, L.C. (1970). Supplementation of mushroom compost. Mushroom News 18(4): 13-21.

Schisler, L.C. (1971). Supplementation of the finished compost at time of spawning, at time of casing, and later. Mushroom News 19(4): 3-13.

Schisler, L.C. (1979). Influence of cultural practices on mushroom yield response to delayed release nutrients. Mushroom News 27(4): 17-23.

Schisler, L.C., Sinden, J.W. (1962). Nutrient supplementation of mushroom compost at spawning. Mushroom Science 5, 150-164.

Schroeder, G.M., Schisler, L.C. (1981). Effect of supplementation at spawning, compost moisture and casing moisture on size, yield and dry weight of mushrooms. Mushroom Science 11(1): 511-521.

Sinden, J.W., Schisler, L.C. (1962). Nutrient supplementation of mushroom compost at casing. Mushroom Science 5, 267-280.

Siqueira, F.G., Dias, E.S., Silva, R., Martosi, E.T., Rinker, D.L. (2009). Cultivation of *Agaricus blazei* ss. Heinemann using different soils as source of casing materials. Sci. Agric. 66:.827-830.

Stoller, B. B. (1974). Mushroom spawn and method of making same. U.S. Patent No. 3,828,470.

Till, O. (1962). Champignonkultur auf sterilisiertem náhrsubstrat und die wiederverwendung von abgetragenem compost. Mushroom Science 5: 127-133.

Vedie, R. (1990). Compte rendu de l'expérimentation. Produits de Supplémentation. Cahier Technique du Champignon Nº 32. Centre Technique du Champignon. St Paterne, France, 16 pp.

Vedie, R., Retailleau, F. (1992). La supplementation des composts avant gobetage. Bull. FNSACC 54: 49-50.

Wach, M.P., Wheeler, D.W. (1998). Mushroom supplements: what they are and why we use them. Mushroom News 46(7): 10-16.

Wang, Q., Li, B.B., Li, H., Han, J.R. (2010). Yield, dry matter and polysaccharides content of the mushroom *Agaricus blazei* produced on asparagus straw substrate. Scientia Hoticulturae 125: 16-18.

Wheeler, D., Wach, M.P. (2006). Re-evaluating supplementation. Mushroom News 54(7): 4-11.

Zadrazil, F. (1980). Influence of ammonium nitrate and organic supplements on the yield of *Pleurotus sajor-caju* (Fr.) Sing. Eur. J. Appl. Microbiol. Biotechnol. 9: 31-35.

Zied, D.C., Pardo, J.E., Pardo, A. Minhoni, Mta. (2009a). Cultivo de Shiitake, *Lentinula edodes* (Berk.) Pegler, en substrato formulado. In: Consejería de Agricultura y Medio Ambiente y Diputación Provincial de Cuenca (Eds.) V Jornadas Técnicas del Champiñón y otros Hongos Comestibles en Castilla-La Mancha (pp. 1-11). Cuenca (in press).

Zied, D.C., Minhoni, M.T.A., Kopytowski Filho, J., Arruda, D.P., Andrade, M.C.N. (2009b). Production of *Agaricus blazei* ss. Heinemann (*A. brasiliensis*) in function of different casing layers and composts. Inci 34:437–442.

Zied, D.C., Minhoni, M.T.A., Kopytowski Filho, J., Andrade, M.C.N. (2010). Production of *Agaricus blazei* ss. Heinemann (*A. brasiliensis*) on different casing layers and environments. World J Microbiol Biotechnol. doi: 10.1007/s11274-010-0367-x.

Assessing Compositional Differences in Soy Products and Impacts on Health Claims

Joyce Boye and Sabine Ribéreau
Food Research and Development Centre
Agriculture and Agri-Food Canada
Canada

1. Introduction

In October 1999, the Food and Drug Administration (USA) approved a health claim for soy indicating that consumption of 25 grams of soy protein a day as part of a diet low in saturated fats and cholesterol may help lower the risk of heart disease. Additionally, soy has officially been approved in the UK, Brazil, Malaysia, Japan, Korea, Philippines, Indonesia and South Africa.

Some countries have, however, been slower to approve a health claim and are requiring more substantive evidence. Questions of concern include the impact of processing on the efficacity of soy protein, information on the normal and acceptable levels of isoflavones in soy products, efficacy of soy proteins in which isoflavones have been removed versus soy proteins containing isoflavones in respect to relationship with cardiovascular disease risk reduction, feasibility of consumers to take in 25 g of soy protein a day, effect of antinutritional factors (e.g., trypsin inhibitors, phytates, lectins, lysinoalanine), heat resistance of trypsin inhibitors found in soybeans and the specific effects of soy protein alone compared to soy protein within a matrix (e.g., whole soybean foods and foods made from soy protein ingredients).

These questions are pertinent as there is a wide variety of soyfoods available on the market with different macro and micro nutrient compositions. Answers to these questions will therefore be useful in identifying the specific conditions required for soyfoods to carry a health claim.

Foods prepared from whole soybeans include soymilk and tofu. Soybean derived ingredients include defatted soy flour, soy protein concentrate, soy protein isolate and soy fibre. Soy protein isolates in particular have been very successful commercially and are used extensively today in the production of a large number of foods. Different techniques can be used for making soy protein isolate and the type of processing treatment used can affect the nutrient and physicochemical composition of the isolate (e.g., composition of residual isoflavones, saponins, trypsin inhibitors, phytic acid and minerals). Isoflavones, saponins, trypsin inhibitors and phytic acid are all biologically active molecules and as such variations in their composition can hinder the ability to determine if a reported health benefit is due to the soy protein alone or the presence of these other compounds.

The chapter will provide a brief update on some of the reported health benefits of soy components. An overview of some of the major soyfoods and soy ingredients currently

available on the market will be provided along with a detailed list comparing the effects of processing on the composition of different soy products. Requirements for obtaining health claims using the Canadian example, as well as impacts of compositional differences on potential health benefits are further presented.

2. Soybeans and health

Cardiovascular disease (CVD) is one of the leading causes of death in the world today. According to the World Health Organization by 2030, almost 23.6 million people will die from CVDs, mainly from heart disease and stroke (www.who.int/mediacentre). Elevated low density lipoprotein (LDL) is a major cardiovascular disease risk factor. LDL transports cholesterol and triglycerides from the liver to peripheral tissues and arteries and regulates cholesterol synthesis. Retention of cholesterol in arteries can result in the formation of arterial plaques which increases the risk of atherosclerosis, peripheral vascular disease, strokes and heart attacks. Foods that decrease LDL levels will, therefore, increasingly be of interest as health foods. Several reports have shown a decrease in LDL levels and an increase in HDL after consumption of soy (Nilavsen & Meinertz, 1998; Potter et al., 1998; Merritt, 2004; Zhuo et al., 2004; Sacks et al., 2006; Harlanda & Haffnerb, 2008; Taku et al., 2008). Earlier studies showing similar results formed the basis of the current soy health claim in many jurisdictions.

Due to apparent inconsistencies in findings, in 2008 the Weston Price Foundation submitted a petition to the FDA in response to the FDA's request for public comment on the issue claiming that soy protein products are not safe and have no long history of use in the food supply. The organization also claimed the evidence on soy protein and heart disease was contradictory and inconsistent, and that no standard of scientific agreement had been met (http://www.physorg.com/news122663958.html).

A systematic study conducted by the US department of Health & Human Services indicated that while the evidence was weak for other disease outcomes, there is a suggestion of a possible dose-response effect for soy protein for LDL reduction (http://www.ahrq.gov/Clinic/epcsums/soysum.htm).

Using predictive equations along with a meta-analysis to determine whether the heart health claim for soy continues to be justified, Jenkins et al. (2010) concluded that low density lipoprotein cholesterol (LDL-C) reduction attributable to the combined intrinsic and extrinsic effects of soy protein foods ranged from 7.9 to 10.3%. They further concluded from their study that soy remains one of a few food components that reduces serum cholesterol (>4%) when added to the diet.

In a more recent study, Onuegbu et al. (2011) fed 500 mL of soymilk daily to 42 apparently healthy young to middle-aged subjects for a period of 21 days and reported that soymilk consumption significantly reduced mean plasma TC by 11% and LDL-C by 25% and increased mean plasma HDL-C by 20%. The authors also concluded that soy drink could be an important non-pharmacological cholesterol-reducing agent.

Furthermore, Bruckert and Rosenbaum (2011) have also recently reported LDL-cholesterol reduction ranging from -3 to -10% for soy protein and have indicated that dietary recommendations may have important impacts on cardiovascular events as they can be implemented early in life and because the sum of the effect on LDL-cholesterol is far from being negligible.

Another major risk factor for developing CVD is elevated blood pressure (EBP). A major contributor to EBP is Angiotensin II which is a potent vasoconstrictor. Vasoconstriction

occurs when renin, an enzyme produced in the kidneys, proteolytically acts on circulating angiotensinogen and converts it to angiotensin I (a decapeptide). In the presence of angiotensin converting enzyme (ACE), angiotensin I is cleaved to the octapeptide, angiotensin II resulting in arterial constriction and EBP. ACE also breaks down bradykinin, a vasodilator, further contributing to the elevation in blood pressure. Inhibition of ACE is, therefore, important for the lowering of blood pressure as this results in a decrease in the concentration of angiotensin II and an increase in the levels of bradykinin (Yang et al., 1970; Erdos, 1975). Various reports have suggested that peptides from soy posses ACE-inhibitory properties (Wu & Ding, 2002; Kuba et al., 2003; Chiang et al., 2006; Hartmann & Meisel, 2007; Yang et al., 2008). These peptides are usually not active when present within the sequence of parent proteins, but are released by enzymatic proteolysis *in vivo* or *in vitro*.

In addition to soy proteins, some reports have attributed the beneficial health effect of soy to phytochemicals found in soybeans such as isoflavones which are naturally occurring non-steroid compounds with weak estrogenic effects and chemical structure similar to estradiol-17β (Fig. 1). Isoflavones are able to bind to estrogen receptors and are capable of triggering estrogen dependent responses physiologically. The main types of isoflavones found in soy are the aglycones (daidzein, genistein, and glycitein), the β-glucosides (daidzin, genistin, and glycitin) and their 6″-O-malonyl-β-glucosides (6OMalGlc) and 6″-O-acetyl-β-glucosides (6OAcGlc) conjugates.

Taku et al. (2010) conducted a meta-analysis to clarify the effects of soy isoflavone extracts on systolic and diastolic blood pressure (SBP and DBP) in adult humans. They reported that soy isoflavone extracts significantly decreased SBP but not DBP in adult humans, however, no dose-response relationship was observed.
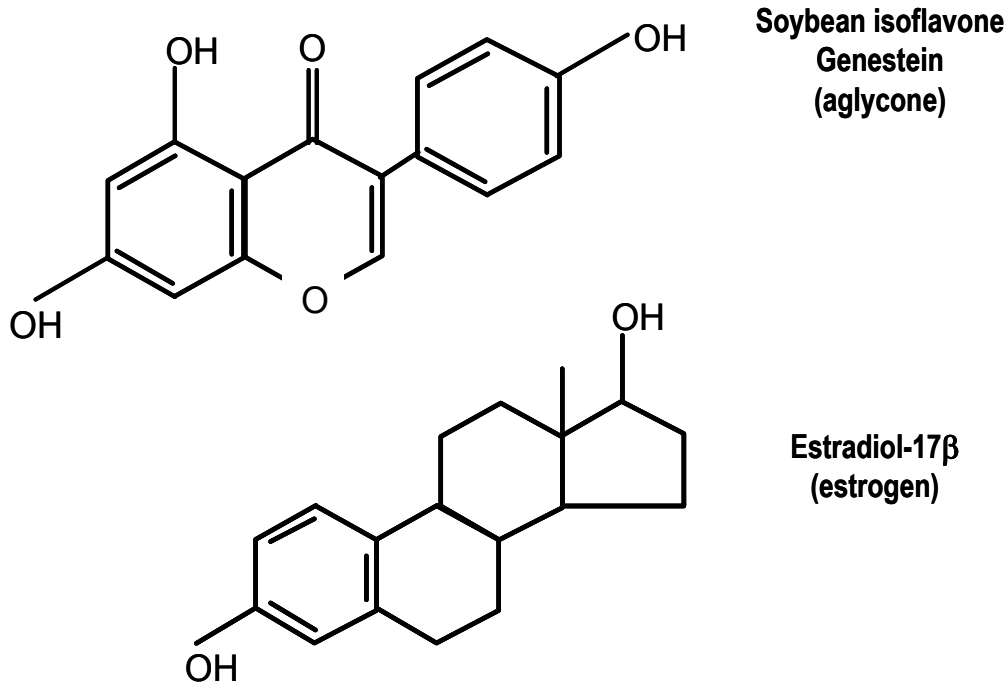

Soybean isoflavone Genestein (aglycone)

Estradiol-17β (estrogen)

Fig. 1. Molecular structure of soy isoflavone genistein compared to estradiol-17β.

In other studies, high dietary intakes of soy isoflavones were found to be associated with lower risk of recurrence among postmenopausal patients with breast cancer positive for estrogen and progesterone receptor and those who were receiving anastrozole as endocrine therapy (Kang et al., 2010a, b) .

Ogborn et al. (2010) have also recently reported that a soy diet ameliorated renal injury in rats. Soy diets preserved normal renal function and reduced relative renal weight, scores for cystic change, fibrosis, tissue oxidized LDL content, inflammation and epithelial cell proliferation. In this study though, alcohol-extracted slow isoflavone soy protein was found to retain its major protective effects and only subtle differences were attributed to isoflavones.

Consumption of tofu containing high levels of isoflavones reportedly exerted positive effects on verbal memory, although not in older men and women, where no or negative effects of these compounds on brain cells and cognition was observed (Hogervorst et al., 2011).

In regards to other health outcomes, Messina (2010) reported that although recent clinical data have not supported the skeletal benefits of isoflavones, 2 large prospective epidemiologic studies found soy intake to be associated with marked reductions in fracture risk. Additionally, soybean isoflavones modestly alleviate hot flashes in menopausal women.

In addition to isoflavones, soy saponins may also exert bioactive effects. Orally administered commercial purified soy saponin at 80 mg/kg body weight/day to spontaneously hypertensive rats for 8 weeks significantly decreased blood pressure (Hiwatashi et al., 2010). In another study on the effect of soy saponins on the growth of human colon cancer cells, Tsai et al. (2010) reported that intake of soy saponin decreased the number of viable cells in a dose-dependent manner. They concluded that soy saponin may be effective in preventing colon cancer by affecting cell morphology, cell proliferation enzymes, and cell growth.

Additionally, phytic acid which is considered to be an antinutritional component in soybean may possess antioxidant effects (Sakač et al., 2010). Recent research studies further suggest that lunasin, lectins, and trypsin inhibitors may have beneficial health properties. Trypsin inhibitors in soy have been of particular concern because, if not destroyed by heat prioir to consumption, they can cause pancreatic hypertrophy/hyperplasia, which ultimately results in growth inhibition (Liener, 1994,1996).

The majority of approved health claim for soy covers only soy proteins as evidence surrounding the effects of isoflavones and other bioactive compouds in soy are more controversial. Overall, although many studies suggest that the beneficial properties of soy may be attributed to the protein fraction, questions remain about potential synergistic or complimentary effects of other soy components.

In the sections below, an attempt will be made to provide a review of some of the different soy products available and their compositional differences and how this could impact their health properties.

3. Commercially available soyfoods and ingredients

Soybean has today become one of the world's most economical and valuable agricultural commodities due to its unique composition. On a wet basis, soybeans contain about 35% protein, 17% oil, 31% carbohydrate and 4.4% ash. The composition varies for different varieties and some cultivars can be found with protein contents of up to 50%. Soy proteins are nutritionally superior among vegetable proteins. Soy protein isolate has a Protein

Digestibility Corrected Amino Acid Score (PDCAAS) of 100% which means that it has all the essential amino acids required to support growth and maintenance. It contains good supplies of essential amino acids, such as lysine, which are normally lacking in other cereals. Fatty acids in soybeans consist of unsaturated fats, such as oleic, linoleic and linolenic acids, which are nutritionally beneficial. Additionally, soybeans also contain fibre and other phytochemicals, such as isoflavones and saponins which may have health benefits.

3.1 Major soyfoods
3.1.1 Soybeans and sprouts
Green soybean and soybean sprouts are two whole soyfoods prepared from soybean seeds. Sprouts are obtained by germinating soybeans for 5-10 days. They may be consumed fresh (e.g., in salads) or used as a vegetable in cooking. Green vegetable soybean, on the other hand, is harvested just before maturity (edamame) and can be cooked and eaten in salads and in soups or as a snack. It is available fresh (in pod or shelled), canned or frozen. The composition of these two products although prepared from the whole seed will vary due to the germination process applied to sprouts.

3.1.2 Soymilk
Soymilk is the liquid extract obtained after cooking, grinding and filtering soybean. It is not a "whole soyfood" per se as the majority of the fibre fraction (okara) is removed during processing. The soymilk extract obtained after filtration has a consistency that is very similar to cow's milk and is frequently used as an alternative to dairy products. There are four major types of soymilk products available (unsweetened, sweetened, flavoured and low fat). Unsweetened soymilk generally contains only water and soybeans. Sweetened soymilk may be sweetened with rice syrup, honey, corn or barley malt extract. Flavoured soymilk may be sweetened or unsweetened, and is often flavoured with cocoa, vanilla, carob or strawberry. Low fat soymilk may also be sweetened or unsweetened, flavoured or unflavoured, but usually contains less fat. Soymilk is frequently fortified with vitamins and minerals to increase its nutrient value. Blends of soymilk made with soybeans and different cereals or fruits are also available as well as "functional soymilk products" (e.g., with added omega 3 or other functional ingredients). Some manufacturers process soymilk using soy protein isolate rather than starting with the bean.

3.1.3 Tofu
Tofu is a curd made from heated soymilk. It is prepared by adding coagulating agents such as glucono-δ-lactone (GDL) or salts (magnesium chloride, calcium chloride, calcium sulphate) to heated soymilk followed by pressing to remove the whey. The final product is a gel with different textures and degrees of hardness depending on the type and amount of coagulant used and processing method (Fig.2). As with soymilk, tofu is not a "whole food" as the fibre is removed in the process of making the soymilk. The composition is also different from the starting soymilk as much of the whey is removed to concentrate the proteins and facilitate gel formation during pressing. Tofu has a soft white texture which is in some respects similar to cheese. On a wet basis, pressed tofu with a moisture content of about 85% contains 7.8% protein, 4.2% lipid, and 2 mg/g calcium (Wang et al., 1983; Liu, 1997). It is important to mention that in addition to compositional differences due to the type of salts used, health outcomes may also vary due to differences in protein digestibility

resulting from the type of network structure induced by the specific salt used in tofu-making.

$$MgCl_2/CaSO_4 \qquad\qquad CaSO_4$$
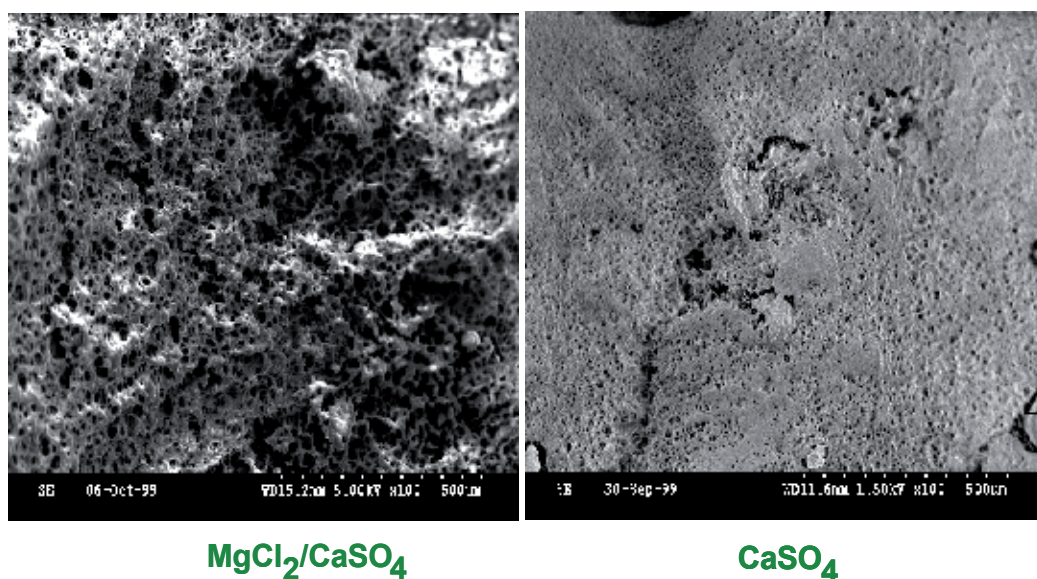
Fig. 2. Microstructure of tofu prepared using different salts.

3.1.4 Other fermented products from soybean

Soy sauce and soy paste (miso) are fermented soy products that are frequently used as condiments and seasoning in foods. Soy sauce and miso are made by fermenting soybean with or without other grains (e.g., wheat, rice, barley) with different types of *Aspergillus.*
Soy sauce is obtained in a liquid form whereas miso is a thick paste. These products are traditionally used in Asian cuisine but have become a mainstay of many modern diets. Tempeh and natto are two other fermented soyfoods but these are less frequently consumed outside of Asia. Tempeh is made by fermenting dehulled and cooked soybeans with *Rhizopus* whereas natto is fermented with *Baccillus subtilis*. The composition of these products will again vary depending on the processing conditions used and the amount of soybean present in the finished product. Furthermore, microorganisms used during fermentation can hydrolyse some phytochemicals such as isoflavones making them more bioactive.

3.1.5 Soy dips/dressings

There is a wide variety of dips/salad dressings etc. made from soybeans, soy flour, tofu, or soy protein isolates available on the market. They contain varying amounts of soy and their composition will similarly vary depending on the other ingredients used in the formulation.

3.1.6 Other soyfoods

The liquid extract during the preparation of soymilk can be further processed into a variety of refrigerated and frozen desserts such as ice cream, soy mousse, and fermented products such as soy yoghurt, soy probiotic beverage, and soy cheese, using processes similar to those

used in the dairy industry. The composition of these products will vary as a function of the ingredients used in processing and the fermentation process including the type of bacteria used during fermentation.

Soybeans can also be roasted in a manner similar to peanuts. The product obtained has a nutlike flavour and a crunchy texture which can be consumed as a snack. As these are made from intact whole bean, the composition will be similar to that of the starting raw material, however, the roasting process can induce changes which may modify digestibility. Roasted soybeans can also be ground to obtain roasted soy flour or roasted soynut butter.

3.2 Major soy ingredients
3.2.1 Soy oil

Today soybean oil is one of the world's leading vegetable oil for human consumption. Soybean oil is extracted from the bean after dehulling and flaking using organic solvents. The extracted oil is downstream processed to obtain a refined oil. Soybean oil is widely used in the manufacture of different foods. It is also frequently used as a salad or cooking oil and in the production of shortening, margarines, mayonnaise and salad dressings. By-products from the processing of soybean oil are also used to produce mono- and diglycerides and lecithin which are commonly used as emulsifying agents in foods.

3.2.2 Soy flour, soy protein concentrates, isolates and hydrolysates

Extraction of oil from soybeans leaves behind the soy meal biomass. Significant effort has been made in the last few decades to process this meal into value-added products. Defatted soy flakes (or flour, grits, meal), soy protein concentrates (SPC) and soy protein isolates (SPI) are the three major products available from soy meal. Additionally a full fat or partially defatted meal or flour can be obtained from the whole soybean.

Defatted soy flakes contain approximately 50% protein while, SPC and SPI generally contain at least 65% and 90% protein on a dry basis, respectively. Full-fat soy flour may be steamed and toasted to inactivate enzymes and enzyme inhibitors or unheated. Unheated, undefatted (or defatted) soy flour gives an enzyme active full-fat soy flour which is used in the bakery industry to bleach flour. Mechanically defatted soy meals with different fat contents are also available and usually sold in the organic category. Micronized soy flour (heat treated with infrared to eliminate anti-nutritional components in soy) are also available as whole beans or ground.

Soy products are widely used in formulated foods, partly because of their nutritional value but especially for the functional properties of the protein, which include gelling, foaming and emulsification which underlie many food sensory attributes. Other food products likely to contain soy ingredients include beverages, nutritional bars, bakery and cereal products, soups, meat products, beverages, confectionery, salad dressings and desserts. A growing use of soy protein concentrates and isolates is in the preparation of texturized food products that are used as meat alternatives or in cereal products. Soy proteins can also be hydrolysed enzymatically or chemically to produce hydrolysed vegetable protein (HVP) which is used as a flavour enhancer in many foods.

3.2.3 Other soy ingredients

In addition to the products listed above, soy dietary fibre, soy hulls, soy isoflavone extracts, vitamin E and soy phytosterols are examples of other ingredients from soybean processing which are used in nutraceutical products.

4. Compositional differences in soy products

The macro and micro nutrient composition of different soy products will vary markedly depending on soybean variety and the attendant biotic and abiotic influences, processing condition, other ingredients used in processing and whether the whole soybean or specific components are used in product development. Tables 1 to 8 provide detailed lists showing some of the compositional differences of a variety of soy products. As can be seen in the tables, the composition of protein, isoflavones, mineral, trypsin inhibitors, phytic acid and saponins can vary markedly for different soy products.

Additionally, it is important to note that even within a particular food category the type of processing technique and conditions used can impact composition and potential health benefits. We have showed in some of our earlier papers that the conditions used for processing of soy protein concentrates and isolates (such as particle size, method of defatting, process pH) can influence final product composition (Russin et al., 2007; L'hocine et al., 2006). Furthermore, Sobral et al. (2010) also reported that methods and conditions of preparation and storage of protein samples and mixtures of proteins were factors that modified their thermal behavior. In some instances higher denaturation temperatures increased thermal stabilization of soybean storage proteins which was attributed to protein-protein interactions occurring during processing. This increased stabilisation can impact digestibility and potentially, bioactivity.

Conditions used during spray drying can affect product composition. An increase of the inlet air temperature during spray drying of fermented soymilk greatly reduced viability and isoflavone aglycone content (Telang & Thorat, 2010). Moreover, denaturation of proteins during processing further reduced product solubility.

Processing of soybeans under severe alkaline conditions could lead to the formation of lysinoalanine, which can have negative impacts on health and decrease the bioavailability of essential amino acids (i.e., lysine). Processing under milder alkaline conditions avoids the formation of lysinoalanine and reduces potential negative side effects (Liener, 1994).

Additionally, the temperature and time used during the germination of soybeans in the production of germinated soybean flour modified the concentrations of bioactive compounds (i.e., isoflavones, saponins, trypsin inhibitors and lectins) (Paucar-Menacho et al., 2010a). At 25 °C, an increase in germination time decreased the concentration of Bowman-Birk inhibitor, lectin and lipoxygenase. Optimal increases in the concentrations of isoflavone aglycones (daidzein and genistein) and saponin glycosides were observed with a 63 h germination time at 30 °C. In a second study the authors found that germination of soybean for 42 h at 25 °C increased lunasin concentration by 62% and decreased the content of lectins by 59% (Paucar-Menacho et al., 2010b). Germination at 25 °C for 42 h resulted in a 32% increase in the concentration of soy saponins.

Similarly, fermentation of soymilk using a variety of probiotic lactic acid bacteria (LAB) resulted in the production of beta-glucosidase which hydrolyzed isoflavone glucosides to the bioactive isoflavone aglycones, genistein and daidzein in the fermented soymilk (Rekha & Vijayalakshmi, 2010a, 2011). Furthermore, decreases in phytic acid and increases in mineral bioavailability (e.g., calcium) were also observed. Huang et al. (2011) prepared sufu, a fermented soybean curd, by ripening salted tofu cubes in *Aspergillus oryzae*-fermented rice-soybean koji mash at 25, 35 or 45 °C for a period of 16 days and found that regardless of temperature, ripening caused a major reduction in the content of β-glucoside and malonylglucoside isoflavones along with a significant increase of aglycone isoflavone

Soy product	Moisture %	Protein %	Fat %	Carb.[4] %	Fiber %	Ash %	Reference
Fresh soybean	68	13	6	11	2	2	Snyder & Know, 1987
Soybean (dry)	7.50-10.1	31.1-36.6	16.3-21.3	6.29	22.0	4.69	Souci et al., 2000
Soybean (dry)	db	39.5-40.1	20.4-21.0	14.7-17.2	21.1-21.7	5.3-5.4	Van der Riet et al., 1987
Soybean sprouts	82	8	2	8	1	1	Snyder & Know, 1987
Soy curd	88	6	3	2	0	0.6	Snyder & Know, 1987
Firm tofu	79.3 - 75.5	10.6-14.3					Shurtleff & Aoyagi, 2000
Edam (cheese)	41.0	28.9	25.0	1.4		3.7	Ono, 2003
Ganmodoki (tofu derivative, td)	63.5	15.3	17.8	1.6		1.8	Ono, 2003
Fried bean curd (td)	75.9	10.7	11.3	0.9		1.2	Ono, 2003
Dried frozen tofu (td)	8.1	49.4	33.2	5.7		3.6	Ono, 2003
Tempeh unfermented soybeans[1]	db	48.3	25.7-28.6	2.0-2.8	17.7-19.3	3.0	Van der Riet et al., 1987
Tempeh fermented (24h)	db	48.7-49.7	25.2-27.9	1.9-2.4	18.3-20.2	2.7-2.8	Van der Riet et al., 1987
Tempeh fermented (48h)	db	48.6-49-7	25.2-26.3	1.9-2.2	16.1-19.3	2.7	Van der Riet et al., 1987
Tempeh fermented (72h)	db	49.3	22.9-23.8	1.7-2.3	15.3-15.7	3.0	Van der Riet et al., 1987
Tempeh (FWB)[2]	64	18	4	13		1	Snyder & Know, 1987
Natto (FWB)[2]	59	17	10	12	2	3	Snyder & Know, 1987
Hamanatto (FWB)[2]	36	26	12	14	3	12	Snyder & Know, 1987
Tempeh Gembus (FSP)[3]	81	5	2	11		1	Snyder & Know, 1987
Oncom ampas tahu (FSP)[3]	84	4	2	8		2	Snyder & Know, 1987
Soy sauce	72	7	0.5	2	0	18	Snyder & Know, 1987
Fermented soy curd	60	17	14	0.1		9	Snyder & Know, 1987
Soy paste	50	14	5	16	2	15	Snyder & Know, 1987
Ko Chu jang (soy paste)	48	9	4	19	4	20	Snyder & Know, 1987

[1]Before fermentation; [2] FWB-Fermented whole soybean; [3] FSP-Fermented soy pulp; [4]Carbohydrate.

Table 1. Composition of different soy foods

Soy product	Moisture %	Protein %	Fat %	Carb.[1] %	Ash %	Reference
Soymilk	94	3	1	1	0.3	Snyder and Know, 1987
Soymilk	88.7	3.20	1.84	5.76	0.48	Souci et al., 2000
Soy milk (full fat) dried	<10	38	18		<7	Garcia et al.,1997
Soy milk (low fat) dried	<5	48	9		<5	Garcia et al.,1997
Soymilk film	9	52	24	12	3	Snyder and Know, 1987
Soy ice cream	91.12 - 92.45	3.12 – 4.08	1.78 – 1.97	1.89 – 2.66	0.46 – 0.85	Sutar et al., 2010

[1]Carbohydrate

Table 2. Composition of soymilk and soymilk derived products

Soy product	Moisture %	Protein %	Fat %	Carbohydrate %	Ash %	Reference
Defatted soy meal		53	1	30		Achouri et al., 2005
Soya flour (full fat)	8.94-9.54	35.9-38.8	19.8-22.1	3.10	4.40	Souci et al., 2000
Soyflour (whole)		50				Hoogenkamp, 2001
Soyflour (natural)		40				Shurtleff & Aoyagi, 1983
Soyflour (full fat) dried	<10	42	21		4.7	Garcia et al.,1997
Soyflour (defatted)	6-8	52-54	0.5-1.0	30-32	5.0-6.0	Endres, 2001
Soyflour (defatted)		51				Shurtleff & Aoyagi, 1983
Soyflour (defatted) dried	6-8	42-52	0.5-1.0		5-6	Garcia et al.,1997
Toasted soy flour	5	38	19	32	5	Snyder & Know, 1987
Soya protein concentrate	4-6	62-69	0.5-1.0		3.8-6.2	Garcia et al.,1997
Soy protein isolate	4-6	86-87	0.5-1.0	3-4	3.8-4.8	Endres, 2001
Soy protein isolate	4-6	86-87	0.5-1.0		3.8-4.8	Garcia et al.,1997

[1]From defatted soybean

Table 3. Composition of selected soy ingredients

Soy product	wb/db	Daidzein family (µg/g)				Genistein family (µg/g)				Glycitein family (µg/g)			
		DEN	DIN	MDN	ADN	GEN	GIN	MGN	AGN	GEIN	GIIN	MGIN	AGIN
Soy bean	db	–	1294	2510	–	–	966	2755	–	–	205	200	–
	db	0	19 - 102	107 - 539	0	0 - 22	22 - 209	26 - 1113	0 - 11	0	31 - 126	110 - 285	0
Roasted soybeans	wb	39	460	45	397	69	551	63	743	52	68	72	102
	wb	44	474	46	40	77	568	65	71	58	70	74	54
Soy flour	wb	5.7	1087	–	–	8.4	1313	672	–	1	211	–	–
	wb	0	122	286	–	24	309	1023	0	–	309	55	–
	wb	4	147	261	–	22	407	–	1	19	41	57	32
Defatted soy flour	wb	80 - 480	480 - 770	–	–	40 - 460	580 - 1540	–	–	0 - 30	60 - 220	–	–
Soy protein concentrate	wb	20 - 200	30 - 760	–	–	10 - 220	40 - 1910	–	–	0 - 40	10 - 220	–	–
	wb	–	–	–	–	–	18	–	1	23	31	–	–
Soy protein isolate	wb	65.1	1326	–	–	47.8	1450	–	–	6	215	–	–
	db	125	44	155	–	246	124	538	61	–	24	34	–
	wb	80 - 210	140 - 300	–	–	50 - 220	550 - 800	–	–	10 - 30	30 - 60	–	–
	wb	11 - 63	0 - 133	18 - 20	6 - 74	36 - 136	137 - 382	88 - 100	0 - 215	22 - 53	34 - 55	36 - 39	33 - 46
Tofu	wb	46	25	159	8	52	84	108	1	12	8	–	29
	wb	116	453	753	54	140	562	788	66	26	130	131	0
	wb	3 - 22	40 - 105	64 - 156	0 - 18	4 - 30	64 - 143	40 - 158	8 - 17	2 - 4	11 - 29	7 - 19	0
Tofu yogurt	wb	–	42	61	–	3	80	79	–	5	12	–	–
Tempeh	wb	137	2	255	11	193	65	164	–	24	14	–	–
	wb	318	117	404	68	518	346	750	76	31	34	34	65
	wb	77 - 85	93 - 105	64 - 66	35 - 49	89 - 103	206 - 226	111	46 - 50	8 - 9	14	8	0
Miso	wb	135.8	81	1180	–	210.0	162	–	–	19	6	–	–
	wb	61	157	14 - 32	0	39	122	979	0	11	37	217	0
	wb	25 - 38	62 - 66	–	8 - 14	29 - 43	89 - 92	17 - 37	20 - 23	12	10 - 13	0 - 8	0 - 9
Soymilk	wb	18	410	690	22	19	710	871	820	10	65	39	89
Soymilk (aseptically processed)	wb	1 - 2	49 - 94	6 - 29	0	1 - 2	70 - 130	10 - 22	0 - 10	4 - 11	6 - 16	0 - 4	0
Soymilk (pasteurized)	wb	2 - 3	20 - 26	38 - 42	0	2 - 4	25 - 34	39 - 45	4 - 5	0 - 1	4 - 6	5	0
Soy beverage	wb	19.4	218	–	–	24.8	430	–	–	1	30	–	–
Tempeh burger	wb	34	36	25	–	96	158	–	1	18	18	–	–
Vegetable burger	wb	0.4	18	–	–	0.3	25	–	–	0.3	3	–	–
Soy molasses	wb	3.8	280	–	–	3	96	–	–	1	63	–	–
Soy hot dog	wb	8	35	12	–	16	67	42	4	8	15	15	14

Source: Reprinted with permission from Shimoni, 2004.
(DEN – daidzein; DIN – daidzin; MDN – malonyl daidzin; ADN – acetyl daidzin; GEN – genistein; GIN – genistin; MGN – malonyl genestin; AGN – acetyl genistin; GEIN – glycitein; GIIN – glycitin; MGIN – malonyl glycitin; AGIN – acetyl genistin)

Table 4. Isoflavone content of various soy foods

Soy product	Potassium	Phosp.[1]	Calcium	Magn.[1]	Iron	Zinc	Magn.[1]	Sodium	Copper	Ref.
	mg/100g									
Soybean	1693-1739	635-830	187-275	247-282	11.6-11.7	4.5-6.0	2.8-4.1	3.5-9.3	1.7-1.8	2
Tempeh unfermented	393-525	609-627	192-296	168-172	7.2-8.0	4.5-6.2	3.3-3.7	13.8-16.1	1.6-1.9	2
Tempeh fermented soybeans (24h)	222-224	688-731	225-310	193-221	8.7-8.9	5.4-7.1	3.5-4.1	14.2-14.6	1.8-1.9	2
Tempeh fermented soybeans (48h)	220-224	731-742	232-333	193-195	9.0	5.5-7.2	3.5-4.1	14.1	1.8-1.9	2
Tempeh fermented soybeans (72h)	218-219	734-795	248-318	193-201	8.7-8.8	5.4-7.4	3.6-4.2	13.8-15.1	1.8-1.9	2
Defatted soy four	2400-2700	700-900	200-300	200-300	10	5	3-4	3-15	1-2	3
Soy protein concentrate	100-2400	600-900	200-400	300	10-20	5	5	2-1200	1-2	3
Soy protein isolate	100-1400	500-800	100-200	30-90	10-20	4-9	2	40-1200	1-2	3

[1] Phosp. – phosphorous, Magn. – magnesium, Mang. - manganese;

[2] Van der Riet et al., 1987;

[3] Endres,2001

Table 5. Mineral content of some soy products

content. The highest increase in aglycone content and greatest decrease in malonylglucosides was observed at 45 °C and increasing ripening time further enhanced the changes observed.

The composition and antioxidant property of tofu is also affected by processing and the type of coagulant used (Rekha & Vijayalakshmi, 2010b). Tofu prepared with natural coagulants (*Citrus limonum, Garcinia indica, Tamarindus indica, Phyllanthus acidus and Passiflora edulis*) had significantly higher antioxidant activity compared to those prepared with salts. Furthermore, higher total crude protein and fat contents were found in some of the tofu prepared using the plant based coagulants (*G. indica and T. indica*).

Differences were reported in the composition and properties of soy whey (aqueous extract of defatted soybean flour) and tofu whey (liquid industrial residue from tofu production) (Sobral & Wagner, 2009), two products that may appear to be similar in properties. Although both byproducts contain primarily carbohydrates, proteins, non-protein nitrogen and salts, tofu whey reportedly had lower amounts of dry matter and proteins, and the antitryptic activity was three times lower than in soy whey. Thermal studies conducted using differential scanning calorimetry (DSC) of soy whey proteins showed endotherms corresponding to lectin and antitryptic factors of Kunitz and Bowman-Birk, whereas the thermogram for tofu whey only showed the presence of antitryptic factors. The authors attributed these differences to the manufacturing processes used. The extent to which these differences can affect health outcomes is unclear.

Soy product	Trypsin inhibitor activity mg/g sample	Reference
Whole soybean	16.7-27.2	Hafez, 1983
Whole soybean	48.2	Miyagi et al., 1997
Raw soy flour	28-32	Rackis et al., 1985
Raw soy flour	52.1	Anderson et al., 1979
Toasted soy flour	7.9-9.4	Rackis et al., 1985
Toasted soy flour	3.2-7.9	Anderson et al., 1979
Soy protein concentrate	5.4-7.3	Peace et al., 1992
Soy protein concentrate	6.3-13.9	Anderson et al., 1979
Soy protein concentrate	4.4-7.3	Peace et al., 1994
Soy protein isolate	1.2-30.0	Peace et al., 1992 Rackis et al., 1985
Soy protein isolate	4.4-11.0	Anderson et al., 1979
Soy based infant formulas	0.3-2.7	Peace et al., 1992
Soy tofu	0.6	Doell et al., 1981
Soy tofu	1.2-3.8	Miyagi et al., 1997
Soy milk	6.3	Miyagi et al., 1997
Soy sauce	0.3	Doell et al., 1981
Soy miso	4.1	Doell et al., 1981
Soy food fiber	6.47	Anderson et al., 1979
Chicken analog*	3.63	Anderson et al., 1979
Ham analog*	5.36	Anderson et al., 1979
Beef analog*	3.42	Anderson et al., 1979
Textured soy flour	5.15	Anderson et al., 1979

* manufactured using soy protein products
Cited from the following sources: Anderson & Wolf, 1995; Snyder and Know, 1987 (calculated on the basis of 1.9TUI = 1 g TI); Gilani et al., 2005.

Table 6. Trypsin inhibitor activity of different soy products

Soy product	Phytic acid % (dry basis)	Reference
Soybean	1.12-1.80	Toda et al., 2006
Soybean	1.00-1.47	Lolas et al., 1976
Soybean	1.32-2.30	Raboy et al., 1984
Whole soybean (dry)	1.07	Sutardi & Buclke,1985a
Soybean, raw	1.41	Sudarmadji & Markakis, 1977
Soaked (24h)	1.69	Sutardi & Buclke,1985a
Soybean, soaked	1.43	Sudarmadji & Markakis, 1977
Soybean, boiled (5 min)	1.68	Sutardi & Buclke,1985a
Soybean, boiled	1.23	Sudarmadji & Markakis, 1977
Soybean soaked (24h)	1.67	Sutardi & Buclke,1985a
Soybean (dehulled)	1.65	Sutardi& Buclke,1985a
Soybean (steamed 30 min)	1.48	Sutardi & Buclke,1985a
Soybean (drained and cooled)	1.47	Sutardi & Buclke,1985a
Full fat soy flour	1.51-1.81	Ranhotra et al., 1974
Defatted soy flour	1.62-1.85	Ranhotra et al, 1974
Defatted soy flour	1.30-1.63	Schuster & Bodwell, 1980
Textured soy flour	1.10-2.02	Davies & Reid, 1979
Textured vegetable protein	0.95-1.63	Harland & Oberleas, 1977
Concentrate	1.25-2.17	Ranhotra et al, 1974
Textured concentrate	1.48-1.50	Harland & Oberleas, 1977
Soy protein isolate	0.97-1.69	Schuster & Bodwell, 1980
Soy protein isolate	1.61-2.00	Honig et al., 1984
Spun isolate fiber	1.48	O'Neill et al., 1980
Soymilk	1.68	Omosaiye & Cheryan, 1979
Soymilk	1.83	Beleia et al., 1990
Tofu	1.5-2.5	Van der Riet et al., 1989
Tofu	1.96-2.88	Schaefer & Love, 1992
Okara (residue from soymilk)	0.5-1.2	Van der Riet et al., 1989
Tempeh	0.69-0.73	Sutardi & Buckle, 1985b
Tempeh unfermented soybeans (before fermentation)	1.0-1.2	Van der Riest et al., 1987
Tempeh fermented soybeans (24h)	0.3-0.6	Van der Riest et al., 1987
Tempeh fermented soybeans (48h)	0.2-0.4	Van der Riest et al., 1987
Tempeh fermented soybeans (72h)	0.1-0.2	Van der Riest et al., 1987
Tempeh (fresh)*	0.68-0.75	Sutardi & Buclke,1985a
Tempeh	0.96	Sudarmadji & Markakis, 1977
Fried fresh Tempeh*	0.35-0.38	Sutardi & Buclke,1985a
Tempeh stored 2wk@5°C*	0.18-0.19	Sutardi & Buclke,1985a
Fried stored tempeh*	0.09-0.10	Sutardi & Buclke,1985a

* Different types of inoculation used in production
Data taken from the following source: Anderson & Wolf, 1995

Table 7. Phytic acid content of different soy products

Soy product	Saponin content %	Reference
Soybean	0.44-0.49	Berhow et al., 2006
Soybean	0.47	Ireland et al., 1986
Soybean	0.225-0.298	Kitagawa et al., 1984
Soybean	5.1	Fenwick & Oakenfull, 1981
Soybean	3.9	Fenwick & Oakenfull, 1983
Soybean	0.46-0.50	Gestetner et al.,1966
Soybean	5.6	Fenwick & Oakenfull, 1981
Toasted, defatted soy flour	0.67	Ireland et al., 1986
Defatted soy flour	2.0	Fenwick & Oakenfull, 1981
Defatted soy flour	0.35	Curl et al.,1985
Defatted soy flour	2.2-2.5	Fenwick & Oakenfull, 1981
Commercial soy flour	0.46	Price et al.,1985
Soya hulls	2.0	Fenwick & Oakenfull, 1981
Full fat, enzyme active soy flour	0.43	Ireland et al., 1986
Full fat, heat treated soy flour	0.49	Ireland et al., 1986
Soya protein isolate	0.76	Ireland et al., 1986
Soy protein isolate : Promine D*	0.3	Fenwick & Oakenfull, 1981
Soy protein isolate : GL-750*	0.8	Fenwick & Oakenfull, 1981
Soy protein isolate : Maxten C*	1.9	Fenwick & Oakenfull, 1981
Soy protein isolate : Maxten E*	2.5	Fenwick & Oakenfull, 1981
Soymilk	0.026	Ireland et al., 1986
Soymilk	0.022	Ireland et al., 1986
Soymilk	0.39	Kitagawa et al., 1984
Yuba (dried soymilk film)	0.41	Kitagawa et al., 1984
Okara (residue of soymilk)	0.10	Kitagawa et al., 1984
Tofu	2.1	Fenwick & Oakenfull, 1981
Tofu	0.30-0.33	Kitagawa et al., 1984
Miso	0.15	Kitagawa et al., 1984
Natto	0.25	Kitagawa et al., 1984
Tonyu (soya milk)	0.047	Kitagawa et al., 1984

*trademark

Table 8. Saponin content of different soy products

In addition to processing, various plant breeding techniques have been used to modify the composition of soybeans for different food applications (Esteves et al., 2010, Brune et al., 2010). Thus depending on the type of variety used, compositional differences can be

expected in macro and micro components (i.e., protein, indispensable, dispensable and total amino acid, lunasin, isoflavones, phytic acid, oxalate and trypsin inhibitors as well as mineral content). The biological activity of soybean products will, therefore, not only depend on the processing technique used but the variety of soybean used.

5. Soyfoods and health claims

An important factor that may contribute to inconsistencies in the evidence of the health benefit of soy may be related to the type of material used for clinical studies. Reinwald et al. (2010) argue the possibility that whole soy may have a more unique effect on health than a select soy component.

Differences in health outcomes related to soy and soy component consumption could be due to varietal and compositional differences, the impact of processing, additive effects of various components (i.e., the whole is greater than the sum of the parts) as well as age and health status. Furthermore, some have argued that the presence or absence of specific gut microflora could also contribute to health outcomes (Patisaul & Jefferson, 2010) as microorganisms may play a critical role in converting physiologically inactive phytochemicals to the bioactive form.

As an example, Reinwald and Weaver (2010) have reported that whereas epidemiological studies in Asia evaluating diets containing traditional whole soyfoods showed a positive association with bone mineral density and fracture protection, smaller scale intervention studies in Western nations mainly using isolated soy protein (SP) and purified or concentrated soy isoflavones (SI) rather than whole soyfoods have produced inconsistent results.

Similarly, Lagari and Levis (2010) found that clinical trials are conflictive regarding the effects of phytoestrogens on bone mineral density and bone turnover markers in premenopausal and postmenopausal women and argue that much of the controversy lies in differences in study design, reporting of results, participants' age and menopausal status, and type and dose of phytoestrogen used.

In Canada, where a health claim for soy has not yet been approved, the ministry responsible for health (Health Canada) requires the following evidence to substantiate health claims:

1. **Causality –** Evidence of high quality and quantity of original research in humans based on randomized controlled intervention and / or prospective observational studies are mandatory to substantiate the health claim of food or food constituents with high level of certainty (statistical significance achieved at $p \leq 0.05$) and the relationship between the amount of food and the health effect.
2. **Generalizability -** The claimed effect of the food of food constituent is biologically / physiologically relevant and expected to benefit the health of the target population, appropriate and validated surrogate marker must be used to ensure the biological relevance and it should be part of the causal pathway between the food and the health outcome.
3. **Quality assurance -** The food is produced according to quality standards and consistently meets predefined specifications.
4. **Safety -** The subject of a health claim application must be for a food approved for safe use; or, if a novel food is the subject of the health claim, a novel food application must

be completed and submitted to Health Canada preceding or concurrent with this application. The adverse effects related to food intake observed in human studies should be addressed and must provide risk management strategies to overcome the adverse effects and or restriction on use of food.

As there is a wide variety of soy products available on the market, the specific type used for clinical studies can impact results.

A major challenge for health claim support studies is the lack of standardized materials and controls for clinical studies as well as the use of appropriate clinical outcomes and surrogate endpoints for different disease risks. Furthermore, very few studies have been conducted to understand what happens to soyfoods and soy components in the gastrointestinal tract and the specific events that occur at the mucosal barrier and how these events influence absorption, distribution, metabolism and excretion. The gastrointestinal mucosa is an interesting and complex system that acts as a barrier between the body and the lumenal environment and is selective in that it allows the transfer of selected nutrients across the epithelium while excluding perceived harmful components in the bolus. This complex interplay between bioactives and the intestinal mucosa also needs to be carefully documented to support any eventual health claim. Matrix interactions can influence the bioactivity of the components of interest and the functional properties of the matrix. Furthermore, for double blind placebo controlled studies which are the gold standard in clinical studies, the effect of the matrices used in the clinical trials need to be carefully evaluated.

As policy makers, food regulators, industry and consumers in other jurisdictions demand more evidence to support current and future health claims, some question of interest that remain include the following:

- What is the relationship reported between the exposure to soy and specific health effects from both observational and intervention studies that meet selection criteria?
- Can a clear distinction be made between the effect of soy protein alone and soy protein within a matrix (e.g., whole soybean foods and foods made from soy protein ingredients)?
- What is the efficacy of soy proteins in which isoflavones have been removed versus soy proteins that contain isoflavones in respect to relationship with cardiovascular disease risk reduction and other health claims?
- How are breakdown products absorbed in the gastrointestinal tract and how do they exert physiological effects in the body?
- How do matrix effects influence adsorption, digestion, metabolism and excretion?
- What is the impact of processing on the efficacy of soy protein and the bioactivity of materials used in clinical trials?
- What are the ideal samples and controls that should be used in clinical trial studies for specific clinical outcomes and what surrogate endpoints should be used?
- What is the feasibility for consuming recommended intake levels for health claims especially in populations that do not consume soy frequently (e.g., is the consumption of 20-25 g of soy protein a day feasible?)?
- What is the documented effect of anti-nutritional factors such as trypsin inhibitors, phytates, lectins and lysinoalanine and the extent of heat resistance of trypsin inhibitors found in soybeans?

6. Conclusion

Soy protein has officially been approved in several countries as a "functional food", making it one of the most valuable vegetable proteins in the world today. As concerns about health, climate change and the impacts of agricultural practices increase, assurance of a diversified sustainable source of nutrition that provides proven health benefits will become increasingly important. Soybeans can contribute to this nutrient biodiversity in an instrumental way. In crop production and rotation, soybeans play a crucial role in nitrogen fixation making them an important component of agricultural sustainability. Furthermore, soy proteins have excellent functional properties that can be exploited in various food applications. Processing technologies are continually being investigated for soy protein fractionation which will allow modifications in protein profile and which could dramatically improve suitability of soy protein products for targeted food, nutraceutical and industrial applications (e.g., enriched 11S, 7S, or 2S soy protein extracts). Breeding efforts to remove or enhance mico nutrients and other bioactive components are also likely to have an impact on improving health outcomes. To support health claims and enhance the benefits of consumption, constant evaluation of the totality of the body of knowledge in regards to potential health benefits and well designed experimental studies using well characterized materials will, therefore, be needed.

7. References

Achouri, A., Boye, J.I., Belanger, D. 2005. Soybean isoflavones: Efficacy of extraction conditions and effect of food type on extractability. *Food Research International*, 38, pp. 1199-1204.

Anderson, R.L., Rachkis, J.J., Tallent, W.H. 1979. Biologically active substances in soy products, In *Soy Protein and Human Nutrition,* Editors: Wilcke H.L., Hopkins D.T. Waggle D.H., eds. Academic Press, New York.

Anderson, R.L., Wolf, W.J. 1995. Overview of soy processing and products: Compositional changes in trypsin inhibitors, phytic acid, saponins and isoflavones related to soybean processing. *Journal of Nutrition*, 125, pp. 581S-588S.

Beleia, A., Ida, E.I., Lethi, T.T. 1990. Phosphorus and pytic acid distribution during soy milk processing. *Brazilian Archives of Biology and Tecnology* (*Arq.Biol. Technol)*, 33, pp. 623-629.

Berhow, M.A., Bin Kong, S., Vermillion, K.E., Duval, M.S. 2006. Complete quantification of group A and group B soyasaponins in soybeans. *Journal of Agricultural and Food Chemistry,* 54 (6), pp. 2035-2044.

Bruckert, E., Rosenbaum, D. 2011. Lowering LDL-cholesterol through diet: Potential role in the statin era. *Current Opinion in Lipidology,* 22(1), 43, pp. 48-64.

Brune, M.F.S.S, Pinto, M.S., Peluzio, M.C.G., Moreira, M.A., de Barros, E.G. 2010. Biochemical and nutritional evaluation of a soybean line lacking the kunitz trypsin inhibitor and lectins. *Ciencia e Tecnologia de Alimentos*, 30(3), pp. 657-63.

Chiang, W. D., Tsou, M. J., Tsai, Z. Y., Tsai, T. C. 2006. Angiotensin I-converting enzyme inhibitor derived from soy protein hydrolysate and produced by using membrane reactor. *Food Chemistry*, 98, pp. 725-732.

Curl, C. L., Price, K.R., Fenwick, G.R. 1985. The quantitative estimation of saponin in pea (*pisum sativum* L) and soya (Glycine max). *Food Chemistry*, 18, pp. 241-250.

Davies, N.T., Reid H. 1979. An evaluation of the phytate, zinc, copper, ion and manganese contents of, and Zn availability from, soya-based textyured-vegetable-protein meat-substitutes or meat-extenders. *British Journal of Nutrition*, 41, pp. 579-589.

Endres, J.E. 2001. Soy protein products – Characteristics, nutritional aspects, and utilization, revised and expanded Edition, a publication of AOCS press, P.O. Box 3489 Champain, IL. 61826-3489 USA - ISBN 1-893997-27-8.

Erdos, E. G. 1975. Angiotensin I-converting enzyme. *Circulation Research*, 36, pp. 247-255.

Esteves, E.A., Martino, H.S.D., Oliveira, F.C.E., Bressan, J., Costa, N.M.B. 2010. Chemical composition of a soybean cultivar lacking lipoxygenases (LOX2 and LOX3). *Food Chemistry,* 122(1), pp. 238-242.

Fenwick D.E., Oakenfull, D. 1981. Saponin content of soya beans and some commercial soya bean products. *Journal of the Science of Food and Agriculture*, 32, pp. 273-278.

Fenwick, D.E., Oakenfull, D. 1983. Saponin content of food plants and some prepared foods, *Journal of the Science of Food and Agriculture*, 34, pp. 186-191.

Garcia, M.C., Torre, M., Marina, M.L., Laorda, F. 1997. Composition and characterization of soyabean and related products, *Critical Reviews in Food Science and Nutrition*, 37(4), pp. 361-391.

Gestetner, B., Birk, Y., Bondi, A., Tencer, Y. 1966. Soya bean saponins – VII. A method for the determination of sapogenin and saponin contents in soya beans. *Phytochemistry,* 5, pp. 803-806.

Gilani, G.S, Cockell, K.A., Sepehr, E. 2005. Special guest editor section: Effets of antinutritional factors on protein digestibility and amino acid availability in foods. Journal of the Association of Analytical Chemists International, 88(3), 967-987.

Harland, B.F., Oberleas, D. 1977. A modified method for phytate analysis using an ion-exchange procedure: application to textured vegetable proteins. *Cereal Chemistry*, 54, pp. 827-832.

Harlanda, J.I., Haffnerb, T.A. 2008. Systematic review, meta-analysis and regression of randomised controlled trials reporting an association between an intake of circa 25g soy protein perd ay and blood cholesterol. *Atherosclerosis,* 200, pp. 13-27.

Hartmann, R., Meisel, H. 2007. Food-derived peptides with biological activity: from research to food applications. *Current Opinion in Biotechnology*, pp. 18, 1-7.

Hiwatashi, K., Shirakawa, H., Hori, K., Yoshiki, Y., Suzuki, N., Hokari, M., Komai, M., Takahashi, S. 2010. Reduction of blood pressure by soybean saponins, renin inhibitors from soybean, in spontaneously hypertensive rats. *Bioscience, Biotechnology and Biochemistry*, 74(11), pp. 2310-2312.

Hogervorst, E., Mursjid, F., Priandini, D., Setyawan, H., Ismael, R.I., Bandelow, S., Rahardjo, T.B. 2011. Soy consumption may be associated with better recall in younger, but not in older, rural indonesian elderly. *Brain Research*, 1379, pp. 206-212.

Honig, D.H., Wolf, W.J., Rackis, J.J. 1984. Phytic acid and phosphorus content of various soybean protein fractions. *Cereal Chemistry*, 61, pp. 523-526.

Hoogenkamp H.W. 2001 Soy protein & meat formulations, In *Lifestyle Marketing Dynamics Coupled With Modern Processing Techniques and Old Time Favorites*, published by Protein Technologies International – A DuPont Business P.O. Box 88940, St Louis, MO 63188.

Huang, Y., Lu, T., Chou, C. 2011. Ripening temperature affects the content and distribution of isoflavones in sufu, a fermented soybean curd. *International Journal of Food Science and Technology*, 46(2), pp. 257-262.

Ireland, P.A., Dziedzic, S.Z., Kearsley, M.W. 1986. Saponin content of soya and some commercial soya products by means of Hight-performance liquid chromatography of sapogenins. *Journal of the Science of Food and Agriculture,* 37, pp. 694-698.

Jenkins, D.J.A., Mirrahimi, A., Srichaikul, K., Berryman, C.E., Wang, L., Carleton, A., Abdulnour, S., Sievenpiper, J.L., Kendall, C.W.C., Kris-Etherton, P.M. 2010. Soy protein reduces serum cholesterol by both intrinsic and food displacement mechanisms. *Journal of Nutrition*, 140(12), pp. 2302S-2311S.

Kang, J., Badger, T.M., Ronis, M.J.J., Wu, X. 2010a. Non-isoflavone phytochemicals in soy and their health effects. *Journal of Agricultural and Food Chemistry*, 58(14), pp. 8119-8133.

Kang, X., Zhang, Q., Wang, S., Huang, X., Jin, S. 2010b. Effect of soy isoflavones on breast cancer recurrence and death for patients receiving adjuvant endocrine therapy. *Canadian Medical Association Journal*, 182(17), pp. 1857-1862.

Kitagawa, I., Yoshikawa, M., Hayashi, T., Taniyama, T. 1984. Quantitative determination of soyasaponins in soybeans of various origins and soybean products by means of high performance liquid chromatography. *Yakugaku Zasshi*, 104, 275-279.

Kuba, M., Tanaka, K., Tawata, S., Takeda, Y., Yasuda, M. 2003. Angiotensin I-converting enzyme inhibitory peptides isolated from tufuyo fermented soybean food. *Bioscience Biotechnology and Biochemistry*, 67, pp. 1278-1283.

Lagari, V.S., Levis, S. 2010. Phytoestrogens and bone health. Current Opinion in Endocrinology. *Diabetes and Obesity,* 17(6), pp. 546-553.

L'Hocine, L., Boye, J.I., Arcand, Y. 2006. Composition and functional properties of soy protein isolates prepared using alternative defatting and extraction procedures. *Journal of Food Science*, 71(3), pp. C137-145.

Liener, I.E. 1994. Implications of antinutritional components in soybean foods. *Critical Reviews in Food Science and Nutrition*, 34(1), pp. 31-67.

Liener, I.E. 1996. Effects of processing on antinutritional factors in legumes: The soybean case. *Archivos latinoamericanos de nutrición,* 44(4 Suppl 1), pp. 48S-54S.

Liu, K. 1997. Soybeans: Chemistry, Technology and Utilization. Aspen Publishers, USA Originally published by Chapman and Hall, New York, USA.

Lolas, G.M., Palamidis, N., Markakis, P. 1976. The phytic acid total phosphorus relationship in barley, oats, soybeans and wheat. *Cereal Chemistry*, 53, pp. 867-871.

Merritt, J. C. 2004. Metabolic syndrome: soybean foods and serum lipids. *Jouranl of the National Medical Association,* 96, pp. 1032–1041.

Messina, M. 2010. Insights gained from 20 years of soy research. *Journal of Nutrition,* 140(12), pp. 2289S-2295S.

Miyagi, Y., Shinjo, S., Nishida, R., Miyagi, C., Takamatsu, K., Yamamoto, T., Yamamoto, S. 1997. *Journal of Nutritional Science and Vitaminology*, 43, pp. 575-580.

Nilavsen, K., Meinertz, H. 1998. Variable response to dietary soy protein in healthy normolipidemic men. *American Journal of Clinical Nutrition,* 68, pp. 1380S-1384S.

O'Neill, J.K., Sargent, M., Trimble, M.L. 1980. Determination of phytate in foods by phophorus 31 Fournier transfrom nuclear magnetic resonance spectrometry. *Analytical Chemistry*, 52, pp. 1288-1291.

Ogborn, M.R., Nitschmann, E., Bankovic-Calic, N., Weiler, H.A., Aukema, H.M. 2010. Dietary soy protein benefit in experimental kidney disease is preserved after isoflavone depletion of diet. *Experimental Biology and Medicine*. 235(11), pp. 1315-1320.

Omosaiye, O., Cheryan, M. 1979. Low-phytate, full fat soy protein product by ultrafiltration of aqueous extracts of whole soybeans. *Cereal Chemistry*, 56, pp. 58-62.

Ono T. 2003. Soy (soya) Cheeses In: *Encyclopedia of Food Sciences and Nutrition*, Second edition, Editors: Caballero B, Trugo L. C. Finglas P. M., Acadamic Press – ISBN 0-12-227055-X, pp. 5398-5402.

Onuegbu, A.J., Olisekodiaka, J.M., Onibon, M.O., Adesiyan, A.A., Igbeneghu, C.A. 2011. Consumption of soymilk lowers atherogenic lipid fraction in healthy individuals. *Journal of Medicinal Food*, 14(3), pp. 257-260.

Patisaul, H.B., Jefferson, W. 2010. The pros and cons of phytoestrogens. *Frontiers in Neuroendocrinology*, 31(4), pp. 400-419.

Paucar-Menacho, L.M., Berhow, M.A., Mandarino, J.M.G., Chang, Y.K., Mejia, E.G.D. 2010a. Effect of time and temperature on bioactive compounds in germinated Brazilian soybean cultivar BRS 258. *Food Research International*, 43(7), pp. 1856-1865.

Paucar-Menacho, L.M., Berhow, M.A., Mandarino, J.M.G., de Mejia, E.G., Chang, Y.K. 2010b. Optimisation of germination time and temperature on the concentration of bioactive compounds in Brazilian soybean cultivar BRS 133 using response surface methodology. *Food Chemistry*, 119(2), pp. 636-642.

Peace, R.W., Sarwar, G., Touchburn, S.P. 1994. Trypsin inhibitor levels in soy-based infant formulas and commercial soy protein isolates and concentrates. *Food Research International*, 25, 137-141.

Potter, S.M., Baum, J.A, Teng, H., Stillman, R.J., Shay, N.F, Erdman J.W. 1998. Soy protein and isoflavones. *American Journal of Clinical Nutrition,* 68, pp. 1375S-1379S.

Price, K.R., Griffiths, N.M., Curl, C.L., Fenwick, G.R 1985. Undesirable sensory propeties of the dried pea. *Food Chemistry*, 25, pp. 105-115.

Raboy, V., Dickinson, D.B., Below, F.E. 1984. Variation in seed total phosphorus, phytic acid, zinc, calcium, magnesium and protein among lines of Glycine max and G. soja. *Crop Science*, 24, pp. 431-434.

Ranhotra, G.S., Loewe, R.J., Puyat, L.V. 1974. Phytic acid in soy and its hydrolysis during breadmaking. *Journal of Food Science*, 39, pp. 1023-1025.

Reinwald, S., Akabas, S.R., Weaver, C.M. 2010. Whole versus the piecemeal approach to evaluating soy. *Journal of Nutrition*, 140(12), pp. 2335S-2343S.

Reinwald, S., Weaver, C.M. 2010. Soy components vs. whole soy: Are we betting our bones on a long shot? *Journal of Nutrition*, 140(12), pp. 2312S-2337S.

Rekha, C.R., Vijayalakshmi, G. 2010a. Bioconversion of isoflavone glycosides to aglycones, mineral bioavailability and vitamin B complex in fermented soymilk by probiotic bacteria and yeast. *Journal of Applied Microbiology*, 109(4), pp. 1198-1208.

Rekha, C.R., Vijayalakshmi, G. 2010b. Influence of natural coagulants on isoflavones and antioxidant activity of tofu. *Journal of Food Science and Technology*, 47(4), pp. 387-393.

Rekha, C.R., Vijayalakshmi, G. 2011. Isoflavone phytoestrogens in soymilk fermented with β-glucosidase producing probiotic lactic acid bacteria. *International Journal of Food Science and Nutrition*, 62(2), pp. 111-120.

Russin, T.A., Arcand, Y., Boye, J.I. 2007. Particle size effect on soy protein isolate extraction. *Journal of Food Processing and Preservation*, 31(3), pp. 308-319.

Sacks, F. M, Lichtenstein, A., Van Horn, L., Harris, W., Kris-Etherton, P., Winston, M. 2006. Soy protein, isoflavones, and cardiovascular health: An American Heart Association Science Advisory for Professionals from the Nutrition Committee. *Circulation*, 113, pp. 1034 – 1044.

Sakač, M., Čanadanovič-Brunet, J., Mišan, A., Tumbas, V., Medič, D. 2010. Antioxidant activity of phytic acid in lipid model system. *Food Technology and Biotechnology*, 48(4), pp. 524-529.

Schaefer, M.J., Love, J. 1992. Relationship between soybean components and tofu texture. *Journal of Food Quality*, 15, pp. 53-66.

Schuster, E.M., Bodwell, C.E. 1980. Phytic acid content of thirty-two commercial soy products. *Federation Proceedings,* 39, pp. 659(abs).

Shimoni, E. 2004. Stability and shelf life of bioactive compound during food processing and storage : Soy isoflavones. *Journal of Food Science: Concise Reviews in Food Science*, 69(6), pp. R160-R166.

Shurtleff, W., Aoyagi, A. 1983. The book of tofu, Protein source of the future …now!, Completed & Unabridged, vol I, Ten Speed Press - A Soyfoods Center book   ISBN 0-89815-095-7.

Shurtleff, W., Aoyagi, A. 2000. Tofu & soymilk Production, The book of tofu, volume II, A Craft and Technical Manual, Soyfoods Center   ISBN 1-928914-04-1.

Snyder, H.E., Know, T.W. 1987. Soybean Utilization. Van Nostrand Rienhold Company Inc, 115th Fifth Avenue New York, New York, 10003 , an AVI book - ISBN 0-442-28216-6.

Sobral, P.A., Palazolo, G.G., Wagner, J.R. 2010. Thermal behavior of soy protein fractions depending on their preparation methods, individual interactions, and storage conditions. *Journal of Agricultural and Food Chemistry*, 58(18), pp. 10092-10100.

Sobral, P.A., Wagner, J.R. 2009. Relationship between the composition and antitryptic activity of soy and tofu wheys and thermal behavior of their isolated protein. *Informacion Tecnologica*, 20(5), pp. 65-74.

Souci F.W., Fachman W., Kaut H. 2000. Food composition and nutrition tables, 6th revised and completed edition, Medphram Scientigfic publishers, CRC press.

Sudarmadji, S., Markakis, P. 1977. The phytate and phytase of soybean tempeh. *Journal of the Science of Food and Agriculture*, 28, pp. 381-383.

Sutar, N., Sutar, P.P., Singh, G. 2010. Evaluation of different soybean varieties for manufacture soy ice cream. *International Journal of Dairy Technology*, 63(1), pp. 136-142.

Sutardi, Buckle, K.A. 1985a. Reduction in phytic acid levels in soybeans during tempeh production, storage and frying. *Journal of Food Science*, 50, pp. 260-263.

Sutardi , Buckle, K.A. 1985b. Phytic acid changes in soybeans fermented by traditional inoculum and six strains of *Rhizopus oligosporus*. Journal of Applied Bacteriology, 58, pp. 539-543.

Taku, K., Lin, N., Cai, D., Hu, J., Zhao, X., Zhang, Y., Wang, P., Melby, M.K., Hooper, L., Kurzer, M.S., Mizuno, S., Ishimi, Y., Watanabe, S. 2010. Effects of soy isoflavone extract supplements on blood pressure in adult humans: Systematic review and meta-analysis of randomized placebo-controlled trials. *Journal of Hypertension*, 28(10), pp. 1971-1982.

Taku, K., Umegaki, K., Ishimi, Y., Watanabe, S. 2008. Effects of extracted soy isoflavones alone on blood total and LDL cholesterol: Meta-analysis of randomized controlled trials. *Journal of Therapeutics and Clinical Risk Management,* 5, pp. 1097-1103.

Telang, A.M., Thorat, B.N. 2010. Optimization of process parameters for spray drying of fermented soy milk. *Drying Technology*, 28(12), pp. 1445-1456.

Tian, J., Zhao, S., Zhai, B., Xu, L. 2010. Biotransformation of group B soybean saponins. 2010 4th International Conference on Bioinformatics and Biomedical Engineering, iCBBE. ISBN 978-1-4244-4712-1, pp. 1-3.

Toda, K., Takahashi, K., Ono, T., Kitamura, K., Nakamura, T. 2006. Variation in phytic acid content of soybeans and its effect on consistency of tofu made from soybean varieties with high protein content. *Journal of the Science of Food and Agriculture*, 86, pp. 212-219.

Tsai, C., Chen, Y., Chien, Y., Huang, W., Lin, S. 2010. Effect of soy saponin on the growth of human colon cancer cells. *World Journal of Gastroenterology*, 16(27), pp. 3371-3376.

Van der Riet, W.B., Wight A. W., Cilliers J.J.L., Datel J.M. 1987. Food chemical analysis of tempeh prepared from South African-grown soybeans. *Food Chemistry*, 25, pp. 197-206.

Van der Riet, W.B., Wight A. W., Cilliers J.J.L., Datel J.M. 1989. Food chemical investigation of tofu and its byproduct okara. *Food Chemistry*, 34, pp. 193-202.

Wang, H.L., Swain, E.W., Kwolek, W.F. 1983. Effect of soybean varieties on the yield and quality of tofu. *Cereal Chemistry,* 60, pp. 245-248.

Wu, J., Ding, X. 2002. Characterization of inhibition and stability of soy-protein-derived angiotensin I-converting enzyme inhibitory peptides. *Food Research International*, 35, pp. 367-375.

Yang, H.Y.T., Erdos, E.G., Levin, Y.A. 1970. A dipeptidyl carboxypeptidase that converts angiotensin I and inactivates bradykinin. *Biochimica et Biophysica Acta*, 214, pp. 374-376.

Yang, H.Y., Yang, S.C., Chen, S.T., Chen, J.R. 2008. Soy protein hydrolysate ameliorates cardiovascular remodeling in rats with L-NAME-induced hypertension. *Journal of Nutritional Biochemistry*, 19, pp. 833-839.

Zhuo, X.G, Melby, M. K., Watanabe, S. 2004. Soy isoflavone intake lowers serum LDL cholesterol: A meta-analysis of 8 randomized controlled trials in humans. *Journal of Nutrition,* 134, pp. 2395-2400.

Edited by Hany El-Shemy

Worldwide, soybean seed proteins represent a major source of amino acids for human and animal nutrition. Soybean seeds are an important and economical source of protein in the diet of many developed and developing countries. Soy is a complete protein and soy-foods are rich in vitamins and minerals. Soybean protein provides all the essential amino acids in the amounts needed for human health. Recent research suggests that soy may also lower risk of prostate, colon and breast cancers as well as osteoporosis and other bone health problems and alleviate hot flashes associated with menopause. This volume is expected to be useful for student, researchers and public who are interested in soybean.

Photo by naito8 / iStock

IntechOpen

ISBN 978-953-51-5172-2

9 789535 151722