Chapter 8

Value - Added Products from Soybean: Removal of Anti-Nutritional Factors *via* Bioprocessing

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Additional information is available at the end of the chapter

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1. Introduction

Soybean is the second largest acreage crop in the United States (29%), right after corn (35%) according to the American Soybean Association [1]. Soybean is widely consumed in the world, particularly in Asian countries. The various soybean products could be separated into non-fermented and fermented soybean products. The non-fermented soybean products include soymilk, tofu, yuba, soybean sprouts, okara, roasted soybeans, soynuts and soy flour, immature soybeans, cooked whole soybeans, and the fermented oriental soybean products include soy paste (Jiang and Miso), soy sauce, Tempeh, Natto, soy nuggets (Douchi), sufu. In the United States, soy oil is often used for food and biodiesel production. The soybean processing process is shown in Figure 1. After the oil extraction, the residue – flaked soy meal, is usually produced into four products (textured soy flour, soy protein concentrate and soy protein isolate, 48% soy meal, soluble soy carbohydrate). The textured soy flour could be used in bakery products, meat products, infant food etc. Soy protein concentrate and isolate could be used in baby food, bakery products, cereals, lunch meat etc. SSPS (soluble soybean polysaccharides) functions as a dispersing agent, stabilizer, emulsifier, and has good adhesion properties [2]. The 48% soy meal is used for animal feed. The portions of different animal usages are poultry (48%), swine (26%), beef (12%), dairy (9%), pets (2%), others (3%) [1]. Poultry and swine usages account for74%.

The popular usage of soybean for food and feed is due to its nutritional profile. Soybean is a good protein source and the only dietary isoflavone source together with other legumes. Anti-nutritional factors in soybean, such as phytic acid, oligosaccharides, trypsin inhibitor etc, limit its usage. Fermentation with GRAS (generally recognized as safe) microorganisms has been used to help degrade these anti-nutritional factors. The nutritional value of fer-

mented soybean and soy meal products with additional nutritional factors is then largely enhanced.

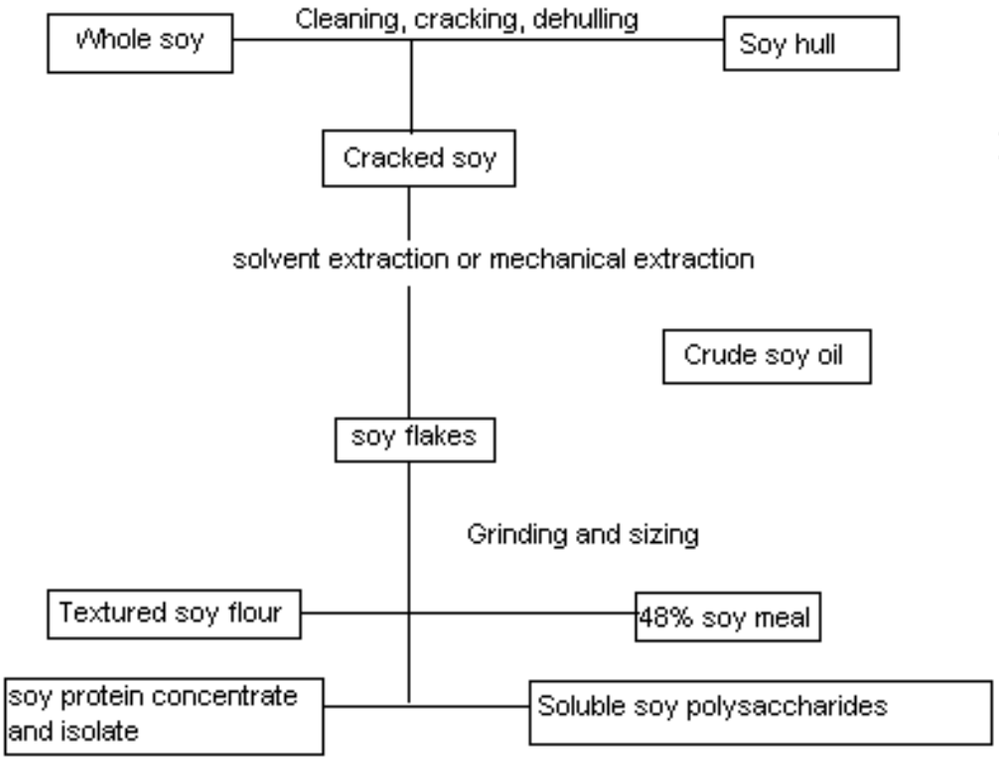

Figure 1. Soybean processing and products

2. Nutritional enhancement of soybean and soy meal via fermentation

2.1. Soy Protein

Protein content in soybean and soy meal are around 40%- 50% respectively. The high protein content makes soybean and soy meal a rich protein source for food and feed. As food source, the Protein Digestibility Corrected Amino Acid Score (PDCAAS), which is adopted by FDA and FAO/WHO, for isolated soy protein is 0.92, soy protein concentrate is 0.99, comparing with beef (0.92) and egg white (1.00). The human subject studies show that well-processed soy protein could serve as the sole source of protein intake for human beings [3]. FDA claims that diets containing 25 g of soy protein can reduce levels of low-density lipoproteins by as much as 10 percent and have considerable value to heart health. The specific reason for the heart protection function is unclear, for there are hundreds of protective compounds in soybean. As feed source, soy protein is high in lysine, but low in sulfur-containing amino acids, with methionine being the most limiting amino acid, followed by threonine [4]. The complementation of soy and corn for lysine and methionine makes them a valuable feed when combined.

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2.1.1. Fermentation increases protein and amino acid content, and degrades protein into small functional peptides.

During fermentation, microorganisms digest the carbohydrates in soybean or soy meal and use for their own growth. The decreased dry matter and increased microorganisms weight ratio result in enhanced protein content[5-7]. In reference [5], fermented soy meal with *S.cerevisae* increased its protein level from 47% to 58%, while with *L. plantarum* and *B. lactis*, protein level increased to 52.08% and 52.14%. Microorganisms used for soybean fermentation have been reported to secret protease during fermentation [8-11]. In Cheonggukjang, the *Bacillus subtilis* fermented traditional soybean food in Korea, the acidic protease activity level could be as high as 590.24±2.92 μg/ml. Neutral protease activity level could achieve 528.13±3.11 μg/ml [9]. Because of protein degradation during fermentation, fermented soybean products are easier to digest.

Four parameters have been often used to evaluate the protein degradation of fermented soybean products. They are trichloroacetic acid (TCA) soluble nitrogen, degree of protein hydrolysis, SDS-PAGE profile, and amino acid content. Usually peptides having 10 or fewer amino acids would dissolve in TCA[12]. During fermentation, the degree of protein hydrolysis increases because of protease hydrolysis [13-14]. Meanwhile, TCA soluble nitrogen and peptide contents could also be enhanced [6, 9, 13-14]. SDS-PAGE analysis shows less large (>70 kDa) and medium (20-60 kDa) peptides and more small (<10 kDa) peptides in soy meal after fermentation of *Lactobacillus plantarum*, *Bifidobacterium lactis*, *Sccharomyces cerevisiae*, or *Aspergillus oryzae* [5, 7, 15]. Reference [13] showed that after 24 hr *Bacillus subtilis* fermentation, soy protein with molecular weight above 20 Kd disappeared from the electrophoretograms. The total amino acid content increased significantly ($p<0.05$) in fermented soy meal or soybean with *Lactobacillus plantarum*, *Bifidobacterium lactis*, *Sccharomyces cerevisiae*, *Bacillus subtilis*, *Aspergillus oryzae*, *Rhizopus oryzae*, *Actinomucor elegans*, *Rhizopus oligosporus* et al. [5, 7-8, 10-11, 13, 16]. *L.plantarum* fermentation of soy flour led to an increase in sulfur amino acids (Met plus Cys), Phe, Tyr, Lys, and Thr [16].

2.1.2. Functional biopeptide

Fermentation degrades large protein molecules into small peptides and amino acids. The biologically active peptides from soybean play an important role as angiotenisin converting enzyme (ACE) inhibitor [17] and as antioxidants [18]. In this section, we will discuss the ACE inhibitor. The antioxidant activity of biopeptides will be discussed in the antioxidant section.

Angiotensin I-converting enzyme (ACE, EC 3.4.15.1) is a dipeptidylcarboxypeptidase associated with the regulation of blood pressure as well as cardiovascular functions [19]. ACE-inhibitory substances are used to lower the blood pressure of hypertensive patients. Various ACE inhibitory peptides have been isolated from traditional fermented soybean foods, like natto, doujiang, soy sauce, and miso paste [20-23].

ACE inhibitory activity of peptides generated by protease is greatly dependent upon fermentation time. ACE inhibitory activity in Textured Vegetable Protein (TVP) fermented by

Bacillus subtilis for 24 and 72hr showed IC50 values of 2.20 and 3.80 mg/ml, respectively [24]. The initial fermentation of TVP resulted in production of effective peptides, but longer fermentation time produced less active peptides as ACE inhibitor. Peptide with ACE inhibitory activity consisted of low molecular weight. Molecular weight of 500-1,000 Da shows the highest ACE inhibitory activity [24]. In [25], oligopeptides generated from soy hydrolysate and fermented soy foods though endoprotease digestion, demonstrated a range of biological activities – angiotensin converting enzyme (ACE) inhibitory, anti-thrombotic, surface tension and antioxidant properties.

2.1.3. Fermentation decreases soy immunoreactivity

Soybean is defined as one of the "big 8" food allergens in the United States [16].The estimated prevalence of soybean allergies is about 0.5% of the total U.S. population [16]. Patients with soy allergy could react subjectively and objectively with 0.21 and 37.2 mg of soy protein, respectively [5]. The principle for food allergy is that epitopes in allergenic protein bind to the immunoglobulin E (IgE) molecules residing in the mast cells and basophils, causing them to release inflammatory mediators, including histamine. Alpha- (72 kDa) and beta- (53 kDa) conglycinin subunits, P34 fraction, and glycinin basic (33 kDa) and acidic (22 kDa) subunits, and trypsin inhibitor (20 kDa) are the main protein components causing plasma immunoreactivity [16]. Glycine was found to stimulate local and systemic immune responses in allergic piglets and had negative effects on piglet performance [26]. The severity of the immune reactions depends on the dose of glycinin; higher doses cause more severe symptoms. The effect of purified beta-conglycinin on the growth and immune responses of rats were investigated [27]. Results showed that purified beta-conglycinin possesses intrinsic immune-stimulating capacity and can induce an allergic reaction. Also, newly weaned pigs with limited stomach acid and enzymatic secretions in the small intestine can have difficulty digesting proteins with complex structures and large molecular weights [28].

Studies have confirmed degradation of soybean allergens during fermentation by microbial proteolytic enzymes in fermented soybean products, such as soy sauce, miso and tempeh [5, 29]. In the fermented soy products, soy protein has been hydrolyzed into smaller peptides and amino acids;therefore the structure of antigen epitopes might be altered, becoming less reactive. The IgE binding potential is reduced and therefore the immunoreactivity is lowered. In soy sauce, proteins are completely degraded into peptides and amino acids after fermentation and allergens are no longer present [29]. The reduction of immunoreactivity by nature and induced fermentation of soy meal with *Lactobacillus plantarum*, *Bifidobacterium lactis*, *Saccharomyces cerevisiae* were evaluated [5]. *S.cerevisiae*, *B.lactis* and *L. plantarum* reduced the immune response77.2%, 77.2%, 78.0%, when using 97.5 kUA/l human plasma and 88.7%, 86.3%, 86.9%, respectively, when the pooled human plasma was used. All three fermented soy meal products showed fewer large (>70 kDa) and medium (20-60 kDa) peptides, and more small (<10 kDa) peptides. Protein hydrolysis reduction of soy protein immunoreactivity was also confirmed through enzyme hydrolysis conducted in reference [30], which showed that after hydrolyzing with three food-grade proteases (Alcalase, Neutrase, Corolase PN-L), no residual antigenicity was observed in resulting soy whey.

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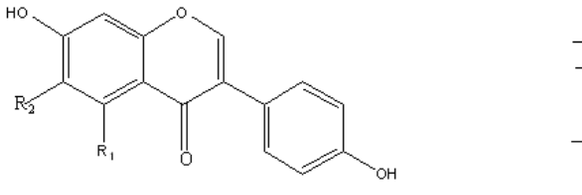
Animal experiments have also confirmed the hypoallergenic properties of fermented soybean or soy meal products. With regard to the soybean allergy, fermentation of soy meal decreased the immune response to soy protein in piglets and the level of serum IgG decreased by 27.2% [31]. Antigenic soybean proteins in the diet of early weaned pigs provoke a transient hypersensitivity associated with morphological changes including villi atrophy and crypt hyperplasia in the small intestine [31]. All of these morphological changes can cause a malabsorption syndrome [26, 33], growth depression, and diarrhea [34, 35]. Differences of the villi condition in such pigs fed soy meal and fermented soy meal were investigated by using scanning electron microscopy [36]. Piglets fed soy meal had shorter, disordered, and broader villi, whereas piglets fed fermented soy meal had long, round, regular, and tapering villi that could better digest and absorb nutrients.

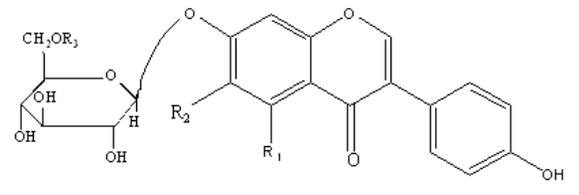
2.2. Isoflavone

One of the acknowledged bioactive compounds in soybean is isolavone. Isoflavones generally exist in soybeans and soy foods as aglycones (daidzein, genistein, and glycitein), beta-glycosides (daidzin, genistin, and glycitin), acetylglycosides (6''-O-acetyldaidzin, 6''-O-acetylgenistin, and 6''-O-acetylglycitin), and malonylglycosides (6''-O-malonyldaidzin, 6''-O—malonylgenistin, and 6''-O-mlonylglycitin). The structures of the 12 isomers are shown in figure 2.Isoflavones physiological effects include their estrogenic activity, antioxidant and antifungal activity, and more importantly, to act as anti-carcinogens. Isoflavones may also help to reduce blood serum cholesterol levels [4].



Aglyconses

R_1	R_2	Compounds
H	H	daidzein
OH	H	genistein
H	OCH_3	glycitein



Glycosides

R_1	R_2	R_3	Compounds
H	H	H	daidzin
OH	H	H	genistin
H	OCH_3	H	glycitin
H	H	$COCH_3$	6"-O-Acetyldaidzin
OH	H	$COCH_3$	6"-O-Acetylgenistin
H	OCH_3	$COCH_3$	6"-O-Acetylglycitin
H	H	$COCH_2COOH$	6"-O-malonyldaidzin
OH	H	$COCH_2COOH$	6"-O-malonylgenistin
H	OCH_3	$COCH_2COOH$	6"-O-malonylglycitin

Figure 2. Chemical structures of 12 isoflavone isomers in soybean [4]

Among soy isoflavones, the relative abundance of genistein including respective derivatives, is the highest – about 50% of isolavone content, followed by daidzein (40%) and glycitein (10%). However, glycitein has been shown to be more bio-available than other

isoflavones, followed by genistein [37]. Most of the isoflavones are associated with proteins in soy, with very little present in the lipid fraction. In their natural state, the majority of isoflavones exists as inactive glycosides (genistin, daidzin, and glycitin) and the remaining as their active aglycone forms (genistein, daidzein, and glycitein, respectively). Glycoside forms are heat sensitive, being converted into malonyl-beta-glycosylated isoflavone when heated. The aglycone forms are quite stable at high temperature [38].

Isoflavones when ingested are metabolized extensively in the intestinal tract, absorbed, transported to the liver, and undergo enterohepatic recycling. Intestinal bacterial glucosidases cleave the sugar moieties and release the biologically active isoflavones as aglycones. Aglycones could be directly absorbed in the adult and these can be further biotransformed by microorganisms to the specific metabolites, equol, desmethylangolensin, and p-ethylphenol. All of these phyto-oestrogens are then eliminated, mainly by the kidney, and therefore share the physiological features and behavior of endogenous estrogens. Among the glycones, beta-glucosides are easier to be hydrolyzed than 6''-O-malonylglucosides and 6''-O-acetylglucosides.

Some microorganisms have been reported to secrete beta-glucosidase, which could convert isoflavones to aglycones. The quantities of melonyl, acetyl, and glycosidyl isoflavonoids decrease during fermentation but those of isoflavonoid aglycones, daidzein, and genistein increase by over 10 to 100 fold. In Meju (long term fermented soybeans), compared with unfermented samples, the total glycosides in 60 hr fermented samples decreased from 1827 ug/g to 487 ug/g, while total aglycones increased from 22 ug/g to 329 ug/g, with daidzein increasing from trace to 152 ug/g, genistein from 16 ug/g to 170 ug/g. However, the quantity of glycitein was not increased [39]. In another study about meju fermented by *Aspergillus oryzae*, malonyl glycosides that initially accounted for 57.2-72.2% in the different soybeans markedly decreased to 7.4-19.9%, while aglycones originally accounted for only 1.1-2.8% in the soybeans, but markedly increased to 34.1-53.2% in miso [40]. In reference [38], total aglycones increased from 12.27 ug/g in whole soy flour to 446.90 ug/g after 48 hr fermentation by *Aspergillus oryzae*(ATCC 22876). Its percentage of total isoflavones increased from 2.67% to 75.51%. Daidzein content increased from trace to 133.07 ug/g, glycitein from trace to 35.56 ug/g, genistein from 12.27 ug/g to 278.27 ug/g. In *Bacillus subtilis* fermented soy paste ChungGuklang (CGJ), about 85% of isoflavones were in the aglycone form in the CGJ, 14% in the glucoside form and acetylglucoside and malonylglucoside forms contributed less than 1% [41]. In *Bacillus pumilus* HY1 fermented Cheonggkjang, the beta glucosidase increased to 24.8 U/g until 36 h. The glycosides and malonylglycosides decreased throughout the fermentation to about 80% - 90% of their starting amount at 60 hr [42]. Part of isoflavonoid aglycones are broken down into secondary metabolites, so the total quantity of isoflavonoids decreased [38, 42].

Aglycones could alleviate the symptoms of type2 diabetes, which the beta-glycosides, acetylglycosides and malonylglycosides forms are not able to do. Type 2 diabetes mellitus emerges from uncompensated peripheral insulin resistance that is associated with unregulated nutrient homeostasis, obesity, peripheral insulin resistance and progressive beta-cell failure. The effect of isoflavones in Meju on alleviating the symptoms of the type2 diabetes

was investigated and four mechanismswere found [39]. Isoflavonoid aglycones could improve insulin-stimulated glucose intake. Also, they could induce PPAR-γ activation to increase insulin-stimulated glucose uptake. The PPAR-γ is the central regulator of insulin and glucose metabolism. It could help improve insulin sensitivity in type 2 diabetic patients and in diabetic rodent models. Besides, aglycones have strong effects for insulin/IGF – 1 signaling through IRS2, which plays a crucial role in beta-cell growth and survival. In this study, aglycones in meju increased GLP-1 secretion. GLP-1 is one of the incretins secreted from enteroendocrine L-cells that augments insulin secretion after the oral intake of glucose and free fatty acids. The induction of its secretion can prevent and/or relieve diabetic symptoms.

2.3. Antioxidant activity

Oxidative stress has been found to be the primary cause of many chronic diseases as well as the aging process itself. Antioxidants could help to delay or prevent oxidative stress. Epidemiological studies show that antioxidants could lower the risk of cardiovascular disease, cancer (overall risk reduction between 30 – 50%), diabetes, neurological diseases, immune diseases, eye diseases et al. [43]. Antioxidant compounds play an important role as a health protecting factor. It is beneficial to eat antioxidant enriched food. Products prepared through the fermentation of soybean including various traditional oriental fermented products of soybean such as natto, tempeh, miso and other fermented products, have been found to exhibit a significantly higher antioxidant activity than their respective non-fermented soybean substrate.

Di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium(DPPH) radical scavenging activity, Fe2+ chelating activity and reducing property have been used to quantify antioxidant activity. DPPH is a stable nitrogen-centered, lipophilic free radical that is used in evaluating the antioxidant activities in a short time. Ferrous ionsare the most effective pro-oxidants in food system. Metal chelation agents prevent metal-assisted homolysis of hydroperoxides and block the formation of chain initiators. IC50 and the relative scavenging effect are the parameters to describe the DPPH radical scavenging and Fe2+ chelating abilities. IC50 is the inhibition concentration of extracts required to decrease initial DPPH radical or Fe2+ concentration by 50%. The relative scavenging effect is calculated by dividing the extract content with the IC50 of the respective extract, and then compare with the scavenging effect of control samples. The reducing property indicates that these compounds are electron donors and can reduce the oxidized intermediates, and therefore, can act as primary or secondary antioxidants. The reduction of Fe3+/ferricyanide complex to ferrous form in presence of antioxidants has been used to test the reducing activity of samples [44].

Antioxidant activity of methanol extracts of soybean koji fermented with *Asp. oryzae*, *Asp. sojae*, *Actinomucor taiwanensis*, *Asp. awamori*, and *Rhizopus* spp. have been investigated [44]. Methanol extracts of soybean koji, which mainly contained phenolic acid, have higher relative DPPH – scavenging effect and Fe2+ chelating effect than the unfermented steamed soybeans. The koji methanol extracts had a relative DPPH - scavenging effect of 2.3-8.9 compared with that of the non-fermented soybean, which was assigned as 1.0. Among them, the *Aspergillus awamori*-soybean koji exhibited the highest DPPH-scavenging effect, at a level

approximately 9.0 fold than that exhibited by the non-fermented steamed soybean [44].The Fe2+ ion chelating ability of soybean increased by 2.1 – 6.7 fold after fermentation, depending on starter organism employed [44]. In Cheonggukjang fermented by *Bacillus pumilus HY1*, the level of DPPH radical scavenging activity increased from 54.5% to 96.2% by 60 hr [42]. In *Bacillus subtilis* fermented soybean kinema, when the methanol extract concentration was 50 mg/ml, 60% DPPH radical scavenging was observed. In the same product with a concentration of 10 mg/ml, the methanolic extract of kinema exhibited 64% metal chelation which was much higher than the activity shown by cooked non-fermented soybean (22%) [45]. Similar findings of enhanced reducing power of fermented bean and bean products were reported from *Bacillus subtilis* fermented soybean kinema[45] and from *Aspergillus oryzae* fermented soybean koji [44].

The enhanced antioxidant activities in fermented soybean products may be due to the increased phenolic compounds contents. Phenolic compounds have been demonstrated to exhibit a scavenging effect for free radicals and metal-chelating ability [46]. Most phenolic acids in cereals primarily occur in the bound form, as conjugates with sugars, fatty acids, or proteins [47].Isoflavones are the predominant phenolics in soybean, and the glucoside form of isoflavones represents 99% of the total isoflavones in soybean [45].This condition lowers the antioxidant activity since the availability of free hydroxyl groups in the phenolic structure is an important characteristic for the resonance stabilization of free radicals. The enhancement of bioactive phenolic compounds by enzymatic hydrolysis from different cereals was reported by Wojdylo [47] and Yuan [48]. Different enzymes during bacteria or fungi fermentation, such as alpha-amylase, alpha and beta-glucosidase, beta-glucuronidase, cellulase et al., have been reported to be involved in the lignin remobilization and phenolic compounds contents enhancement [49]. Fermented soybean products have higher amount of phenolic compounds [42, 44]. In Cheonggukjang fermented by *Bacillus pumilus HY1*, total phenolics increased markedly from the starting amount of 253 g/kg to 9586 g/kg at the end of fermentation (60hr) [42]. In *Bacillus subtilis* fermented kinema, total phenol content of kinema was 144% higher than that of cooked non-fermented soybean. The total phenol content was positively correlated ($p<0.001$) with radical- scavenging, reducing power, metal-chelating activity in *Bacillus subtilis* fermented kinema [45].

The other reason is the short chain peptides generated by fungi or bacterial protease. Antioxidant activities of peptides have been reported [50-52]. In [51], five different proteases were used to hydrolyze soybean β-conglycinin and the hydrolysates from three of them had significant enhanced antioxidative activities. Peptide antioxidant activity is related, but not limited, to the amino acid composition and its sequence. In reference [51], peptides isolated from the antioxidative beta-conglycinin hydrolysate contain histidine, proline or tyrosine residue in their sequences and hydrophobic amino acids, valine or leucine at the N terminus. The constituent amino acids had no antioxidant activity when mixed at the same concentration as the peptides. Anti-oxidative activities of peptides with different amino acid composition or sequences have different antioxidant mechanisms. Reference [52] studied the anti-oxidative properties of combinatorial tri-peptides. Tri-peptides Tyr-His-Tyr, Xaa-Xaa-

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Trp/Tyr, and Xaa-Xaa-Cys (SH) had a strong synergistic effect with phenolic antioxidants, a high radical scavenging activity, and a high peroxynitrite scavenging activity, respectively.

2.4. Phytic acid

Phytate is the calcium-magnesium-potassium salt of inositol hexaphosphoric acid commonly known as phytic acid [Figure 3]. Phytate and phytic acid are also referred to as phytin in some literature.

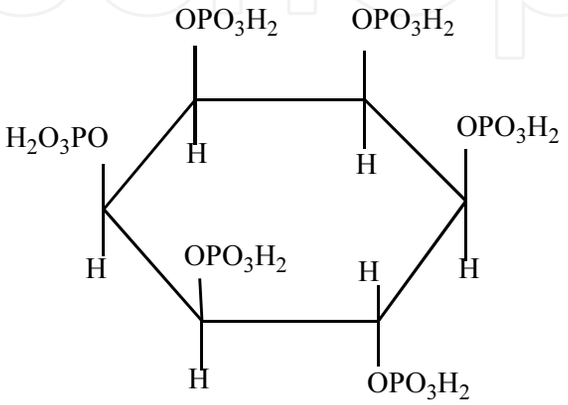

Figure 3. Basic structure of phytic acid [52]

Phytate is the main storage form of phosphorous in soybean. Its content in soybean ranged from 1.00 to 1.47% on a dry matter basis [4].Phytate is known to be located in the protein bodies, mainly within their globoid inclusions. Phosphorous in the phytate form could not be absorbed by monogastric animals, because they lack phytase, the digestive enzyme required to release phosphorus from the phytate molecule. Phytic acid could form protein-phytate or protein-phytate-protein complexes; these have more resistance to digestion by proteolytic enzymes; thus, utilization of dietary protein is reduced. Also, phytic acid has a strong binding affinity to important minerals such as calcium, magnesium, iron, and zinc. When a mineral binds to phytic acid, it becomes insoluble, precipitates, and is not absorbable in the intestines. In food industry, the presence of phytic acid in high concentration is undesirable. In feed industry, the unabsorbed phytate passes through the gastrointestinal tract of monogastric animals, elevating the amount of phosphorus in the manure. Excess phosphorus excretion can lead to environmental problems such as eutrophication. With the pressure on the swine industry to reduce the environmental impact of pork production, it is important to use feed ingredients that can minimize this influence.

The ability of the molds for oriental fermented soybean food to produce phytase has been investigated. Phytase is the enzyme hydrolyzing phytic acid to inositol and phosphoric acid and thereby removing the metal chelating property of phytic acid. There are two strains of *Rhizopus oligosporus* used for tempeh fermentation, two strains of *Aspergillus oryzae* used for soy sauce and six strains of *Aspergillus oryzae* used for miso fermentation, all the ten strains were reported to be able to secret both extracellular and intracellular phytases [54]. The

phytic acid content of soybeans was reduced by about one-third as a result of *Rhizopus oligosporus* NRRL 2710 fermentation [55-56]. That was from original 1.27% to 0.61% after 48 hr fermentation [56]. Animal test showed that fermentation of soy meal increased phosphorus availability [57-58] and zinc availability [59] and reduced phosphorus excretion without affecting growth of chicks. Using fermented soy meal as substitute for regular soy meal saved 0.2% of dietary inorganic phosphorous [60].

2.5. Oligosaccharides

Galacto-oligosacchrides (GOS) generally represent approximately 4 to 6% of soybean dry matter. In soy meal produced at 10 commercial processing plants in the United States, concentrations of stachyose, raffinose, and verbascose ranged from 41.0 to 57.2, 4.3 to 9.8, and 1.6 to 2.4 mg/g DM, respectively [61]. Oligosaccharides in the carbohydrate fraction, particularly raffinose and stachyose [figure 4], could lead to flatulence and abdominal discomfort for monogastric animals [62-63] because of the deficiency of alpha-galactosidase.

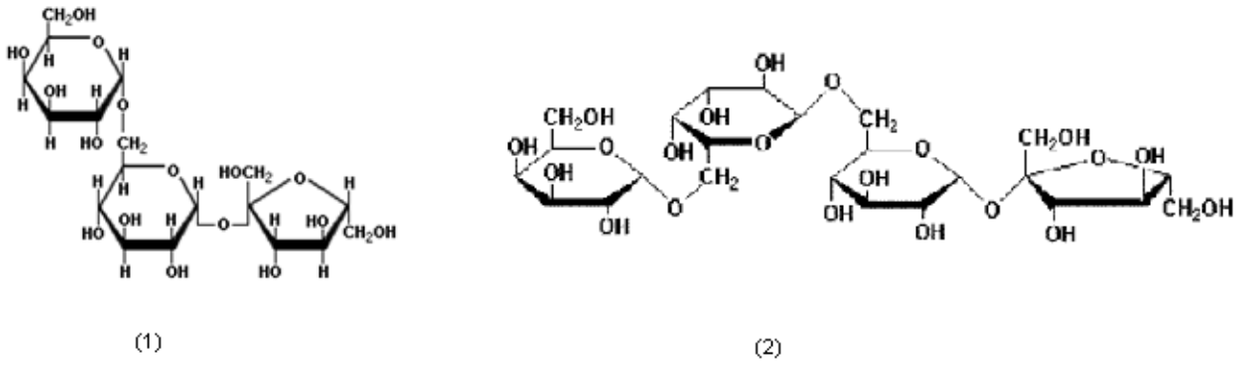

Figure 4. Structure of raffinose and stachyose (1) raffinose; (2) stachyose

Galacto-oligosacchrides are digested to some extent in the small intestine (76 to 88% for stachyose, 31 to 65% for raffinose, and 32 to 55% for verbascose), resulting in the production of carbon dioxide and hydrogen [64]. In some cases, the accumulation of flatulent rectal gas provokes gastrointestinal distress such as abdominal pain, nausea, and diarrhea. Weanling pigs fed a GOS-free diet supplemented with 2% stachyose or fed a diet containing soy meal had increased incidence of diarrhea compared with pigs fed a control diet [63]. Additionally, fermentation of GOS has been implicated to have negative effects on nutrient digestibilities and energy availability of soy meal. Roosters fed soy meal with low oligosaccharide concentrations had higher total net metabolizable energy values (2931 kcal/kg dm) than those fed conventional soy meal (2739 kcal/kg DM) [65]. The removal of polysaccharides from soy foods and feed is, therefore, a major factor in improving their nutritive value. To reduce non-digestible oligosaccharides, fermentation with fungi, yeast, and bacteria with alpha-galactosidase secreting ability has been attempted over the years.

The enzyme alpha-D-Gal (alpha-D-galactoside galactohydrolase, EC 3.2.1.22) is of interest for hydrolyzing the raffinose-type sugars found in soybeans. *Rhizopus oligosporus*, *Lactobacil-*

lus curvatus R08, *Leuconostoc mesenteriodes*, *Lactobacillus fermentum*, *Bifidobacterium* sp. et al. have been reported to be able to produce alpha-galactosidase [56, 66-70]. These microorganisms have been applied for soybean fermentation to reduce the oligosaccharides [56, 67-68, 71]. In [56],stachyose and raffinose decreased by 56.8% and 10%, respectively, in soybean during 48 hr fermentation by *Rhizopus oligosporus*. In *Leu.mesenteriodes JK55* and *L.curvatus R08* fermented soymilk, the non-digestive oligosaccharides were completely hydrolyzed after 18-24 h of fermentation [67].

Alpha-galactosidase has been isolated from plant and microbial sources, and its properties have been documented. In general, alpha-galactosidase acts upon gal-gal bonds in the tetrasaccharide stachyose, releasing galactose and raffinose, and also acts upon gal-glu bonds with the release of sucrose. Sucrose is, in turn, split by invertase, producing fructose and glucose [72]. So, α-galatosidase activity is noticeably dependent on the type of sugar. The type and concentration of the carbon source are known to be nutritional factors that regulate the synthesis of bacterial galactosidase. Reference [67] found that the existence of glucose and fructose inhibit the alpha-galatosidase expression both in *Lactobacillus curvatus R08* and *Leucomostoc mesenteriodes*.

2.6. Trypsin inhibitor

Protease inhibitors constitute around 6% of soybean protein [73]. Two protein protease inhibitors have been isolated from soybeans. The Kunitz trypsin inhibitor has a specificity directed primarily toward trypsin and a molecular weight of about 21.5 kDa. The Bowman-Birk (BB) inhibitor is capable of inhibiting both trypsin and chymotrypsin at independent reactive sites and has a molecular weight of 7.8 kDa [74]. Trypsin and chymotrypsin, the two major proteolytic enzymes produced in the pancreas, belong to the serine protease class.

Trypsin inhibitors present in soybeans are responsible for growth depression by reducing proteolysis and by an excessive fecal loss of pancreatic enzymes rich in sulfur-containing amino acid which can not be compensated by dietary soy protein [75]. Trypsin inhibitors account for 30-50% of the growth inhibition effect [76]. Rats fed a raw soybean extract from which trypsin inhibitors had been inactivated showed improved growth performance when compared with control rats fed diets containing raw soybeans from which inhibitors had not been inactivated [77].

Diets with a trypsin inhibitor concentration of 0.77 mg/g and less did not reduce the growth of pigs according to reference [7]. And, research showed that after fermentation with *Aspergillus oryzae GB-107*, the trypsin inhibitor in soy meal was reduced from 2.70 mg/g to 0.42 mg/g [7]. In the *in vivo* experiment of reference [15], the activities of total protease and trypsin at the duodenum and jejunum of piglets fed with fermented soy meal increased because of the inactivation of trypsin inhibitor. Just as was mentioned above, protease produced during fermentation could degrade protein molecules into peptides and amino acids. The trypsin inhibitor may be degraded or modified during fermentation and lose its activity binding to trypsin.

2.7. Vitamin

The increased content of some vitamins or provitamins, both water-soluble and fat-soluble vitamins, such as riboflavin, niacin, vitamin B6, β-carotene et al., which are due to fungal metabolic activities,is one of the healthy and nutritional advantages of fermented soybean products and has been extensively examined.

Vitamin or provitamin formation during tempeh fermentation by *Rhizopus oligosporus*, *R.arrhizus*, *R. oryzae* and *R.slolonifer*, respectively, were studied [11, 78]. In ref [11], all of the fourteen Rhizopus strains used in the research could form riboflavin, vitamin B6, nicotinic acid, nicotinamide, ergosterol, with isolates of *R.oligosporus* the best vitamin formers. In ref [78], six of 14 Rhizopus strains were able to form β-carotene in significant amounts. Five of these six strains belonged to the species of *R.oligosporus* and one was identified as *R.stolonifer*. A newly fourfold increase in β-carotene from 0.6 ug/g dw to 2.2 ug/g dw could be detected between 34 and 48hr in fermentations with *R.oligosporus* strain. During this period the content of total carotenoids increased from 9.1 ug/g dw to 11.2 ug/g dw in the fermentations with *R.oligosporus* strain. Ergosterol is the vitamin D2 precursor. Vitamin D can be derived from two naturally occurring compounds: ergocalciferol (D2) and cholecalciferol (D3). Both forms have equal biological activities in humans. The fourteen strains could produce ergosterol. The ergosterol concentration could be up to 1610 ug/g dw after 96 hr fermentation.

Vitamin K is an essential cofactor for the posttranslational conversion of glutamic acid residues of specific proteins in the blood and bone into γ-carboxyglutamic acid (Gla). There are two naturally occurring forms of vitamin K, vitamins K1 and K2. Vitamin K1 (phylloquinone) is formed in plants. Vitamin K2 (menaquinone, MK) is primarily synthesized by bacteria [79].Menaquinone (MK) plays an important role in blood coagulation and bone metabolism [80]. Japanese fermented soybean product, Natto, has been regarded as a high content of MK source (about 6- 9 ug/g) and is found in everyday products. Natto produced by a mutated *B. subtilis* strain showed a much higher content of MK up to 12.98 ug/g [81].Aromatic amino acids (phenylalanine, tyrosine, and trypstophan) could slow down the MK synthesis rate in cheonggukjang by using *Bacillus amyloliquefaciens KCTC11712BP*, while the supplement of 4% glycerol could signicantly increase its yield [82].

3. Conclusion

After fermentation by GRAS microorganisms, the anti-nutritional factors in soybean or soy meal are totally degraded, including oligosaccharides, trypsin inhibitor and phytic acid. Fermentation could also degrade large soy protein into peptides and amino acids, therefore, removing the allergenic effect of soy protein. Nutritional factors are formed during fermentation along with removal of undesirable factors. Functional peptides, such as peptides with ACE inhibitory activity are created by protein degradation. Isoflavones are converted to their functional forms, the aglycones. Antioxidant activity is enhanced, contributed by the increase of short chain peptides and phenolic compounds. Certain vitamins or provi-

tamins are formed such as riboflavin, β-carotene, vitamin K2 and ergosterol. Total nutritional profiles of soybean and soy meal are greatly enhanced by fermentation.

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