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

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

BOOK CITATION INDEX
CLARIVATE ANALYTICS
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

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

Soy Isoflavones as Bioactive Ingredients of Functional Foods

Lutz Mariane
Centro de Investigación y Desarrollo de Alimentos Funcionales (CIDAF),
Universidad de Valparaíso and Centro Regional de Estudios de Alimentos
Saludables (CREAS), Valparaíso
Chile

1. Introduction

Soybean (*Glycine max*) is one of the most important agricultural commodities. It contains considerable amounts of nutrients (proteins, fatty acids, minerals, vitamins) and constitutes a well known source of bioactive phytochemicals with health-promoting effects, including oligosaccharides, lectins, trypsin inhibitors, saponins, phytates, phytosterols, and isoflavones (IFs). This legume contains the highest amount of IFs, in the range of 0.1-0.4% dry weight (Liu, 2006), the main species being daidzin (4′,7-dihydroxyisoflavone), genistin (4′,5,7-trihydroxyisoflavone), and glycitin (7,4′-dihydroxy-6-methoxy-isoflavone), the β-glucosides of daidzein, genistein, and glycitein, respectively. These compounds may be found in the free or the conjugate forms: glucosides, acetylglucosides, and malonylglucosides. In the acetylglucoside form, IFs are named as 6′′-*O*-acetyldaidzin, 6′′-*O*-acetylgenistin, and 6′′-*O*-acetylglycycitin. In the malonylglucoside form, their respective names are 6′′-*O*-malonyldaidzin, 6′′-*O*-malonylgenistin, and 6′′-*O*-malonylglycitin.

Since IFs are phytoalexins, their concentrations increase in times of plant stress, such as reduced moisture, and are influenced by the environmental conditions. The IF levels and distribution of isomers in soybeans depend largely on the genotypes of the soybean variety, the crop year and environmental factors such as the location and sowing/harvesting periods (Eldridge & Kwolek, 1983; Kitamum et al., 1991; Wang & Murphy, 1994a; Aussenac et al., 1998). Total IF content in soybean has been described in the range of 300 µg/g to greater than 3,000 µg/g among the United States Department of Agriculture (USDA) soybean germoplasm collection (www.ars-grin.gov/var/apache/cgi-bin/npgs/html). Considerable data on the IFs content of foods are available from USDA (1999) and Jackson & Gilani (2002). In general, the amounts of IFs range from *ca.* 1 to 4 mg/g in soybeans and 0.5 to 2.6 mg/g in traditional soy foods such as tofu (Wang & Murphy, 1994b).

A universal definition for the term "Functional Food" has not been established, and various countries and groups of countries apply different meanings to the term. However, it is accepted that functional foods are aimed to promote health and well being of the consumers. While in some countries foods that supply high amounts of nutrients are considered as functional, as well as natural unprocessed foods such as fruits and vegetables, there is a trend towards the use of the term exclusively for those foods that exert beneficial effects beyond their nutrients only. Consequently, the bioactive components should impart

health benefits beyond basic nutrition. The beneficial effects of functional foods are attributed to the presence of putative bioactive compounds, and their benefits should be demonstrated by means of adequate laboratory and clinical trials. The consumers have the right to know what bioactive compounds are contained in a functional food, the amounts per serving and how it should be consumed, as well as if the compounds are absorbed and exert their action via blood distribution into target organs or if they act locally in the gastrointestinal tract, among other issues. It is the role of the governmental agencies to analyze, accept or reject the proposed health claims, based on the significant scientific agreement standard of evidence available (ADA, 2009; ILSI Europe, 2010)

The bioactive properties of soy IFs have been evaluated by a variety of *in vitro*, *in vivo* and clinical studies providing the rationale for their use in the formulation of various functional foods directed towards the reduction of risk factors of chronic diseases such as cancer, cardiovascular diseases, hypertension, osteoporosis, and neurodegenerative diseases. A series of concentrated and purified soybean products are found in the market, including pure forms of the putative health-promoting IFs. However, the results of the studies designed to demonstrate the beneficial effects of soy IFs represent a challenge due to a series of complications that may lead to unresolved issues. The aim of this chapter is to describe some of the considerations that should be taken into account when analyzing the formulation of novel functional foods containing soy IFs in order to be able to sustain their beneficial effects and accept health claims to communicate them to the consumer.

2. Bioavailability of soy IFs

2.1 Absorption of soy IFs

The absorption of IFs differs among populations due to factors such as the composition of the intestinal microflora (Xu et al., 1995), dietary habits, and ethnic background (Zubik & Meydani, 2003). The first phase of IF absorption, up to one hour, is impaired in lactose malabsorbers, which suggests a role for lactase, but overall this is compensated by microbial hydrolysis, and Tamura et al. (2008) observed that total absorption was not significantly affected by lactose malabsorption. The bioavailability of IFs is further influenced by their chemical form in foods, their hydrophobicity and susceptibility to degradation, and the food matrix (Birt et al., 2001). Izumi et al. (2000) and Kano et al. (2006) found a greater bioavailability of daidzein and genistein, but not their glucosides, whereas Setchell et al (2001) reported a more efficient use of glucosides. Other studies reported that the absorption of aglycones and glucosides was similar (Tsunoda et al., 2002; Richelle et al., 2002; Zubik & Meydani, 2003). It seems that IF aglycones are absorbed faster than glucosides, due to their greater hydrophobicity and a smaller molecular weight, whereas glucosides have lower absorbability and must be converted to aglycones.

The absorption of IFs is highly dependent on their chemical form, and the β-glucosides require hydrolysis to aglycones to be absorbed by the gut and exert their potentially protective effects (Setchell et al., 2002a; Zheng et al., 2003). In fact, free IFs reach peak plasma levels before the corresponding glycosylated forms (Setchell et al., 2001). Hydrolysis occurs along the entire length of the intestinal tract by the action of both the brush border membrane and the bacterial β-glucosidases and β-glucuronidases (Day et al., 1998; Manach et al., 2004), while β-glucuronidases and sulphatases participate in the reabsorption process of the hepatic conjugates and biliary excretion (Xu et al., 2000). The aglycones and bacterial metabolites are absorbed from the intestinal tract to undergo enterohepatic recycling

(Sfakianos et al., 1997). IFs undergo extensive biotransformation catalyzed by hepatic cytochrome P450 (Kulling et al., 2001), producing metabolites that exert antioxidant activity (Rüfer & Kulling, 2006).

The colonic microflora plays important roles in the metabolism of IFs. Daidzein may be metabolized to form two IFs: equol (7-hydroxy-3-(4′-hydroxyphenyl)-chroman) and O-desmethylangolensin (Xiao, 2008). Only near 30% soy consumers produce equol (Lampe et al., 1998; Setchell et al., 2002b). The ability to produce equol may be determinant of the beneficial effects of IFs, since subjects that are able to form this metabolite exhibit enhanced responses to diets containing IFs (Duncan et al., 2000; Setchell et al., 2003a; Akaza et al., 2004; Vafeiadou et al., 2006).

De Pascual et al. (2006) determined the effect of food matrix on the levels of IFs attained in serum and urine in healthy postmenopausal women given an oral dose of three different foods containing 50 mg IFs on three separate occasions. They observed a lower total urinary recovery of genistein following ingestion of juice (61%) in comparison with solid foods (66% and 70% for bars and cookies, respectively). The levels of daidzein were not altered by food matrix and none of the volunteers appeared capable of converting this precursor to equol. Serum peak genistein concentrations were attained earlier following consumption of liquid matrix, although the differences were not statistically significant. When the authors compared the IF concentrations after the technological processing of the different test foods, they only found differences in aglycone levels.

Bacterial species of bacteroides, bifidobacteria and lactobacilli have the highest β-glucosidase activity (Xu et al., 1995). Furthermore, an increase of the intestinal β-glucosidase activity with the chronic ingestion of soy has been observed (Wiseman et al., 2004). Nielsen & Williamson (2007) summarized data from 16 studies on factors affecting the bioavailability of IFs, reporting that it increases with a rapid gut transit time and low fecal digestion rates and decreases with dietary fiber. Dietary prebiotics (such as fructooligosaccharides, FOS) increase the intestinal bioavailability and affect the metabolism of IFs in rats (Uehara et al., 2001), and increase microbial production of equol in mice (Ohta et al., 2002). However, Sung & Choi (2008) assayed different doses of IFs in rats (up to 500 mg/kg diet) and observed that an increased plasma equol level did not offer additional protection to that provided by FOS against colon carcinogenesis. These results indicate that equol production is not directly related to a health promoting effect, since an inhibitory effect on colon cancer in a favorable gut ecosystem was not observed. Moreover, Larkin et al. (2007) observed that the addition of neither probiotic bacteria nor resistant starch (prebiotic) to a soy diet significantly affect IFs absorption or metabolism. The authors propose that since the probiotics *L. acidophilus* and *B.bifidus* do not increase β-glucuronidase activity in the human gastrointestinal tract, the lack of effects observed in the study may indicate that β-glucuronidase activity is more important than β-glucosidase activity in IFs bioavailability.

An important factor affecting the efficiency of cellular uptake by passive diffusion is the affinity of the IF molecules to the cellular membrane. Increased order of affinity to liposomal membranes have been reported as genistin = daidzin < daidzein < genistein < flavonoid aglycones (Murota et al., 2002).

2.2 Bioavailability of IFs

One of the requisites a functional food must comply is the demonstration of the bioavailability of the putatively active compounds it contains in order to allow their

adequate distribution in body tissues and target cells in physiological concentrations. This is currently assessed by measuring the bioactives in blood (plasma) and/or their urinary excretion. In fact, the rate of excretion of a compound in urine is usually directly proportional to the systemically bioavailable fraction. In general terms, although the oral bioavailabilities of flavonoids are low (Hu, 2007), the concentrations of their phase II metabolites, in particular glucuronides in the body are still appreciable and some of these metabolites are also demonstrated to be bioactive (Zhang et al., 2007).

IF levels in urine and plasma have been widely used as biomarkers of IF intake (Yamamoto et al., 2001; Nagata et al., 2006). Franke et al. (2009) reported a good correlation between peak concentrations of plasma daidzein and genistein and their concentrations in the first 24 h urine following soy consumption. Elimination of IFs is predominantly via the urine (mainly equol), although total recovery by mass balance is less than 50%, suggesting that unrecognized metabolites are being formed. In populations that consume small to moderate amounts of soy only occasionally, adherence should be assessed because urinary IFs reflect primarily the intake within the past 48 h. Turner et al. (2003) emphasize the role of the gut microflora on the bioavailability of IFs by stating that studies involving the metabolism of functional food components should focus on the bacteria inhabiting the small intestine, where absorption takes place, in order to facilitate the manipulation of factors influencing their activity and hence bioavailability of IFs.

Setchell et al. (2003a) observed that IFs are absorbed relatively quickly, attaining maximum serum concentrations from 2 to 8 h after ingestion, with means for daidzein and genistein of 6.1 and 5.0 h, respectively. The bioavailability, obtained from the area under the curve (AUC) of plasma concentration *vs.* time, showed a curvilinear relationship with increasing levels of IFs ingested, especially in the dose range of 0.4–1.8 mg/kg body weight. The authors attribute the reduced systemic availability to reduced absorption of IFs with increasing levels of intake. The pharmacokinetics and systemic bioavailability of β-glycosides has been found to be greater than the corresponding aglycones in dose-normalized AUCs, and relatively small proportions of aglycones appear in plasma even after an ingestion of high amounts of these compounds (Setchell et al., 2001). The actual composition of most IF–rich ingredients to be used in functional foods and dietary supplements are not completely known, and it would be very advantageous to direct the chemical composition of the formulation towards the expected clinical effect expected. A review of the analytical methods available for the analysis of IFs in foods, supplements and biological samples was recently published by Hsu et al. (2010).

Zhou et al. (2008) demonstrated that the absorption, biotransformation, and excretion of genistein show a nonlinear dose-dependent relationship at high doses in rats. They observed that genistein (free and glucuronidated) can be detected in plasma within 5 min after oral administration, indicating that this compound can be quickly absorbed and metabolized in the gastrointestinal tract. The authors reported that the primary form of genistein in plasma is the glucuronidated form due to the action of the gut microflora (Sfakianos et al., 1997). In effect, the majority of metabolite is genistein-7-O-β-D-glucuronide; other metabolites include genistein-4'-O-sulfate and genistein-4'-O-sulfate-7-O-β-D-glucuronide, which are formed in smaller amounts (Yasuda et al., 1996; Prasain et al., 2006). Since glucuronidated genistein can be further deconjugated by glucuronidase in the intestine, the released genistein can be absorbed, metabolized, and excreted for a second time (enteric recycling and enterohepatic circulation). Thus, the exposure time of the body to genistein is prolonged. However, its bioavailability is low, due to its poor absorption and its significant first-pass metabolism (glucuronidation and sulfation) (Chen et al., 2005).

Urinary IFs are frequently used as biomarkers of their potential bioavailability from foods or supplements rich in these compounds (Faughnan et al., 2004). Urine is easy to collect and contains 100-fold higher concentrations of IFs as the plasma levels (Setchell et al. 2001, 2003b). It is usually collected for 24 h and correlates well with serum assessment of systemic bioavailability of IFs (Setchell et al. 2003a). Additionally, quantitative assessment in urine provides information on the extent of intestinal metabolism of IFs and subject compliance in intervention trials.

Daidzein and genistein are the two main IFs excreted in the urine after soy foods are consumed, and daidzein is always excreted in greater amounts than genistein in the urine of adults (Kirkman et al., 1995). However, only a small proportion of dietary IFs are excreted in urine (1–25%). Therefore, they may be not absorbed from the gut, absorbed and released in bile followed by faecal excretion, or metabolized by gut microflora or the liver (Scalbert & Williamson, 2000).

On the other hand, Lampe et al. (1999) observed higher urinary lignan and phytoestrogen excretion in individuals consuming higher amounts of fruits and vegetables as well as sex differences in IF excretion, since men exhibit higher urinary excretion of genistein and daidzein. In fact, the urinary recovery appears to be influenced by gender and the food matrix (Lu & Anderson, 1998), with longer half-lives for daidzein and genistein in females compared with males. Moreover, the production of equol differs among postmenopausal women populations, and Chanteranne et al. (2008) classified the population of different countries in three groups, according to the magnitude of equol production, as high, medium and very low. The authors observed that French volunteers were the main equol producers (42%), in opposite to Italy (30%) or the Netherlands (21%). In each country, daidzein concentrations in plasma were lower than those of genistein, which reflect the ingested proportions, and the reverse was observed in urine.

As stated by Martin et al. (2008), collectively current data suggest that the bioavailability of soy IFs is in the range of 20-30% in both animals and humans, reaching plasma concentrations in the range of high nanomolar to low micromolar in both animals and humans.

2.3 Types of studies

In order to substantiate health protective functions claims, appropriate human-intervention trials and other clinical studies must be made. Intervention studies are quite complex, since they demand the participants to restrain completely from certain foods or drinks and to be randomly assigned to consume a test product or placebo even for several years, among other requisites. To overcome the need for long-term intervention studies, alternatives include the use of validated biomarkers to predict certain disease risk factors, which represents a challenge. In the latter years, metabolomic techniques that help to identify the response of each individual to the dietary intake of bioactives constitute a promising method for studying mechanisms of action (Gibney et al., 2005; Fardet et al., 2008).

Many conflicting results have been obtained stating the relationship between soy IFs and health-related endpoints. This is often associated to a reductionist approach to the study, assuming that the effects of feeding a soy food reflect the activity of one or a few related soy components; that the activity of a purified soy component reflects the effects of eating whole soy foods; or that soy foods equal IFs that are either estrogenic or antiestrogenic, which explains the biological effects observed. Animal studies demonstrate that these assumptions are false (Naciff et al., 2005; Badger et al., 2008; Chen et al., 2008; 2009; Singhal et al., 2009).

Bioavailability and potential modes of action of various soy constituents differ, and most ingredients act via multiple mechanisms (van Ee, 2009). In addition, soy constituents may potentially interact synergistically in maintaining/obtaining study endpoints.

In vitro assays are usually performed to explore bioactivity. The main flaw of these studies is due to the use of supraphysiological levels of isolated IFs or mixtures of IFs in cell cultures, over- or underestimating *in vivo* effects (Stevenson & Hurst, 2007). Therefore, the relevance of these assays to human situations is uncertain and the results should be interpreted with caution, although they may be helpful in determining the mechanisms by which soy IFs may exert their effects at cellular and molecular levels (Erdman et al., 2004). On the other hand, animal models are not completely comparable to humans. IF metabolism in rodents and nonhuman primates differ markedly from that of humans (Gu et al., 2006). Moreover, animals may be fed with very high amounts of soy, at levels exceeding what can be administered in clinical trials. If the IFs are administered to animals by injection, they would bypass the gastrointestinal tract and liver and may also exceed the exposure of human consumption.

The bioavailability of IFs depends upon factors such as solubility, partition coefficient, permeability, metabolism, excretion, target tissue uptake, and disposition of the bioactives (Karakaya, 2004), all of which make the results of the studies controversial. It is critical that IF blood levels (total and aglycone) be evaluated and be comparable to the blood levels observed in human populations consuming IF-containing products. Besides, the intestinal microflora of animals may be more efficient at producing equol and consequently, results of studies in these species may not predict the effect of soy consumption in humans.

In the US, the National Institutes of Health (NIH) launched the Justification for Clinical Research Guidance (http://grants.nih.gov), while the Food and Drug Administration (FDA) published the Clinical Trials Guidance Documents (http //www.fda.gov/RegulatoryInforma tion/Guidances/ucm122046.htm), which establish the variables to be taken into consideration in cell culture, animal and clinical studies as well as product characterization, stability, and analytical methods used to determine product integrity, among others. For instance, although epidemiological studies of Asian populations provide the background for many clinical studies, the type of soy Asians consume often differs from that consumed by other populations. Also, few dose-response trials have been conducted in humans, so it is difficult to estimate with confidence the threshold amount of soy needed to exert various physiologic effects *in vivo*.

The NIH commissioned the National Center for Complementary and Alternative Medicine and the Office of Dietary Supplements to review the evidence-based scientific reports on the effects of soy intake, through the Agency for Health Care Research and Quality (AHRQ) (Balk et al., 2005). The document, titled "Effects of Soy on Health Outcomes," summarized the formulations of soy products and/or soy food used in clinical trials and the current evidence of the health effects of soy and its constituents on cardiovascular disease, menopausal symptoms, endocrine function, cancer, bone health, reproductive health, kidney function, cognitive function, and glucose metabolism. Specifically, the NIH launched the Soy Research Guidelines, a document that addresses the following items to be considered in a study: 1) The need for sound justification for studying the health effects of soy in humans; 2) Approaches to understanding and ensuring product composition and integrity; 3) Methods for assessing exposure to non-study soy and intervention adherence; 4) Some appropriate analytical methods to test the products; 5) The importance of understanding how soy is processed and how it acts in the body: and 6) The role that genetic makeup may play in the health effects of soy (Klein et al., 2010).

An important source of conflicting results is the use of diverse forms of soy products. The soybean contains 12 forms of IF isomers, including the 3 aglycones, their respective β-glycosides, and 3 β-glucosides, each esterified with either malonic or acetic acid. The type and concentrations of these isomers in foods will vary depending on the plant part from which they are derived and the method by which they are processed (Coward et al., 1993; Erdman et al., 2004; Choi & Rhee, 2006). For instance, some soy products are designed to be very bland and are made from soy flour that has been treated with hot aqueous ethanol. Since this solvent extracts the IFs, these products are essentially IF-free (Barnes, 2008). Total IF content has been reported in the range of 60 to 340 mg/100 g for soy ingredients such as defatted and whole soy flours (90-95% glycosylated), soy protein isolates (20-55% aglycones) and textured soy proteins (90-95% glycosylated, but 15-25% acetylglycosides) (Genovese & Lajolo, 2010).

Since the IF content of foods is often reported without indicating whether it refers to aglycone or glycoside, Klein et al. (2010) propose the use of the term "aglycone IF equivalents" to describe the bioactive form of IFs, since cleavage of the glycosides is probably required before the compounds can be absorbed. As a result, IF values could be converted to aglycone equivalents if desired.

3. Health claims

3.1 Cardiovascular health

The beneficial effects of fruits and vegetables have been largely ascribed to polyphenols, since these bioactives affect dyslipidemia and atherosclerosis; endothelial dysfunction and hypertension; platelet activation and thrombosis; the inflammatory process associated with the induction and perpetuation of cardiovascular diseases (Fraga et al., 2010).

In spite of the great amount of scientific reports demonstrating the beneficial effects of IFs in a variety of assays *in vitro, in vivo* and clinical studies, as well as their mechanisms of action, the only health claim currently approved by the US FDA relates the intake of soy protein to the protection of cardiovascular health, establishing that an amount of 25 g of soy protein should be consumed on a regular daily basis (FDA, 1999). The exact mechanism by which soy lowers blood lipids remains unclear, but in 1999 the FDA approved the health claim stating that the inclusion of soy protein into a diet low in saturated fat and cholesterol may reduce the risk of coronary heart disease by lowering blood cholesterol levels. Due to the inconsistency of the results of the studies available, the National Center for Complementary and Alternative Medicine and the Office of Dietary Supplements, both of NIH, reviewed the evidence-based literature and published a report through the Agency for Health Care Research and Quality (AHRQ) (Balk et al., 2005).

The European Food Safety Authority analyzed the claimed effect for soy protein stating that "reduces blood cholesterol and may therefore reduce the risk of (coronary) heart disease" (EFSA, 2006). Clinical studies were provided to sustain the claim, most of which were randomized controlled trials. Meta-analyses and a review of possible mechanisms by which soy protein might exert the claimed effect were all examined. However, most of these studies were not appropriately designed to test the effect of soy protein *per se*, but were conducted using either soy protein isolate (SPI, by definition contains 90% protein) or soy foods containing other constituents that may exert an effect on blood cholesterol in human intervention studies (e.g., fat and fatty acids, fiber, IFs). The Panel considered that the design of the studies on SPI did not address the effects of the food constituent that is the subject of

the health claim on LDL-cholesterol concentrations. Then new intervention studies were included in a new meta-analysis which aimed to address the effects of soy protein *per se* on blood cholesterol concentrations, and there was a statistically significant dose-response relationship between the intake of IFs and the decrease in total and LDL-cholesterol concentrations. One study was designed to assess the effects of IF-containing and of IF-free SPI on biomarkers of cardiovascular risk, including blood lipids. No significant differences were observed between the SPI with no IFs (or the SPI with IFs) and the control group with respect to changes in total or LDL-cholesterol concentrations during the study. The EFSA Panel concluded that this study did not support an effect of the protein component of soy on LDL-cholesterol concentrations.

In weighing the evidence, the EFSA Panel took into account that the results from human intervention studies identified as being controlled for the macronutrient composition of the test products did not support an effect of the protein component of soy on LDL-cholesterol concentrations, and that the proposed mechanism by which the protein component of soy would exert the claimed effect is not supported by available scientific evidence. Consequently, a cause and effect relationship was not established between the consumption of soy protein and the reduction of LDL-cholesterol concentrations (Scientific Opinion on the substantiation of a health claim related to soy protein and reduction of blood cholesterol concentrations pursuant to Article 14 of the Regulation (EC) No 1924/2006, published: 30 July 2010).

The "Dietary Approaches to Stop Hypertension" (DASH) study (Sacks et al., 2001) showed that blood pressure levels may be lowered with a healthy eating plan that includes fruits and vegetables, is low in total fat, saturated fat, and cholesterol. Antihypertensive effects of soy IFs have been reported for over a decade, although the results of the clinical studies and the ability of specific dietary compounds to lower blood pressure is still controversial. Nestel et al. (1997) observed that IFs improve systemic arterial compliance. A cardioprotective effect of genistein was observed in association with its ability to lower blood pressure in postmenopausal women (Teede et al., 2001). However, the same authors (Teede et al., 2006) did not report any beneficial effects on arterial function after three months of soy protein dietary supplementation containing IFs in hypertensive men and postmenopausal women.

A number of the cardiovascular protective actions of IFs have been associated to their effects on thromboxane A_2 (TxA_2), a pro-atherogenic metabolite of arachidonic acid, since the stimulation of TxA_2 receptors activates a series of cell signals involved in the development of atherogenesis (Huang et al., 2004). Genistein inhibits TxA_2-mediated platelet responses (Nakashima et al., 1991), acting as an antagonist of TxA_2 receptors, and the same action has been described for equol (Munoz et al., 2009). These molecules compete with TxA_2 receptors and decrease their density (Garrido et al., 2006). Genistein and daidzein inhibit platelet adhesion and aggregation (Sargeant et al., 1993; Gottstein et al., 2003; Borgwardt et al., 2008) and inhibit the secretory activity of platelets (Guerrero et al., 2005; Munoz et al., 2009).

IFs also exert anti-inflammatory effects, and Huang et al. (2005) observed a 66.7% reduction of TNF-α in postmenopausic healthy women who consumed soymilk containing 112.2 mg IFs for 16 weeks, while Chan et al. (2008) observed a reduction of C-reactive protein with IFs supplement in patients with ischaemic stroke, improving their endothelial function. Soy IFs have been shown to inhibit TNF-α induced NF-κB activation (a transcription factor that regulates genes involved in inflammation, cytokine response, and cell proliferation and survival) in cultured human lymphocytes, growth control and its supplementation in

healthy men was shown to prevent NF-κB activation by TNF-α in blood lymphocytes (Davis et al., 2001). Choi et al. (2011) demonstrated that soybean and two Korean traditional fermented soybean products modulate inflammation-related NF-κB activation in Sprague–Dawley rats fed a high-fat diet. The authors report that the expressions of NF-κB related proinflammatory genes, notably COX-2, iNOS, and that of the adhesion molecule VCAM-1, increased with the feeding of a high-fat diet, but that soybean and fermented soybean products modulated these gene expressions.

Besides, several studies have shown that endothelial nitric oxide synthase (eNOS) expression is increased following treatment with dietary soy (Mahn et al., 2005) or genistein (Squadrito et al., 2003; Si et al., 2008), increasing nitric oxide (NO) production, thus improving vascular function. Joy et al. (2006) showed that rapid activation of eNOS with IFs includes interaction between multiple signaling pathways, involving activation of the ERK1/2 pathway, and activation of the PI3 kinase/Akt pathway (Tissier et al., 2007), resulting in phosphorylation of eNOS and subsequent association of eNOS with heat shock protein 90, which participates in the activation of this enzyme. Mann et al. (2007) reported that an important aspect of the vascular response to IFs involves increased expression of components of cellular antioxidant mechanisms, since IFs may amplify NO signaling increasing NO bioavailability by directly quenching reactive oxygen species. Moreover, IFs also reverse vascular contraction through inhibitory interactions with a number of vascular constriction mechanisms (Joy et al., 2006).

Other actions of soy IFs have been reported that may aid in the cardiovascular protection, such as a decrease of body fat in older ovariectomized mice (Naaz et al., 2003), and rats (Kim et al., 2006), an inhibitory effect on the enlargement of adipose tissue (Ørgaard & Jensen, 2008), and the reduction of fasting blood glucose and lipid levels (Park et al., 2006), contributing to prevent obesity-associated diseases. However, care should be taken when describing these actions, since many of the studies have been performed *in vitro* or animal studies using concentrations that are unexpected to be reached from a dietary intake of IFs.

3.2 Bone health and menopausal symptoms

IFs possess estrogenic activity in animals at concentrations lower than 0.1 µM based on the direct interactions between IFs and estrogen receptors (ERs): ERα and ERβ, providing these polyphenols the ability to act as estrogen agonists or antagonists (Messina, 2010a). This chapter does not describe the effects of soy IFs on circulating levels of estrogens and other hormones in women, and the extensive review and meta-analysis of the literature to examine these effects published by Hooper et al. (2009) is recommended. Since a variety of functional foods especially formulated for women are marketed considering the putative beneficial effects of IF intake on bone health and menopausal symptoms, these subjects are briefly described.

Epidemiological Asian studies have found that postmenopausal women with the highest intake of IF-rich soy foods have the highest bone mineral density (BMD) in the lumbar spine compared with women with low intakes (Somekawa et al., 2001; Mei et al., 2001). The evidence of a bone health protective effect of IFs is associated to their ability to bind selectively to estrogen receptors (Kuiper et al., 1997). These compounds stimulate osteoblasts and inhibit osteoclast activity *in vitro*, effects that are consistent with reduced bone turnover (Rassi et al., 2002; Chen et al., 2003). Besides, animal studies have shown bone-sparing effects of soy protein or IFs (Setchell & Lydeking-Olsen, 2003), while short-term human studies have demonstrated that IFs can reduce bone loss in postmenopausal

women (Potter et al., 1998; Scheiber et al., 2001). In a model of ovariectomized rats, Al-Nakkash et al. (2010) after 2 weeks of genistein treatment (250 mg/kg body weight) observed increased uterine weight, femur weight, and femur-to-body weight ratio, estrogen-like effects that were not associated to oxidative stress. According to Lydeking-Olsen et al. (2004), soy foods with IFs can prevent bone loss of the lumbar spine in postmenopausal women, who may otherwise lose 1.5–3% of bone/year. This prevention of bone loss, if continued into old age, could translate into a decrease in lifetime risk of osteoporosis and a lowering of fracture rates.

The North American Menopause Society (2000; 2006) stated that "the role of IFs in the management of short-term menopausal symptoms as well as diseases related to menopause/aging is still uncertain…" These reports, however, were retired. In a review on the subject of the anti/proestrogenic effects of IFs in breast cancer, Hasler & Kundrat (2002) emphasize that clinical trials are needed in order to clearly establish the potential beneficial effects. Although a series of subsequent papers report beneficial effects of the intake of soy IFs on BMD (Atkinson et al., 2004; Kreijkamp-Kaspers et al., 2004), the situation remains unclear. Tests have been performed using pharmaceutical forms also, and Marini et al. (2007) administered 54 mg/day tablets of purified genistein to postmenopausal women, evidencing a significant increase in BMD in certain locations *vs*. placebo.

More recently, the North American Menopause Society (2010) stated that the evidence of the benefits of the consumption of IFs on bone health is weak, regardless of the food source. In agreement with this statement, the EFSA analyzed the claimed effect of IFs on bone health, related to bone mass, BMD, and bone structure, all of which contribute to bone strength. However, although significant effects on markers of bone turnover and/or on spine BMD have been described in some short-term randomized trials in relation to the dietary intake of soy IFs, longer-term interventions do not support a sustained effect of soy IF intake on markers of bone health. The Panel also took into account the lack of a clear dose-response relationship between the dietary intake of soy IFs and the claimed effect, and the different results obtained depending on the source and nature of the IFs used. In conclusion, it stated that the evidence provided was not sufficient to establish a cause and effect relationship between the consumption of soy IFs and the maintenance of BMD in post-menopausal women (EFSA Scientific Opinion on the substantiation of health claims related to soy IFs and maintenance of bone mineral density (ID 1655) pursuant to Article 13(1) of Regulation (EC) No 1924/2006, published: 1 October 2009).

3.3 Antioxidant effects

The antioxidant capacity of IFs has been observed mostly *in vitro* (Ruiz-Larrea et al., 1997; Rüfer & Kulling, 2006). However, as it has been described to polyphenols in general, IFs may act as indirect antioxidants by up-regulating endogenous antioxidant enzymes (Stevenson & Hurst, 2007; Kampkotter et al., 2008). Borras et al. (2006) observed that genistein at low micromolar physiological levels up-regulates the expression of longevity-related genes in a manner similar to 17 β-estradiol, involving interactions with estrogen receptors, activation of ERK1/2 and NFκB and up-regulation of longevity-related gene expression. *In vivo*, Wiseman et al. (2000) observed that dietary soy IFs decrease $F_{(2)}$-isoprostane concentrations (a biomarker of oxidation) and increase the resistance of LDL to oxidation in humans. Among the various beneficial effects attributed to their antioxidative properties, dietary soy IFs have been described as neuroprotective in transient focal cerebral ischemia in male and ovariectomized female rats. Consequently, IFs may protect the brain

via increases in endogenous antioxidant mechanisms and reduced oxidative stress (Ma et al., 2010).

With regard to properties such as "protection of DNA, proteins and lipids from oxidative damage", claimed as antioxidant health, the target population was assumed to be individuals performing physical exercise. In the context of the proposed wording, the EFSA Panel assumed that the claimed effect refers to the protection of DNA, proteins and lipids from oxidative damage caused by free radicals that are generated during physical exercise. However, no conclusions could be drawn from the two human studies provided for the scientific substantiation of the claimed effect because they do not distinguish between the effects of soy protein (which is the subject of the health claim) and those of soy IFs on lipid peroxidation. On the basis of the data presented, a cause and effect relationship was not established between the consumption of soy protein and the protection of DNA, proteins and lipids from oxidative damage (Scientific Opinion on the substantiation of health claims related to soy protein and contribution to the maintenance or achievement of a normal body weight (ID 598), maintenance of normal blood cholesterol concentrations (ID 556) and protection of DNA, proteins and lipids from oxidative damage (ID 435) pursuant to Article 13(1) of Regulation (EC) No 1924/2006, published: 19 October 2010).

3.4 Cancer

It has been suggested that a high intake of soy products in Asian populations may have contributed to cancer protection, mainly by lowering the risk at certain locations such as colorectal and breast cancer. The cancer protective effects of flavonoids have been attributed to a wide variety of mechanisms, including free radical scavenging, the modification of enzymes that activate or detoxify carcinogens, and inhibition of the induction of the transcription factor activator protein-1 (AP-1) activity by tumor promoters (Shih et al., 2000). Soy constituents have also been shown to have other anticancer effects, including the inhibition of DNA topoisomerases I and II, proteases, tyrosine kinases, inositol phosphate, and angiogenesis, as well as the boost of immune response and antioxidative effects (Adlercreutz & Mazur, 1997; Taylor et al., 2009).

Genistein is an effective inhibitor of DMBA-induced DNA damage in MCF-7 cells by inhibiting CYP1A1 and CYP1B1. In fact, it inhibits recombinant human CYP1A1 and CYP1B1 (Chan & Leung, 2003). IFs could reduce xenobiotic-induced CYP1A1 and 1B1 mRNA expression through interference with xenobiotic responsive elements (XRE)-dependent transactivation (Moon et al., 2006). XRE are enhancer elements located in the promoter regions of xenobiotic responsive genes, which include genes encoding for CYP1A1 and 1B1, and their expression can be regulated through pathway involving aryl hydrocarbon receptor (AhR). However, Kishida et al. (2004) reported that dietary soy IFs had no effect on the hepatic mRNA abundance of CYP1A1 and 1A2 in rats, determined by real-time quantitative RTPCR. This indicates that dietary IFs may not be able to induce CYPs in either the transcriptional step or through post-transcriptional mRNA stabilization.

Genistein also exerts anticancer properties by modulating genes that regulate the cell cycle and apoptosis (Sarkar et al., 2002; Banerjee et al., 2008). As an antioxidant, genistein decreases reactive oxygen species levels, and it also induces the expression of the antioxidant enzymes superoxide dismutase (SOD) and catalase, which are associated with AMP-activated protein kinase (AMPK) and phosphatase and tensin homolog deleted from chromosome 10 (PTEN) pathways (Park et al., 2010). Genistein has been proposed as a natural alternative to estrogen replacement due to its ability to act via estrogen receptor-

dependent mechanisms, utilizing the phosphatidylinositol 3-kinase /Akt pathway (Tissier et al., 2007). The PTEN protein is a lipid phosphatase and has been suggested to act as a tumor suppressor owing to its inhibition of the PI3K/Akt signalling pathway, and genistein has been reported to promote apoptosis in mammary epithelial cells by inducing PTEN (Dave et al., 2005), accompanied by a decrease in mammary tumorigenesis.

3.4.1 Colorectal cancer

Evidence from *in vitro* and animal studies has implicated soy and soy IFs in colorectal cancer protection. Moreover, a series of *in vivo* studies performed in laboratory animals have shown that soy diets inhibit chemically induced colon tumorigenesis, and IFs may be the putative bioactives playing a role in this inhibitory process (Raju et al., 2009). However, care should be taken when the results of these studies are analyzed, since interactions between soy protein and IFs may occur as well as different effects from purified extracts of IFs and other components such as saponins, in their anticancer effects. Soy saponins may be important anticancer compounds present in soy due to their ability to inhibit tumor cell growth without altering normal colon morphology (MacDonald et al., 2005).

The human studies reported present a series of methodological limitations, particularly with regard to dietary measurement issues, such as incomplete assessment of soy intake, inadequate quantification, and inappropriate time period for cancer prevention as well as inadequate adjustment for confounders (Spector et al., 2003). Yang et al. (2009) in a population-based, prospective cohort of Chinese women with high but varied soy intake found that the risk of colorectal cancer decreased with increasing soy food intake, mainly in postmenopausal women. The authors reported that the risk was 30% lower among women in the upper third of soy food intake level compared with women in the bottom third of soy food intake level.

More recently, Yan et al. (2010) developed a meta-analysis of 11 epidemiologic studies that assessed the association of soy consumption with colorectal cancer incidence in humans. In two of these studies soy intake was found to be associated with a significant reduction in colorectal cancer risk in women, whereas in the other nine studies no such association was observed in either women or men. The authors conclude that consumption of soy foods is not associated with the risk of colorectal cancer or colon or rectal cancer separately.

3.4.2 Breast cancer

An inverse relationship between soy consumption and breast cancer incidence has been observed in Asian populations and attributed to the high intake of soy foods. However, it is noteworthy that Japanese incidence rates of breast cancer have increased markedly during the last decades, along with Westernization of the diet and culture, while simultaneously breast cancer has become the number one type of cancer among Japanese women (http://www.mc.pref.osaka.jp/ocr/). The results of the epidemiological studies published are confusing, and evidence suggests that plasma or serum concentrations of IFs may be a more sensitive predictor of the possible protective effects of soy foods against breast cancer than the assessment of dietary IFs intake (Messina & Wu, 2009), mainly due to the marked differences in IF metabolism that exist among individuals, as well as differences in sources of IFs, differences in biological response to IFs among ethnic groups, interactions with background diet, among others. Consequently, plasma IF levels are more reliable biomarkers compared with dietary IFs intake for evaluating the potential of soy to reduce breast cancer risk.

One of the more intriguing subjects is that the protective effect of soy IFs observed in Asian studies results from lifelong exposure or exposure to IFs early in life. Although the preclinical models have limitations, these studies provide useful information that supports clinical and epidemiological studies, as is the case of the observation of a protective effect from early life exposure to soy IFs against breast cancer (Russo & Russo, 2006; Warri et al., 2008; Lee et al., 2009). Undoubtedly, the most conclusive evidence of health-promoting effects of IFs is obtained through well designed clinical trials, and a better understanding of the mechanisms by which IFs may affect the development of breast cancer is still needed.

It is noteworthy that a controversial question remains associated with the roles of IFs on cancer prevention. In moderation, IFs may exert beneficial effects; however, for some cancers, increased risk has been reported when IF intakes are high (Gee et al., 2000; Daly et al., 2007). In fact, Petrakis et al. (1996) demonstrated that consumption of soy protein isolate had stimulatory effects on the breast tissue of premenopausal women, reporting greater numbers of hyperplastic epithelial cells, while Allred et al. (2001) reported that soy protein isolates stimulated the growth of MCF-7 tumors in a dose-dependent manner as the concentration of genistein increased. Consequently, the IFs have paradoxical effects that should be taken into consideration when dosage and timing of administration are defined. For example, prepubertal exposure to genistein appears to be protective against the development of breast cancer, but consumption of the IFs in either pure form or in soy protein isolate, after development of an estrogen-dependent breast cancer may enhance the growth of that tumor (Allred et al., 2001). Shu et al. (2009) in a population based study of breast cancer survival demonstrated that soy food intake was associated with improved breast cancer survival. The authors showed that women who had the highest level of soy food intake and did not take tamoxifen had a lower risk of mortality and a lower recurrence rate than women who had the lowest level of soy food intake and used tamoxifen, suggesting that high soy food intake and tamoxifen use may have a comparable effect on breast cancer outcomes.

4. Patents

Patents related to soy and/or IFs may be classified into two main groups: a) those dealing with processes developed to obtain extracts and other forms as ingredients suitable to the formulation of functional foods, dietary supplements and/or nutraceuticals, and b) those dealing with the health-promoting effects of innovative products. Among the first group, a series of diverse processes have been developed, and just a few are mentioned in order to exemplify the diversity of subjects covered. The patented processes include, among a wide variety, an encapsulated soy extract that includes IF derivatives: daidzin, glycitin, genistin, daidzein, glycitein and genistein, encapsulated with cyclodextrins or combinations of various oligosaccharides (PCT/US2007/083323); a process to produce a composition containing a high concentration of aglycone IFs using microorganisms which are generally recognized as safe (GRAS), that can express or produce β-glycosidase on a soy-based substrate (Serial No. 358938, Taiwan); a soy protein isolate that has increased amounts of IFs and saponins and a high Nitrogen Solubility Index ("NSI") produced by a process that involves ultrafiltration and the avoidance of isoelectric precipitation. The soy protein isolate has at least about 90.0 wt % protein of total dry matter; an IF content of at least about 1.0 mg/g IFs of total dry matter, and a NSI of at least about 75% (US Patent 7,306,821). Methods of producing from natural soybeans, soybean materials (i.e. tofu dregs, soy molasses) and

other plant sources are also described (Appl. No. 11/622,468, Hong Kong), as well as the recovery of conjugated IFs of residues and sub-products of food industries based on the use of soy and its derivatives, including foods containing IFs and from genetically modified *Aspergillus oryzae* ATCC 22786 (RIB 430), involving a process of conversion of conjugated IFs (malonate and acetates), in glucosylated IFs, which through fermentative and enzymatic processes are transformed into aglycones (US Patent Application 20100048689).

A series of companies have developed various soy-based products marketed to the treatment of various diseases and exhibit expanded patent portfolios, that include uses such as US patent 6,399,072 for the "Method of Preparing and Using Isoflavones for the Treatment of Alcoholism" and US patent 6,391,310 describing the "Method of Preparing and Using Isoflavones for the Treatment of Neurological Symptoms". One of the developed products claims a series of benefits that are a direct result of their patented, natural concentration process (US Patent 6,482,448 "Soy formulas and their use for promoting health"). Products presented as protein shakes or protein bars provide about the same amount of soy IFs found in 6 cups of a typical soymilk (~160 mg of soy). In the US, these products as well as others available should be labeled *"These statements have not been evaluated by the Food and Drug Administration. (Name of brand) ® foods and dietary supplements are not intended to diagnose, treat, cure or prevent any disease. Individual results vary."*

A series of patents describing the applications of IFs in the prevention of diseases have been published, mainly in the late 1990's and early 2000's, including situations such as cancer (Thurn & Juang, 1999), heart disease (Potter et al., 1999), macular degeneration (Jenks, 1999), as well as to prevent hair loss and maintain hair integrity (Segelman, 2000), to improve deficient skin conditions (Lanzendorfer et al., 1999), to inhibit Alzheimer's disease and related dementias, for preserving cognitive function (Clarkson et al., 1999), to inhibit gram-negative bacterial cytotoxicity (Fleiszig & Evans, 1999), and to treat cystic fibrosis (Hwang et al., 1999), among others.

Many patents deal with the relief of menopausal symptoms, as is the case of preparations such as a composition claimed for the relief and/or prevention of climacteric and menopausal disorders affecting women in pre-, peri- or post-menopause, comprising soy IFs and viable lactic acid bacteria aimed to enhance the absorption of soy IFs (US Patent 7,025,998). In this case, as in most of the patents available, the compositions are provided in dosage forms for oral administration (which constitute actual nutraceutical products, since they are presented as pharmaceutical dosage forms and are not consumed as part of the normal diet, a requisite for a functional food), and some commercial preparations announce pharmacological effects. This is the case of a soy formulation claimed to "promote the health of an individual, preferably utilizing the soy formulations, dietary supplements, food products and/or pharmacological compositions of the invention" (US Patent Application 20030021859). A list of US patents related to soy and soy IF can be seen at http:// patft.uspto.gov/while in Europe the list is available at http://www.epo.org/patents /patent-information.html, a website that also allows to search Asian patents at http://www.epo.org/patents/patent-information/east-asian.html. The Google website http://www.google.com/patents may also be visited.

Additionally, due to all the available information about soy IFs that in many cases may be confusing to the consumer, the NIH's Office of Dietary Supplements published the fact sheet available at:

http://ods.od.nih.gov/Health_Information/Information_About_Individual_Dietary_Suppl ements.aspx

5. Functional foods containing soy IFs

A series of soy based functional foods are currently marketed in many countries, most of which attribute beneficial effects to their IFs content. The so-called functional soy foods include a variety of products e.g. those traditionally fermented such as miso, tempeh, natto, tofu. It should be noted that fermentation of soybeans changes the amount of IFs in them. Amounts of IFs in the fermented soybean products miso (bean paste) and natto (fermented soybeans) are significantly different than those in unfermented soybeans (Fukutake et al., 1996). The amount of genistein in the fermented soybean products is higher than in soybeans and soybean products such as soymilk and tofu. There is a wide variety of IF composition in the different soybean products commercially available as a source of bioactive IFs (Nurmi et al., 2002).

Setchell et al. (2001) analyzed 33 phytoestrogen supplements and extracts available at that time and observed differences in the content from that claimed by the manufacturers. Novel fermented products include those fermented by probiotics that have the potential to reduce the levels of some carbohydrates responsible for gas production in the intestinal system and to change the bacterial composition towards a healthy population (Champagne et al., 2009). The most traditional concentrated and purified soy IFs are produced mostly from soy molasses, soy germ, and defatted soy flakes (Liu, 2004). Other soybean functional foods include dairy products that highlight the absence of cholesterol, lactose and milk proteins; bakery products; substitutes for meat, poultry or fish; and beverages (Jooyandeh, 2011).

Boniglia et al. (2009) observed different "fingerprints" in 14 soy-based dietary supplements intended to help alleviate perimenopausal and menopausal symptoms on sale in Italy, probably on account of different sources of the soy raw materials and the methods of processing and preparation of extracts. These authors reported total IF levels ranging from 33.75 to 80.00 of the values given by the manufacturers, while Stürtz et al. (2008) quantified the intact IFs (glycosides forms) in different supplements and observed values higher than those declared on the labels. These and other studies show that the IF contents are extremely variable and many times far below the values (34–150 mg) that appear to have some beneficial effects. For this reason it is important to standardize the amount of IFs present in these products. To overcome these inconsistencies, after analyzing a series of commercial products, Collison (2008) recommended an analytical method to be adopted as Official First Action for analysis of total soy IFs in dietary supplements, dietary supplement ingredients, and processed soy foods containing at least 0.5 mg/g total IFs.

One important question that should be addressed when selecting a functional food is the variety and amount of bioactive ingredients it contains. Functional foods containing IFs should be effective to provide the benefits by consuming regular amounts of the products in the diet. Slavin et al. (1998) in a dose-response study suggest that the IFs in soy-protein isolate are bioavailable at amounts as low as 9 mg/day, or about the amount found in 28.4 g tofu (standard serving size), while Frank et al. (1999) reported mean total IF levels ranging from 35 ppm (soy milk) to 7,500 ppm (dietary supplements) in a variety of 25 soy-based foods and dietary supplements. It is important to keep in mind that health outcomes in relation to soy intake may be dependent, to some extent, on the timing and duration of soy exposure, the hormonal status of the individual, the tissue(s) affected, and the amount and/or composition of the soy consumed, and adverse effects should also be reported (Song et al., 2007). While traditional soy foods are comprised of a unique and complex blend of protein, lipids, vitamins, minerals, IFs, and other bioactive compounds that may act

individually and/or synergistically to exert healthful physiologic effects, there are many IF supplements or nutraceuticals currently available that manufacturers claim contain 1000 mg of genistein per dosage form (usually tablets) but for which the efficacy is unknown (Reinwald et al., 2010). These products should not be considered as part of a regular diet, however in many countries they are sold as nutritional aids.

Additionally, all functional foods should be innocuous and secure. Thus, toxicological studies are a requisite for functional ingredients. The NOAEL (no observed adverse effect level) of genistein has been established in 50 mg/kg body weight/day by McClain et al. (2006), who carried out hazard analyses for acute, subchronic and chronic safety of genistein in rats. The US Center for the Evaluation of Risks to Human Reproduction of the National Toxicology Program expert review panel expressed negligible concern for adverse effects in the general population of consuming dietary sources of genistein, concluding that under current exposure conditions, adults would be unlikely to consume sufficient daily levels of genistein to cause adverse reproductive and/or developmental effects (Rozman et al., 2006). Klein & King (2007) reviewed the literature on the potential genotoxicity or cellular effects of high doses of genistein (> 5 µM) *in vitro*, an amount that is not likely to be found *in vivo* due to the low uptake and bioavailability of this compound.

6. Conclusions

Consumers demand the food industry to produce functional foods that contribute to maintain health and prevent diseases, mainly those related to the aging process. However, it is of major importance to provide adequate information in order to protect the consumer's interests by using adequate health claims that are based upon solid scientific support obtained by means of *in vitro, in vivo* and clinical studies that substantiate the bioactivity of the functional ingredients contained in the products.

Many health benefits have been attributed to soy IFs. However, to date there is only one accepted health claim related to dietary soy intake: it refers to soy protein, and is not associated to the various bioactive phytochemicals that are contained in this legume, including IFs. As stated by Messina (2010b) in an extensive review on the evidence of the health-protective effects of soy intake, there is almost no credible evidence to suggest that traditional soy foods exert clinically relevant adverse effects in healthy individuals when consumed in amounts consistent with Asian intake.

Since the actual Western dietary IFs intake is low, an increase in dietary intake of IFs constitutes an interesting target and an alternative is the design of functional foods. A wide diversity of soy ingredients have been developed as a source of IFs that may be used as functional food ingredients. However, the various processes used affect the IF content and profile. The validation of the health claims associated to the dietary intake of putative bioactive IFs is a crucial issue. However, a thorough understanding of their bioavailability from different food products is critical to achieve the desired biological efficacy of these compounds. Clinical studies should be performed, although they may be costly and highly complex due to the various factors involved. Biological effects require sufficient delivery of IFs from the site of administration (the gastrointestinal tract) to the sites of target organs and receptors. One major factor is the bioavailability of IFs, which depends upon factors such as solubility, partition coefficient, permeability, metabolism, excretion, target tissue uptake, and disposition of the bioactives. Moreover, the poor bioavailability of polyphenols makes it even more difficult to conduct relevant but smaller clinical trials because large exposure differences are expected among the participants.

In spite of a myriad of patented processes and products containing soy IFs, including functional foods currently available for health-conscious consumers, most of the metabolic mechanisms of the specific health benefits attributed to IF consumption have not been clearly established and require further research. Consequently, no health claims should be declared for functional foods containing IFs, since they still lack scientific sound evidence.

7. References

Adlercreutz, H. & Mazur, W. (1997). Phyto-oestrogens and Western diseases. *Annals of Medicine*, Vol. 29, No. 2, (April 1997), pp. 95-120, ISSN 0003-4819.

Akaza, H., Miyanaga, N., Takashima, N., Naito, S., Hirao, Y., Tsukamoto, T., Fujioka, T., Mori, M. & Kim, W.J. (2004). Comparisons of percent equol producers between prostate cancer patients and controls: case-controlled studies of isoflavones in Japanese, Korean and American residents. *Japanese Journal of Clinical Oncology*, Vol. 34, No. 2, (February 2004), pp. 86-89, ISSN 0368-2811.

Allred, C.D., Allred, K.F., Ju, Y.H., Virant, S.M. & Helferich, W.G. (2001). Soy diets containing varying amounts of genistein stimulate growth of estrogen dependent (MCF-7) tumors in a dose-dependent manner. *Cancer Research*, Vol. 61, No. 13, (July 2001), pp. 5045–5050, ISSN 0008-5472.

Al-Nakkash, L., Markus, B., Batia, L., Prozialeck, W.C. & Broderick, T.L. (2010). Genistein induces estrogen-like effects in ovariectomized rats but fail to increase cardiac GLUT4 and oxidative stress. *Journal of Medicinal Food*, Vol. 13, No. 6, (December 2010), pp. 1369-1375, ISSN 1096-620X.

American Dietetic Association. (2009). Position of the American Dietetic Association: Functional Foods. *Journal of the American Dietetic Association*, Vol. 109, No. 4, (April 2009), pp.735-746, ISSN 0002-8223.

Atkinson, C., Compston, J.E., Day, N.E., Dowsett, M. & Binghmam, S.A. (2004). The effects of phytoestrogens isoflavones on bone density in women: a double-blind, randomized, placebo-controlled trial. *American Journal of Clinical Nutrition*, Vol. 79, No. 2, (February 2004), pp. 326-333, ISSN 0002-9165.

Aussenac, T., Lacombe, S. & Dayde, J. (1998). Quantification of isoflavones in soybean seeds by capillary zone electrophoresis: effects of variety and environment. *American Journal of Clinical Nutrition*, Vol. 68, No. 6 Suppl, (December 1998), pp. 1480S-1486S, ISSN 0002-9165.

Badger, T.M., Ronis, M.J.J., Wolff, G., Stanley, S., Ferguson, M., Shankar, K., Simpson, P. & Jo, C.H. (2008). Soy protein isolate reduces hepatosteatosis in yellow Avy/a mice without altering coat color phenotype. *Experimental Biology and Medicine (Maywood)*, Vol. 233, No. 10, (October 2008), pp. 1242–1254, ISSN 1535-3702.

Balk, E., Chung, M., Chew, P., Ip, S., Raman, G., Kupelnick, B., Tatsioni, A., Sun, Y., Wolk, B., DeVine, D. & Lau, J. (2005). Effects of soy on health outcomes. Evidence Report/Technology Assessment No. 126. Tufts-New England Medical Center Evidence-Based Practice Center, Contract No. 290–02–0022, AHRQ Publication No. 05–E024–2. Rockville (MD): Agency for Healthcare Research and Quality. Available from: http://www.ahrq.gov/clinic/epcsums/soysum.pdf and http://www.ahrq.gov/clinic/tp/soytp.htm.

Banerjee, S., Li, Y., Wang, Z. & Sarkar, F.H. (2008). Multi-targeted therapy of cancer by genistein. *Cancer Letters,* Vol. 269, No. 2, (October 2008), pp. 226–242, ISSN 0304-3835.

Barnes, S. (2008). Nutritional genomics, polyphenols, diets, and their impact on dietetics. *Journal of the American Dietetic Association,* Vol.108, No. 11, (November 2008), pp. 1888–1895, ISSN 0002-8223.

Birt, F., Hendrich, S. & Wang, W. (2001). Dietary agents in cancer prevention: flavonoids and isoflavonoids. *Pharmacology & Therapeutics,* Vol. 90, No. 2-3, (May-June 2001), pp. 157-177, ISSN 0009-9236.

Boniglia, C., Carratù, B., Gargiulo, R., Giammarioli, S., Mosca, M. & Sanzini, E. (2009). Content of phytoestrogens in soy-based dietary supplements. *Food Chemistry*, Vol. 115, No. 4, (August 2009), pp. 1389–1392, ISSN 0308-8146.

Borgwardt, K., Bonifatius, S. & Gardemann, A. (2008). Acidic peptides enhanced genistein-dependent inhibition of human platelet aggregation: potential protective effect of digestible peptides plus genistein against atherosclerosis. *Nutrition Research*, Vol. 28, No. 8, (August 2008), pp. 523-531, ISSN 0271-5317.

Borras, C., Gambini, J., Góimez-Cabrera, M.C., Sastre, J., Pallardo, F.V., Mann, G.E. & Viña, J. (2006). Genistein, a soy isoflavone, up-regulates expression of antioxidant genes: involvement of estrogen receptors, ERK1/2, and NFkB. The *FASEB Journal,* Vol. 20, No. 12, (December 2006), pp. 2136-2138, ISSN 0892-6638.

Champagne, C.P., Green-Johson, J., Raymond, Y., Barrete, J. & Buckley, N. (2009). Selection of probiotic bacteria for the fermentation of a soy beverage in combination with Streptococcus thermophilus. *Food Research International*, Vol. 42, No. 5-6, (June-July), pp.612-621, ISSN 0963-9969.

Chan, H.Y. & Leung, L.K. (2003). A potential protective mechanism of soya isoflavones against 7,12-dimethylbenz[a]anthracene tumour initiation. *British Journal of Nutrition*, Vol. 90, No. 2, (August 2003), pp.457–465, ISSN 0007-1145.

Chan, Y. H., Lau, K.K., Yiu, K.H., Li, S.W., Chan, H.T., Fong, D.Y.T., Tam, S., Lau, C.P. & Tse, H.F. (2008). Reduction of C-reactive protein with isoflavone supplement reverses endothelial dysfunction in patients with ischaemic stroke. *European Heart Journal*, Vol. 29, No. 22, (September 2008), pp. 2800-2807, ISSN 0195-668x.

Chanteranne, B., Branca, F., Kaardinal, A., Wahala, K., Braesco, V., Ladroite, P., Brouns, F. & Coxam, V. (2008). Food matrix and isoflavones bioavailability in early post menopausal women: A European clinical study. *Clinical Interventions in Aging,* Vol. 3, No. 4, (December 2008), pp. 711–718, ISSN 1176-9092.

Chen, X., Garner, S.C., Quarles, L.D. & Anderson, J.B. (2003). Effects of genistein on expression of bone markers during MC3T3-E1 osteoblastic cell differentiation. *The Journal of Nutritional Biochemistry*, Vol. 14, No. 6, (June 2003), pp. 342-349, ISSN 0955-2863.

Chen, J., Lin, H. & Hu, M. (2005). Absorption and metabolism of genistein and its five isoflavone analogs in the human intestinal Caco-2 model. *Cancer Chemotherapy and Pharmacology*, Vol. 55, No. 2, (July 2005), pp. 159–169, ISSN 0344-5704.

Chen, J.R., Singhal, R., Lazarenko, O.P., Liu, X., Hogue, W.R., Badger, T.M. & Ronis, M.J.J. (2008). Short term effects on bone quality associated with consumption of soy protein isolate and other dietary protein sources in rapidly growing female rats.

Experimental Biology and Medicine (Maywood), Vol. 233, No. 11, (November 2008), pp. 1348–1358, ISSN 1535-3702.

Chen, J.R., Lazarenko, O.P., Blackburn, M.L., Badeaux, J.V., Badger, T.M. & Ronis, M.J.J. (2009). Infant formula promotes bone growth in neonatal piglets by enhancing osteoblastogenesis through bone morphogenic protein signaling. *Journal of Nutrition,* Vol. 139, No. 10, (October 2009), pp. 1839–1847, ISSN 0022-3166.

Choi, M-S. & Rhee, K.C. (2006). Production and processing of soybeans and nutrition and safety of isoflavone and other soy products for human health. *Journal of Medicinal Food*, Vol. 9, No 1, (Spring 2006), pp. 1-10, ISSN 1096-620X.

Choi, J., Kwon, S-H., Park, K-Y., Yu, B.P., Kim, N.D., Jung, J.H., Chung, H.Y. (2011). The anti-inflammatory action of fermented soybean products in kidney of high-fat-fed rats. *Journal of Medicinal Food,* Vol. 14, No. 3, (February 2011), pp. 232–239, ISSN 1096-620X.

Clarkson, T.B., Jr., Anthony, M.S., Pan, Y., Adams, M.R. & Waggle, D.H. (1999). Method for inhibiting the development of Alzheimer's disease and related dementias and for preserving cognitive function. US patent 5,952,374. September 14.

Collison, M.W. (2008). Determination of total soy isoflavones in dietary supplements, supplement ingredients, and soy foods by high-performance liquid chromatography with ultraviolet detection: collaborative study. *Journal of the AOAC International*, Vol. 91, No. 3, (May-June 2008), pp. 489-500, ISSN 1060-3271.

Coward, L., Barnes, N.C., Setchell, K.D.R. & Barnes, S. (1993). Genistein, daidzein, and their β-glycoside conjugates: antitumor isoflavones in soybean foods from American and Asian diets. *Journal of Agricultural and Food Chemistry,* Vol. 41, No. 11, (November 1993), pp. 1961–1967, ISSN 0021-8561.

Daly, K.T., Tracy, A.C., Malik, M., Wang, T., Francke-Carroll, S. & Magnuson, B.A. (2007). Enhanced estrogenic responses and sensitivity to azoxymethane following dietary soy isoflavone supplementation in older female rats. *Food and Chemical Toxicology,* Vol. 45, No. 4, (April 2007), pp. 628–637, ISSN 0278-6915.

Dave, B., Eason, R.R., Till, S.R., Geng, Y., Velarde, M.C., Badger, T.M. & Simmen, R.C. (2005). The soy isoflavone genistein promotes apoptosis in mammary epithelial cells by inducing the tumor suppressor PTEN. *Carcinogenesis,* Vol. 26, No. 10, (October 2005), pp. 1793–1803, ISSN 0143-3334.

Davis, J.N., Kucuk, O., Djuric, Z. & Sarkar, F.H. (2001). Soy isoflavone supplementation in healthy men prevents NF-kappa B activation by TNF-alpha in blood lymphocytes. *Free Radical Biology and Medicine*, Vol. 30, No. 11, (June 2001), pp. 1293–1302, ISSN 0891-5849.

Day, A. J., Du Pont, M. S., Ridley, S., Rhodes, M., Rhodes, M. J., Morgan, M. R. & Williamson, G. (1998). Deglycosylation of flavonoid and isoflavonoid glycosides by human small intestine and liver beta-glucosidase activity. *Federation of European Biochemical Societies (FEBS) Letters*, Vol. 436, No. 1, (September 1998), pp. 71–75, ISSN 0014-5793.

De Pascual, S., Hallund, J., Talbot, D., Schroot, J., Williams, C.M., Bugel, S. & Cassidy, A. (2006). Absorption of soflavones in humans: effects of food matrix and processing. *Journal of Nutritional Biochemistry*, Vol. 17, No. 4, (April 2006), pp. 257–264, ISSN 0955-2863.

Duncan, A.M., Merz-Demlow, B.E., Xu, X., Phipps, W.R. & Kurzer, M.S. (2000). Premenopausal equol excretors show plasma hormone profiles associated with lowered risk of breast cancer. *Cancer Epidemiology, Biomarkers and Prevention*, Vol. 9, No.6, (June 2000), pp. 581-586, ISSN 1055-9965.

European Food Safety Agency. (2006). Scientific Opinion on the substantiation of a health claim related to soy protein and reduction of blood cholesterol concentrations pursuant to Article 14 of the Regulation (EC) No 1924/2006, Published: 30 July 2010.

Eldridge, A.C. & Kwolek, W.F. (1983). Soybean isoflavones: effects of environment and variety on composition. *Journal of Agricultural and Food Chemistry*, Vol. 31, No. 2, (March 1983), pp. 394-396, ISSN 0021-8561.

Erdman, J.W. Jr, Badger, T.M., Lampe, J.W., Setchell, K.D.R. & Messina, M. (2004). Not all soy products are created equal: caution needed in interpretation of research results. *Journal of Nutrition*, Vol. 134, No. 5, (May 2004), pp. S1229–S1233, ISSN 0022-3166.

Fardet, A., Llorach, R., Orsoni, A., Martin, J-F., Pujos-Guillot, E., Lapierre, C. & Scalbert, A. (2008). Metabolomics provide new insight on the metabolism of dietary phytochemicals in rats. *Journal of Nutrition*, Vol. 138, No. 7, (July 2008), pp. 1282–1287, ISSN 0022-3166.

Faughnan, M.S., Hawdon, A., Ah-Singh, E., Brown, J., Millward, D.J. & Cassidy, A. (2004). Urinary isoflavone kinetics: the effect of age, gender, food matrix and chemical composition. *British Journal of Nutrition*, Vol. 91, No. 4, (April 2004), pp. 567–574, ISSN 0007-1145.

Fleiszig, S.M.J. & Evans, D.J. (1999). Methods for inhibiting bacterial cytotoxicity. US patent 5,948,815. September 7.

Food and Drug Administration. (1999). Health and Human Services: Food labeling: health claims; soy protein and coronary heart disease. Final rule. *Federal Register,* Vol. 64, No. 206, (October 1999), pp. 57700–57733, ISSN 0097–6326.

Fraga, C.G., Galleano, M., Verstraeten, S.V. & Oteiza, P.I. (2010). Basic biochemical mechanisms behind the health benefits of polyphenols. *Molecular Aspects of Medicine,* Vol. 31, No. 6, (December 2010), pp. 435–445, ISSN 0098-2997.

Frank, A.A., Hankin, J.H., Yu, M.C., Maskarinec, G., Low, S.H. & Custer, L.J. (1999). Isoflavone level in soy foods consumed by multiethnic populations in Singapore and Hawaii. *Journal of Agricultural and Food Chemistry*, Vol. 7, No. 3, (March 1999), pp. 977-986, ISSN 0021-8561.

Franke, A.A., Halm, B.M., Kakazu, K., Li, X. & Custer, L.J. (2009). Phytoestrogenic isoflavonoids in epidemiologic and clinical research. *Drug Testing and Analysis*, Vol. 1, No.1, (January 2009), pp.14–21, ISSN 1942-7603.

Fukutake, M., Takahashi, M., Ishida, K., Kawamura, H., Sugimura, T. & Wakabayashi, K. (1996). Quantification of genistein and genistin in soybeans and soybean products. *Food and Chemical Toxicology*, Vol. 34, No. 5, (May 1996), pp. 457–461, ISSN 0278-6915.

Garrido, A., De la Maza, M.P., Hirsch, S. & Valladares, L. (2006). Soy isoflavones affect platelet thromboxane A2 receptor density but not plasma lipids in menopausal women. *Maturitas*, Vol. 54, No. 3, (June 2006), pp. 270-276, ISSN 1521-690X.

Gee, J.M., Noteborn, H.P.J.M., Polley, A.C.J. & Johnson, I.T. (2000). Increased induction of aberrant crypt foci by 1,2-dimethylhydrazine in rats fed diets containing purified genistein or genistein-rich soya protein. *Carcinogenesis,* Vol. 21, No. 12, (December 2000), pp.2255–2259, ISSN 0143-3334.

Genovese, M.I. & Lajolo, F. (2010). Soy Protein Ingredients as Isoflavone Sources for Functional Foods. K. R. Cadwallader & S.K.C. Chang (eds). Chemistry, Texture, and Flavor of Soy. ACS Symposium Series, Vol. 1059, pp. 189–200, ISBN 9780841225619, Washington, D.C.

Gibney, M.J., Walsh, M., Brennan, L., Roche, H.M., German, B. & van Ommen, B. (2005). Metabolomics in human nutrition: opportunities and challenges. *American Journal of Clinical Nutrition,* Vol. 82, No. 3, (September 2005), pp. 497-503, ISSN 0002-9165.

Gottstein, N., Ewins, B.A., Eccleston, C., Hubbard, G.P., Kavanagh, I.C., Minihane, A.M., Weinberg, P.D. & Rimbach, G. (2003). Effect of genistein and daidzein on platelet aggregation and monocyte and endothelial function. *British Journal of Nutrition*, Vol. 89, No. 5, (May 2003), pp. 607-616, ISSN 0007-1145.

Gu, L., House, S.E., Prior, R.L., Fang, N., Ronis, M.J.J., Clarkson, T.B., Wilson, M.E. & Badger, T.M. (2006). Metabolic phenotype of isoflavones differ among females rats, pigs, monkeys, and women. *Journal of Nutrition*, Vol. 136, No. 5, (May 2006), pp. 1215–1221, ISSN 0022-3166.

Guerrero, J.A., Lozano, M.L., Castillo, J., Benavente-García, O., Vicente, V. & Rivera, J. (2005). Flavonoids inhibit platelet function through binding to the thromboxane A2 receptor. *Journal of Thrombosis and Haemostasis*, Vol. 3, No. 2, (February 2005), pp. 369-376, ISSN 1538-7933.

Hasler, C.M. & Kundrat, S. (2002). Soy isoflavones as functional ingredients in women's health. G.S. Gilani & J.J.B. Anderson (eds). *Phytoestrogens and Health.* AOCS Press, pp. 32-50, ISBN 1893997324, USA.

Hooper, L., Ryder, J.J., Kurzer, M.S., Lampe, J.W., Messina, M.J., Phipps, W.R. & 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. *Human Reproduction Update*, Vol.15, No.4, (April 2009), pp. 423–440, ISSN 1460-2369.

Hsu, B-Y., Inbaraj, B.S. & Chen, B-H. (2010). Analysis of soy isoflavones in foods and biological fluids: an overview. *Journal of Food and Drug Analysis*, Vol. 18, No. 3, (March 2010), pp. 141-154, ISSN 1021-9498.

Hu, M. (2007). Commentary: Bioavailability of flavonoids and polyphenols: call to arms. *Molecular Pharmaceutics*, Vol. 4, No. 6, (December 2007), pp. 803–806, ISSN 1543-8384.

Huang, J.S., Ramamurthy, S.K., Lin, X. & Le Breton, G.C. (2004). Cell signalling through thromboxane A2 receptors. *Cell Signaling*, Vol. 16, No. 5, May 2004), pp. 521-533, ISSN 1945-0877.

Huang, Y., Cao, S., Nagamani, M., Anderson, K.E., Grady, J.J. & Lu, L.J.W. (2005). Decreased circulating levels of tumor necrosis factor-alpha in postmenopausal women during consumption of soy-containing isoflavones. *Journal of Clinical Endocrinology and Metabolism*, Vol. 90, No. 7, (July 2005), pp. 3956-3962, ISSN 0021-972X.

Hwang, T.C., Smith, A.L., Konig, P., Clarke, L.L., Price, E.M. & Cohn, L.A. (1999). Genistein for the treatment of cystic fibrosis. US patent 5,948,814. September 7.

ILSI Europe (2010). *Beyond PASSCLAIM – Guidance to Substantiate Health Claims on Foods.* Summary Report of a Workshop held in December 2009. Report Series. (May 2010), pp. 1-28. ISBN: 9789078637219, Belgium.

Izumi, T., Piskula, M.K., Osawa, S., Obata, A., Tobe, K., Saito, M., Kataoka, S., Kubota, Y. & Kikuchi, M. (2000). Soy isoflavone aglycones are absorbed faster and in higher amounts than their glucosides in humans. *Journal of Nutrition,* Vol. 130, No. 7, (July 2000), pp. 1695–1699, ISSN 0022-3166.

Jackson , C.J.C. & Gilani, G.S. (2002). Tables of isoflavone, coumestan, and lignan data. In: *Phytoestrogen and Health.* G.S. Gilani, J.J.B. Anderson, Eds. AOCS Press, pp. 124-146, ISBN 1893997324, USA.

Jenks, B.H. (1999). Method for inhibiting or reducing the risk of macular degeneration. US patent 6,001,368. December 14.

Joy, S., Siow, R.C., Rowlands, D.J., Becker, M., Wyatt, A.W., Aaronson, P.I., Coen, C.W., Kallo, I., Jacob, R. & Mann, G.E. (2006). The isoflavone Equol mediates rapid vascular relaxation: Ca2+-independent activation of endothelial nitric-oxide synthase/Hsp90 involving ERK1/2 and Akt phosphorylation in human endothelial cells. *Journal of Biological Chemistry*, Vol. 281, No. 37, (September 15, 2006), pp. 27335-27345, ISSN 0021-9258.

Jooyandeh, H. (2011). Soy products as healthy and functional foods. *Middle-East Journal of Scientific Research*, Vol. 7, No. 1, (January 2011), pp. 71-80, ISSN 1990-9233.

Kampkötter, A., Chovolou, Y., Kulawik, A., Röhrdanz, E., Weber, N., Proksch, P. & Wätjen, W. (2008). Isoflavone daidzein possesses no antioxidant activities in cell-free assays but induces the antioxidant enzyme catalase. *Nutrition Research*, Vol. 28, No. 9, (September 2008), pp. 620-628, ISSN 0271-5317.

Kano, M., Takayanagi, T., Harada, K., Sawada, S. & Ishikawa, F. (2006). Bioavailability of isoflavones after ingestion of soy beverages in healthy adults. *Journal of Nutrition*, Vol. 136, No. 9, (September 2006), pp. 2291-2296, ISSN 0022-3166.

Karakaya, S. (2004). Bioavailability of phenolic compounds. *Critical Reviews in Food Science and Nutrition*, Vol. 44, No. 6, (November-December 2004), pp. 453–464, ISSN 1040-8398.

Kim, H-K., Nelson-Dooley, C., Della-Fera, M.A., Yang, J-Y., Zhang, W., Duan, J., Hartzell, D.L., Hamrick, M.W., & Baile, C.A. (2006). Genistein decreases food intake, body weight, and fat pad weight and causes tissue apoptosis in ovariectomized female mice. *Journal of Nutrition,* Vol. 136, No. 2, (February 2006), pp. 409-4124, ISSN ISSN 0022-3166.

Kirkman, L. M., Lampe, J. W., Campbell, D. R., Martini, M. C. & Slavin, J. L. (1995). Urinary lignan and isoflavonoid excretion in men and women consuming vegetable and soy diets. *Nutrition and Cancer,* Vol. 24, No. 1, (January 1995), pp. 1–12, ISSN 0163-5581.

Kishida (2004). Lack of an inducible effect of dietary soy isoflavones on the mRNA abundance of hepatic cytochrome P-450 isozymes in rats. *Bioscience, Biotechnology, and Biochemistry*, Vol. 68, No. 3, (March 2004), pp.508-515, ISSN 0916-8451.

Kitamum, K., Igita, K., Kikuchi, A., Kudou, S. & Okubo, K. (1991). Low isflavone content in some early maturing cultivars, so-called `Summer-type Soybeans' (Glycine max (L) Merrill). *Japan Journal of Breeding*, Vol. 41, No. 4, (October 1991), pp. 651-654, ISSN 0536-3683.

Klein, C.B. & King, A.A. (2007). Genistein genotoxicity: Critical considerations of *in vitro* exposure dose. *Toxicology and Applied Pharmacology,* Vol. 224, No. 1, (October 2007), pp. 1-11, ISSN 0041-008X.

Klein, M.A., Nahin, R.L., Messina, M.J., Rader, J.I., Thompson, L.U., Badger, T.M., Dwyer, J.T., Kim, Y.S., Pontzer, C.H., Starke-Reed, P.E. & Weaver, C.M. (2010). Guidance from an NIH Workshop on designing, implementing, and reporting clinical studies of soy interventions. *Journal of Nutrition*, Vol. 140, No. 6, (June 2010), pp. 1192S-1204S, ISSN 0022-3166.

Kreijkamp-Kaspers, S., Kok, L., Grobbee, D.E., de Haan, E.H.F., 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 postmenopoausal women: a randomized controlled trial. *Journal of the American Medical Association*, Vol. 292, No. 1, (July 2004), pp. 65-74, ISSN 0098-7484.

Klein, M.A., Nahin, R.L., Messina, M.J., Rader, J.I., Thompson, L.U., Badger, T.M., Dwyer, J.T., Kim, Y.S., Pontzer, C.H., Starke-Reed, P.E. & Weaver, C.M. (2010). Guidance from an NIH Workshop on Designing, Implementing, and Reporting Clinical Studies of Soy Interventions. *Journal of Nutrition*, Vol. 140, No. 6, (June 2010), pp. 1192S–1204S, ISSN 0022-3166.

Kuiper, G.G., Carlsson, B., Grandien, K., Enmark, E., Haggblad, J., Nilsson, S. & Gustafsson, J.A. (1997). Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. *Endocrinology,* Vol. 138, No. 3, (March 1997), pp. 863–870, ISSN 0013-7227.

Kulling, S. E., Honig, D. & Metzler, M. (2001). Oxidative metabolism of the soy isoflavones daidzein and genistein *in vitro* and *in vivo*. *Journal of Agricultural and Food Chemistry*, Vol. 49, No. 6, (June 2001), pp. 3024-3033, ISSN 0021-8561.

Lampe, J.W., Karr, S.C., Hutchins, A.M. & Slavin, J.L. (1998). Urinary equol excretion with a soy challenge: influence of habitual diet. *Proceedings of the Society of Experimental Biology and Medicine*, Vol. 217, No. 3, (March 1998), pp. 335–339, ISSN 0037-9727.

Lampe, J.W., Gustafson, D.R., Hutchins, A.M., Martín, M.C., Li, S., Wähälä, K., Grandits, G.A., Potter, J.D. & Slavin, J.L. (1999). Urinary isoflavonoid and lignan excretion on a Western diet: Relation to soy, vegetable, and fruit intake. *Cancer Epidemiology, Biomarkers and Prevention*, Vol. 8, No. 8, (August 1999), pp. 699-707, ISSN 1055-9965.

Lanzendorfer, G., Stab, F. & Untiedt, S. (1999). Agents acting against hyperactive and hypoactive, deficient skin conditions and manifest dermatitides. US patent 5,952,373. September 14.

Larkin, T.A., Price, W.E. & Astheimer, L.B. (2007). Increased probiotic yogurt or resistant starch intake does not affect isoflavone bioavailability in subjects consuming a high soy diet. *Nutrition*, Vol. 23, No. 10, (October 2007), pp. 709–718, ISSN 0899-9007.

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.

Liu, K-S. (2004). Edible soybean products in the current market. Liu, K-S. (ed.), *Soybeans as Functional Foods and Ingredients.* AOCS Press, pp. 23-51, ISBN 1-893997-33-2, Champaign, Illinois, U.S.A.

Lu, L.J. & Anderson, K. E. (1998). Sex and long-term soy diets affect the metabolism and excretion of soy isoflavones in humans. *American Journal of Clinical Nutrition*, Vol. 68, No. 6, (December 1998), pp. 1500S-1504S, ISSN 0002-9165.

Lydeking-Olsen, E., Beck-Jensen, J.E., Setchell, K.D.R. & Holm-Jensen, T. (2004). Soymilk or progesterone for prevention of bone loss-a 2 year randomized, placebo-controlled trial. *European Journal of Nutrition*, Vol. 43, No. 4, (August 2004), pp. 246-257, ISSN 1436-6207.

Ma, Y., Sullivan, J.C. & Schreihofer, D.A. (2010). Dietary genistein and equol (4', 7 isoflavandiol) reduce oxidative stress and protect rats against focal cerebral ischemia. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology*, Vol. 299, No. 3, (September 2010), pp. R871-R877, ISSN 0363-6119.

MacDonald, R.S., Guo, J-Y., Copeland, J., Browning, J.D., Sleper, D., Rottinghaus, G.E. & Berhow, M.A. (2005). Environmental influences on isoflavones and saponins in soybeans and their role in colon cancer. *Journal of Nutrition, Vol.* 135, No. 5, (May 2005), pp. 1239–1242, ISSN 0022-3166.

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 1, 2005), pp. 1755-1757, ISSN 0892-6638.

Manach, C., Scalbert, A., Morand, C., Rémésy, C. & Jiménez, L. (2004). Polyphenols: food sources and bioavailability. *American Journal of Clinical Nutrition*, Vol. 79, No. 5, (May 2004), pp. 727–747, ISSN 0002-9165.

Mann, G.E., Rowlands, D.J., Li, F.Y., de Winter, P. & Siow, R.C. (2007). Activation of endothelial nitric oxide synthase by dietary isoflavones: role of NO in Nrf2-mediated antioxidant gene expression. *Cardiovascular Research*, Vol. 75, No. 2, (July 15, 2007), pp. 261-274, ISSN 0008-6363.

Marini, H., Minutoli, L., Polito, F., Bitto, A., Altavilla, D., Atteritano, M., Gaudio, A., Mazzaferro, S., Frisina, A., Frisina, N., Lubrano, C., Bonaiuto, M., D'Anna, R., Cannata, M.L., Corrado, F., Adamo, E.B., Wilson, S. & Squadrito, F. (2007). Effects of the phytoestrogens genistein on bone metabolism in osteopenic postmenopausal women: a randomized trial. *Annals of Internal Medicine,* Vol. 146, No. 12, (June 2007), pp. 839-847, ISSN 0003-4819.

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.

McClain, R.M., Wolzm, E., Davidovich, A., Pfannkuch, F., Edwards, J.A. & Bausch, J. (2006). Acute, subchronic and chronic safety studies with genistein in rats. *Food and Chemical Toxicology,* Vol. 44, No. 1, (January 2006), pp. 56-80, ISSN 0278-6915.

Mei, J., Yeung, S.S. & Kung, A.W. (2001) High dietary phytoestrogen intake is associated with higher bone mineral density in postmenopausal but not premenopausal women. *Journal of Clinical Endocrinology and Metabolism*, Vol. 86, No. 11, (November 2001), pp.5217–5221, ISSN 0021-972X.

Messina, M. & Wu, A.H. (2009). Perspectives on the soy–breast cancer relation. *American Journal of Clinical Nutrition*, Vol. 89, No. 5, (May 2009), pp. 1673S-1679S, ISSN 0002-9165.

Messina, M. (2010a). A brief historical overview of the past two decades of soy and isoflavone research. *Journal of Nutrition*, Vol. 140, No. 7, (July 2010), pp. 1350S–1354S, ISSN 0022-3166.

Messina, M. (2010b). Insights gained from 20 years of soy research. *American Journal of Clinical Nutrition*, Vol. 140, No. 12, (December 2010), pp. 2289S-2295S, ISSN 0002-9165.

Moon, Y.J., Wang, X. & Morris, M.E. (2006). Dietary flavonoids: Effects on xenobiotic and carcinogen metabolism. *Toxicology in Vitro*, Vol. 20, No. 2, (March 2006), pp. 187–210, ISSN 0887-2333.

Munoz, Y., Garrido, A. & Valladares, L. (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, (March 2009), pp. 740-744, ISSN 0049-3848.

Murota, K., Shimizu, S., Miyamoto, S., Izumi, T., Obata, A., Kikuchi, M., & Terao, J. (2002). Unique uptake and transport of isoflavone aglycones by human intestinal Caco-2 cells: Comparison of isoflavones and flavonoids. *Journal of Nutrition*, Vol. 132, No. 7, (July 2002), pp. 1956–1961, ISSN 0022-3166.

Naaz, A., Yellayi, S., Zakroczymski, M.A., Bunick, D., Doerge, D.R., Lubahn, D.B., Helferich, W.G. & Cooke, P.S. (2003). The soy isoflavone genistein decreases adipose deposition in mice. *Endocrinology*, Vol. 144, No. 8, (August 2003), pp. 3315-3320, ISSN 0013-7227.

Naciff, J.M., Hess, K.A., Overmann, G.J., Torontali, S.M., Carr, G.J., Tiesman, J.P., Foertsch, L.M., Richardson, B.D., Martinez, J.E. & Daston, G.P. (2005). Gene expression changes induced in the testis by transplacental exposure to high and low doses of 17a-ethynyl estradiol, genistein, or bisphenol A. *Toxicological Sciences*, Vol. 86, No. 2, (August 2005), pp. 396–416, ISSN 1096-6080.

Nagata, C., Iwasa, S., Shiraki, M., Ueno, T., Uchiyama, S., Urata, K., Sahashi, Y. & Shimizu, H. (2006). Associations among maternal soy intake, isoflavone levels in urine and blood samples, and maternal and umbilical hormone concentrations (Japan). *Cancer Causes and Control*, Vol. 17, No. 9, (November 2006), pp. 1107–1113, ISSN 0957-5243.

Nakashima, S., Koike, T. & Nozawa, Y. (1991). Genistein, a protein tyrosine kinase inhibitor, inhibits thromboxane A2-mediated human platelet responses. *Molecular Pharmacology*, Vol. 39, No. 4, (April 1991), pp. 475-480, ISSN 0026-895X.

Nestel, P.J., Yamashita, T., Sasahara, T., Pomeroy, S., Dart, A., Komesaroff, P., Owen, A. & Abbey, M. (1997). Soy isoflavones improve systemic arterial compliance but not plasma lipids in menopausal and perimenopausal women. *Arteriosclerosis, Thrombosis, and Vascular Biology*, Vol. 17, No. 12, (December 1997), pp. 3392-3398, ISSN 10795642.

Nielsen, I.L.F. & Williamson, G. (2007). Review of the factors affecting bioavailability of soy isoflavones in humans. *Nutrition and Cancer*, Vol. 57, No. 1, pp. 1-10, ISSN 0163-5581.

North American Menopause Society (2000). The role of isoflavones in menopausal health: Consensus Opinion of the North American Menopause Society. *Menopause,* Vol. 7, No. 4, (July-August 2000), pp. 215–229, ISSN 1072-3714.

North American Menopause Society (2006). Management of osteoporosis in postmenopausal women: 2006 position statement of the North American Menopause Society. *Menopause*, Vol.13, No. 3, (June 2006), pp. 340-367, ISSN 1072-3714.

North American Menopause Society (2010). Management of osteoporosis in postmenopausal women: 2010 position statement of The North American Menopause Society. *Menopause*, Vol. 17, No. 1, (January 2010), pp. 25-54, ISSN 1072-3714.

Nurmi, T., Mazur, W., Heionen, S., Kokkonen, J. & Adlercreutz, H. (2002). Isoflavone content of the soy based supplements. *Journal of Parrmaceutical and Biomedical Analysis*, Vol. 28, No. 1, (April 2002), pp. 1-11, ISSN 0731-7085.

Ohta, A., Uehara, M., Sakai, K., Takasaki, M., Adlercreutz, H., Morohashi, T. & Ishimi, Y. (2002). A combination of dietary fructooligosaccharides and isoflavone conjugates increases femoral bone mineral density and equol production in ovariectomized mice. *Journal of Nutrition*, Vol. 132, No. 7, (July 2002), pp. 2048-2054, ISSN 0022-3166.

Ørgaard 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 0022-3166.

Park, S.A., Choi, M-S., Cho, S-Y., Seo, J-S., Jung, U.J., Kim, M-J., Sung, M-K., Park, Y.B. & Lee, M-K. (2006). Genistein and daidzein modulate hepatic glucose and lipid regulating enzyme activities in C57BL/KsJ-*db/db* mice. *Life Sciences*, Vol. 79, No. 12, (August 15, 2006), pp. 1207-1233, ISSN 0024-3205.

Park, C.E., Yun, H., Lee, E-B., Min, B-I., Bae, H., Choe, W., Kang, I., Kim, S-S. & Ha, J. (2010). The antioxidant effects of genistein are associated with AMP-activated protein kinase activation and PTEN induction in prostate cancer cells. *Journal of Medicinal Food* , Vol. 13, No. 4, (August 2010), pp. 815–820, ISSN 1096-620X.

Petrakis, N. L., Barnes, S., King, E. B., Lowenstein, J., Wiencke, J., Lee, M. N., Miike, R., Kirk, M. & Coward, L. (1996). Stimulatory influence of soy protein isolate on breast fluid secretion in pre- and postmenopausal women. *Cancer Epidemiology, Biomarkers, and Prevention*, Vol. 5, No., (October 1996), pp. 785–794, ISSN 1055-9965.

Potter, S.M., Baum, J.A., Teng, H., Stillman, R.J., Shay, N.F. & Erdman, J.W. Jr. (1998). Soy protein and isoflavones: Their effect on blood lipids and bone density in postmenopausal women. *American Journal of Clinical Nutrition*, Vol. 68, No. 6 (suppl December 1998), pp.1375S–1379S, ISSN 0002-9165.

Potter, S.M., Henley, E.C. & Waggle, D.H. (1999). Method for decreasing LDL-cholesterol concentration and increasing HDL-cholesterol concentration in the blood to reduce the risk of atherosclerosis and vascular disease. US patent 5,855,892. January 5.

Prasain, J.K., Xu, J., Kirk, M., Smith Johnson, M., Sfakianos, J. & Barnes, S. (2006). Differential biliary excretion of genistein metabolites following intraduodenal and intravenous infusion of genistin in female rats. *Journal of Nutrition*, Vol. 136, No. 12, (December 2006), pp. 2975–2979, ISSN 0022-3166.

Raju, J., Bielecki, A., Caldwell, D., Lok, E., Taylor, M., Kapal, K., Curran, I., Cooke, G.M., Bird, R.P. & Mehta, R. (2009). Soy isoflavones modulate azoxymethane-induced rat colon carcinogenesis exposed pre- and postnatally and inhibit growth of DLD-1 human colon adenocarcinoma cells by increasing the expression of estrogen receptor-beta. *Journal of Nutrition, Vol.* 139, No. 3, (March 2009), pp. 474–481, ISSN 0022-3166.

Rassi, C.M., Lieberherr, M., Chaumaz, G., Pointillart, A. & Cournot, G. (2002). Down-regulation of osteoclast differentiation by daidzein via caspase 3. *Journal of Bone and Mineral Research*, Vol. 17, No. 4, (April 2002), pp. 630 -638, ISSN 0884-0431.

Reinwald, S., Akabas, S.R., & Weaver, C.M. (2010). Whole versus the piecemeal approach to evaluating soy. *Journal of Nutrition*, Vol. 140, No. 12, (December 2010), pp. 2335S–2343S, ISSN 0022-3166.

Richelle. M., Pridmore-Merten, S., Bodenstab, S., Enslen, M. & Offord, E.A. (2002). Hydrolysis of isoflavone glycosides to aglycones by β-glycosidase does not alter plasma and urine isoflavone pharmacokinetics in postmenopausal women. *Journal of Nutrition,* Vol. 132, No. 9, (September 2002), pp. 2587–2592, ISSN 0022-3166.

Rozman, K.K., Bhatia, J., Calafat, A.M., Chambers, C., Culty, M., Etzel, R.A., Marty, S., Hansen, D.K., Flaws, J.A., Chambers, C., Thomas, J.A., Hoyer, P.B. & Umbach, D. (2006). NTP-CERHR expert panel report on the reproductive and developmental toxicity of genistein. *Birth Defects Research* (Part B), Vol. 77, No. 6, (December 2006), pp. 485-638, ISSN 1542-9741.

Rüfer, C.E. & Kulling, E. (2006). Antioxidant activity of isoflavones and their major metabolites using different *in vitro* assays. *Journal of Agricultural and Food Chemistry*, Vol. 54, No. 8, (March 2006), pp. 2926-2931, ISSN 0021-8561.

Ruiz-Larrea, M.B., Mohan, A.R., Paganga, G., Miller N.J., Bolwell, G.P. & Rice-Evans, C.A. (1997). Antioxidant activity of phytoestrogenic isoflavones. *Free Radical Research,* Vol. 26, No. 1, (January 1997), pp. 63-70, ISSN 1071-5762.

Russo, J. & Russo, I.H. (2006). The role of estrogen in the initiation of breast cancer. *Journal of Steroid Biochemistry and Molecular Biology*, Vol. 102, No. 1-5, (December 2006), pp. 89–96, ISSN 0960-0760.

Sacks, F.M., Svetkey, L.P., Vollmer, W.M., Appel, L.J., Bray, G.A., Harsha, D., Obarzanek, E., Conlin, P.R., Miller 3rd, E.R., Simons-Morton, D.G., Karanja, N. & Lin, P.H. (2001). DASH-Sodium Collaborative Research Group. Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. DASH Sodium Collaborative Research Group. *New England Journal of Medicine,* Vol. 344, No. 1, (January 2001), pp. 3–10, ISSN 0028-4793.

Sargeant, P., Farndale, R.W. & Sage, S.O. (1993). ADP-and Thapsigargin-evoked Ca2+ entry and protein-tyrosine phosphorylation are inhibited by the tyrosine kinase inhibitors genistein and methyl-2,5-dihydroxycinnamate in fura-2-loaded human platelets. *Journal of Biological Chemistry*, Vol. 268, No. 24, (August 1993), pp. 18151-18156, ISSN 0021-9258.

Sarkar, F.H. & Li, Y. (2002). Mechanisms of cancer chemoprevention by soy isoflavone genistein. *Cancer and Metastasis Reviews,* Vol. 21, No. 3-4, (December 2002), pp. 265–280, ISSN 0167-7659.

Scalbert, A. & Williamson, G. (2000). Dietary intake and bioavailability of polyphenols. *Journal of Nutrition, Vol.* 130, No. 8, (August 2000), pp. 2073S–2085S, ISSN 0022-3166.

Scheiber, M.D., Liu, J.H., Subbiah, M.T., Rebar, R.W. & Setchell, K.D.R. (2001). Dietary inclusion of whole soy foods results in significant reductions in clinical risk factors for osteoporosis and cardiovascular disease in normal postmenopausal women. *Menopause*, Vol. 8, No.5, (September-October 2001), pp.384–392, ISSN 1072-3714.

Segelman, A.B. (2000). Use of isoflavones to prevent hair loss and preserve the integrity of existing hair. US patent 6,017,893. January 25.

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, ISSN 0022-3166.

Setchell, K.D.R., Brown, N.M., Desai, P., Zimmer-Nechemias, L., Wolfe, B.E., Brashear, W.T., Kirschner, A.S., Cassidy, A. & Heubi, J.E. (2001). Bioavailability of pure isoflavones in healthy humans and analysis of commercial soy isoflavone supplements. *Journal of Nutrition,* Vol. 131, No. 4, (April 2001), pp. 1362S–1375S, ISSN 0022-3166.

Setchell, K.D.R., Brown, N.M., Zimmer-Nechemias, L., Brashear, W.T., Wolfe, B.E., Kirschner, A.S. & Heubi, J.E. (2002a). Evidence for lack of absorption of soy isoflavone glycosides in humans, supporting the crucial role of intestinal metabolism for bioavailability. *American Journal of Clinical Nutrition*, Vol. 76, No. 2, (August 2002), pp. 447–453, ISSN 0002-9165.

Setchell, K.D.R., Brown, N.M. & Lydeking-Olsen, E. (2002b). The clinical importance of the metabolite equol-a clue to the effectiveness of soy and its isoflavones. *Journal of Nutrition*, Vol.132, No. 12, (December 2002), pp. 3577-3584, ISSN 0022-3166.

Setchell, K.D.R., Faughnan, M. S., Avades, T., Zimmer-Nechemias, L., Brown, N. B., Wolfe, B., Brashear, W. T., Desai, P., Oldfield, M. F., Botting, N. P. & Cassidy, A. (2003a). Comparing the pharmacokinetics of daidzein and genistein using [13C]labeled tracers in premenopausal women. *American Journal of Clinical Nutrition*, Vol. 77, No.2, (February 2003), pp.411-419, ISSN 0002-9165.

Setchell, K.D.R., Brown, N.M., Desai, P.B., Zimmer-Nechimias, L., Wolfe, B., Jakate, A.S., Creutzinger, V. & Heubi, J.E. (2003b). Bioavailability, disposition, and dose-response effects of soy isoflavones when consumed by healthy women at physiologically typical dietary intakes. *Journal of Nutrition*, Vol. 133, No. 4, (April 2003), pp. 1027-1035, ISSN 0022-3166.

Setchell, K.D.R. & Lydeking-Olsen, E. (2003). Dietary phytoestrogens and their impact on bone-evidence from in vitro and in vivo, human observational and dietary intervention studies. *American Journal of Clinical Nutrition*, Vol. 78, No. (suppl September 2003), pp. 593S–609S, ISSN 0002-9165.

Sfakianos, J., Coward, L., Kirk, M. & Barnes, S. (1997). Intestinal uptake and biliary excretion of the isoflavone genistein in rats. *Journal of Nutrition,* Vol. 127, No. 7, (July 1997), pp. 1260–1268, ISSN 0022-3166.

Shih, H., Pickwell, G.V. & Quattrochi, L.C. (2000). Differential effects of flavonoid compounds on tumor promoter-induced activation of the human CYP1A2 enhancer. *Archives of Biochemistry and Biophysics,* Vol. 373, No. 1, (January 2000), pp. 287–294, ISSN 0003-9861.

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 0022-3166.

Singhal, R., Shankar, K., Badger, T.M. & Ronis, M.J. (2009). Hepatic gene expression following consumption of soy protein isolate in female Sprague-Dawley rats differs from that produced by 17b-estradiol treatment. *Journal of Endocrinology*, Vol. 202, No. 1, (January 2009), pp. 141–152, ISSN 0022-0795.

Slavin, J.L., Karr, S.C., Hutchins, A.M. & Lampe, J.W. (1998). Influence of soybean processing, habitual diet, and soy dose on urinary isoflavonoid excretion. *American Journal of Clinical Nutrition*, Vol. 68, No. 6 suppl, (December 1998), pp. 1492S–1495S, ISSN 0002-9165.

Squadrito, F., Altavilla, E., 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. *American Journal of Medicine*, Vol. 114, No. 6, (April 2003), pp. 470-476, ISSN 0002-9343.

Somekawa, Y., Chiguchi, M., Ishibashi, T. & Aso, T. (2001). Soy intake related to menopausal symptoms, serum lipids, and bone mineral density in postmenopausal Japanese women. *Obstetrics and Gynecology*, Vol. 97, No. 1, (January 1997), pp. 109–115, ISSN 0029-7844.

Song, W.O., Chun, O.K., Hwang, I., Shin, H.S., Kim, B-G., Kim, K.S., Lee, S-L., Shin, D. & Lee, S.G. (2007). Soy isoflavones as safe functional ingredients. *Journal of Medicinal Food*, Vol. 10, No. 4, (December 2007), pp. 571-580, ISSN 1096-620X

Spector, D., Anthony, M., Alexander, D. & Arab, L. (2003). Soy consumption and colorectal cancer. *Nutrition and Cancer,* Vol. 47, No. 1, (January 2003), pp. 1–12, ISSN 0163-5581.

Stevenson, D.E. & Hurst, R.D. (2007). Polyphenolic phytochemicals – just antioxidants or much more? *Cellular and Molecular Life Sciences,* Vol. 64, No. 22, (November 2007), pp. 2900-2916, ISSN 1420-682X.

Stürtz, M., Lander, V., Schmid, W., & Winterhalter, P. (2008). Quantitative determination of isoflavones in soy based nutritional supplements by high performance liquid chromatography. *Journal für Verbraucherschutz und Lebensmittelsicherheit*, Vol. 3, No. 2, (August 2008), pp. 127–136, ISSN 1661-5751.

Sung, H.Y. & Choi, Y.S. (2008). Dose-response assessment of the anti-cancer efficacy of soy isoflavones in dimethylhydrazine-treated rats fed 6% fructooligosaccharide. *Nutrition Research and Practice*, Vol. 2, No. 2, (Summer issue), pp. 55-61, ISSN 1976-1457.

Tamura, A., Shiomi, T., Hachiya, S., Shigematsu, N. & Hara, H., (2008). Low activities of intestinal lactase suppress the early phase absorption of soy isoflavones in Japanese adults. *Clinical Nutrition*, Vol. 27, No. 2, (April 2008), pp. 248–253, ISSN 0261-5614.

Taylor, C.K., Levy, R.M., Elliott, J.C. & Burnett, B.P. (2009). The effect of genistein aglycone on cancer and cancer risk: a review of in vitro, preclinical, and clinical studies. *Nutrition Reviews*, Vol. 67, No. 7, (July 2009), pp. 398-415, ISSN 0029-6643.

Teede, H.J., Dalais, F.S., Kotsopoulos, D., Davis, S.R., Liang, Y.L. & McGrath, B.P. (2001). Soy protein dietary supplementation containing phytoestrogens improves lipid profiles and blood pressure: a double blind, randomised, placebo controlled study in men and postmenopausal women. *Journal of Clinical Endocrinology and Metabolism,* Vol. 86, No. 7, (July 2001), pp. 3053–3060, ISSN 0021-972X.

Teede, H.J., Giannopoulos, D., Dalais, F.S., Hodgson, J. & McGrath, B.P. (2006). Randomized, controlled, cross-over trial of soy protein with isoflavones on blood pressure and arterial function in hypertensive subjects. *Journal of the American College of Nutrition*, Vol. 25, No. 6, (June 2006), pp. 533-540, ISSN 0731-5724.

Thurn, M.J. & Juang, L.J. (1999). Methods for treating cancer with legume plant extracts. US patent 6,004,558. December 21.

Tissier, R., Waintraub, X., Couvreur, N., Gervais, M., Bruneval P., Mandet C., Zini, R., Enriquez, B., Berdeaux, A. & Ghaleh, B. (2007). Pharmacological postconditioning with the phytoestrogen genistein. *Journal of Molecular and Cellular Cardiology,* Vol. 42, No. 1, (January 2007), pp. 79-87, ISSN 0022-2828.

Tsunoda, N., Pomeroy, S. & Nestel, P. (2002). Absorption in humans of isoflavones from soy and red clover is similar. *Journal of Nutrition,* Vol. 132, No. 8, (August 2002), pp. 2199–2201, ISSN 0022-3166.

Turner, N.J., Thomson, B.M. & Shaw, I.C. (2003). Bioactive isoflavones in functional foods: the importance of gut microflora on bioavailability. *Nutrition Reviews*, Vol. 61, No. 6, (June 2003), pp. 204-213, ISSN 0029-6643.

Uehara, M., Ohta, A., Sakai, K., Suzuki, K., Watanabe, S. & Adlercreutz, H. (2001). Dietary fructooligosaccharides modify intestinal bioavailability of a single dose of genistein and daidzein and affect their urinary excretion and kinetics in blood of rats. *Journal of Nutrition,*Vol. 131, No.3, (March 2001), pp. 787-795, ISSN 0022-3166.

United States Department of Agriculture. (1999). *Database of isoflavone content of foods*. Available from:
http://www.nal.usda.gov/fnic/foodcomp/Data/isoflav/isfl_tbl.pdf.

van Ee, J.H. (2009). Soy constituents: modes of action in low-density lipoprotein management. *Nutrition Reviews,* Vol. 67, No. 4, (April 2009), pp. 222–234, ISSN 0029-6643.

Vafeiadou, K., Hall, W.L. & Williams, C.M. (2006). Does genotype and equol-production status affect response to isoflavones? Data from a pan-European study on the effects of isoflavones on cardiovascular risk markers in post-menopausal women. *Proceedings of the Nutrition Society*, Vol. 65, No. 1, (February 2006), pp. 106-115, ISSN 0029-6651.

Wang, H.J. & Murphy, P.A. (1994a). Isoflavone composition of American and Japanese soybeans in Iowa: effect of variety, crop year and location. *Journal of Agricultural and Food Chemistry*, Vol. 42, No. 8, (August 1994), pp. 1674-1677, ISSN 0021-8561.

Wang, H.J. & Murphy, P.A. (1994b). Isoflavone content in commercial soybean foods. *Journal of Agricultural and Food Chemistry*, Vol. 42, No. 8, (August 1994), pp. 1666 –1673, ISSN 0021-8561.

Warri, A., Saarinen, N.M., Makela, S. & Hilakivi-Clarke, L. (2008). The role of early life genistein exposures in modifying breast cancer risk. *British Journal of Cancer*, Vol. 98, No. 9, (May 2008), pp. 1485–1493, ISSN 0007-0920.

Wiseman, H., O'Reilly, J. D., Adlercreutz, H., Mallet, A. I., Bowey, E. A., Rowland, I. R. & Sanders, T. A. (2000). Isoflavone phytoestrogens consumed in soy decrease F(2)-isoprostane concentrations and increase resistance of low density lipoprotein to oxidation in humans. *American Journal of Clinical Nutrition*, Vol. 72, No. 2, (August 2000), pp.395-400, ISSN 0002-9165.

Wiseman, H., Casey, K., Bowey, E.A., Duffy, R., Davies, M., Rowland, I.R., Lloyd, A.S., Murray, A., Thompson, R. & Clarke, D.B. (2004). Influence of 10 wk of soy consumption on plasma concentrations and excretion of isoflavonoids and on gut microflora metabolism in healthy adults. *American Journal of Clinical Nutrition*, Vol. 80, No. 3, (September 2004), pp. 692–699, ISSN 0002-9165.

Yamamoto, S., Sobue, T., Sasaki, S., Kobayashi, M., Arai, Y., Uehara, M., Adlercreutz, H., Watanabe, S., Takahashi, T., Iitoi, Y. Iwase, Y., Akabane, M. & Tsugane, S. (2001). Validity and reproducibility of a self-administered food-frequency questionnaire to assess isoflavone intake in a Japanese population in comparison with dietary records and blood and urine isoflavones. *Journal of Nutrition*, Vol. 131, No. 10, (October 2001), pp. 2741–2747, ISSN 0022-3166.

Yang, G., Shu, X-O., Li, H., Chow, W-H., Cai, H., Zhang, X., Gao, Y-T. & Zheng, W. (2009). Prospective cohort study of soy food intake and colorectal cancer risk in women. *American Journal of Clinical Nutrition*, Vol. 89, No. 2, (February 2009), pp. 577–583, 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 0022-3166.

Xu, X., Wang, H.J., Murphy, P.A., Cook, L. & Hendrich, S. (1994). Daidzein is a more bioavailable soymilk isoflavone than is genistein in adult women. *Journal of Nutrition*, Vol. 124, No. 6, (June 1994), pp. 825-832, ISSN 0022-3166.

Xu, X., Harris, K.S., Wang, H.J., Murphy, P.A. & Hendrich, S. (1995). Bioavailability of soybean isoflavones depends upon gut microflora in women. *Journal of Nutrition*, Vol. 125, No. 1, (September 1995), pp. 2307-2315, ISSN 0022-3166.

Xu, X., Wang, H.J., Murphy, P.A. & Hendrich, S. (2000). Neither background diet nor type of soy food affects short-term isoflavone bioavailability in women. *Journal of Nutrition*, Vol. 130, No. 4, (April 2000), pp. 798-801, ISSN 0022-3166.

Yan, L., Spitznagel, E.L. & Bosland, M.C. (2010). Soy consumption and colorectal cancer risk in humans: A Meta-Analysis. *Cancer Epidemiology Biomarkers and Prevention*, Vol. 19, No. (January 2010), pp. 148-158, ISSN 1055-9965.

Yasuda, T., Mizunuma, S., Kano, Y., Saito, K. & Oshawa, K. (1996). Urinary and biliary metabolites of genistein in rats. *Biological and Pharmaceutical Bulletin*, Vol. 19, No. 3, (March 1996), pp. 413–417, ISSN 0918-6158.

Zhang, L., Zuo, Z. & Lin, G. (2007). Intestinal and hepatic glucuronidation of flavonoids. *Molecular Pharmaceutics*, Vol. 4, No. 6, (November 2007), pp. 833–845, ISSN 1543-8384.

Zheng, Y., Hu, J., Murphy, P.A., Alekel, D.L., Franke, W.D. & Hendrich, S. (2003). Rapid gut transit time and slow fecal isoflavone disappearance phenotype are associated with greater genistein bioavailability in women. *Journal of Nutrition*, Vol. 133, No. 10, (October 2003), pp. 3110–3116, ISSN 0022-3166.

Zhou, S., Hu, Y., Zhang, B., Teng, Z., Gan, H., Yang, Z., Wang, O., Huan, M. & Mei, O. (2008). Dose-dependent absorption, metabolism, and excretion of genistein in rats. *Journal of Agricultural and Food Chemistry*, Vol. 56, No. 18, (August 2008), pp. 8354–8359, ISSN 0021-8561.

Zubik, L. & Meydani, M. (2003). Bioavailability of soybean isoflavones from aglycone and glucoside forms in American women. *American Journal of Clinical Nutrition*, Vol. 77, No. 6, (June 2003), pp. 1459–1465, ISSN 0002-9165.
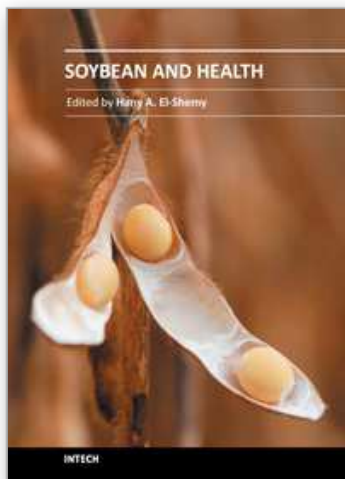
Soybean and Health

Edited by Prof. 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.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

INTECH
open science | open minds