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REVIEW PAPER

Content and Profile of Isoflavones in Soy-Based Foods as a Function of the Production Process

Ana Villares · Mauricio A. Rostagno · Ana García-Lafuente · Eva Guillamón · J. Alfredo Martínez

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Abstract Soy has been traditionally incorporated in diet as processed foods, such as soymilk, tofu, miso, tempeh, etc., and the consumption is commonly associated with a reduction of the development of chronic diseases due to their antioxidant, anti-inflammatory, and anti-allergic properties, among others. Many of the health benefits of soy have been attributed to isoflavones. They comprise a group of naturally occurring flavonoids consisting of heterocyclic phenols. Soy contains three types of isoflavones in four chemical forms: the aglycones daidzein, genistein, and glycitein; the β-glucosides daidzin, genistin, and glycitin; their 6″-*O*-malonyl-β-glucosides (6OMalGlc); and their 6″-*O*-acetyl-β-glucosides (6OAcGlc) conjugates. Industrial processing methods of soy-based food products commonly lead to the loss of isoflavones through removal of undesirable fractions. On the other hand, isoflavones can be transformed into different conjugates, which may have significant effects on the food texture and on the bioavailability and pharmacokinetics of the isoflavones. This article reviews the effect of a number of soybean processing treatments on the isoflavone content and profile. The preparation and manufacturing of different soy-based food and food ingredients, fermented and non-fermented, has been analyzed in terms of content and distribution of the three major isoflavone derivatives, daidzein, genistein, and glycitein, and their respective conjugates.

A. Villares (✉) · M. A. Rostagno · A. García-Lafuente · E. Guillamón · J. A. Martínez
Centro para la Calidad de los Alimentos, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Campus Universitario c/José Tudela s/n, 42071 Soria, Spain
e-mail: villares.ana@inia.es

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Introduction

Nowadays, there is a growing interest in phytochemicals because they may provide interesting properties within a healthy human diet. In addition to the basic functions of supplying nutrients, "functional foods" are designed to promote specific health aspects and to reduce the risk of developing chronic diseases such as type 2 diabetes, cardiovascular disease, or some cancers (Clavel et al. 2005; Charalampopoulos et al. 2002; Holzapfel and Schillinger 2002; Ranalli et al. 2007; Roberfroid 2000). In this field, legumes may play an important role in the daily human intake of bioactive phytocompounds because they are an important source of proteins, vitamins and minerals, saponins, phytosterols, complex carbohydrates, isoflavones, etc. (Adhami et al. 2008; Cantoral et al. 1995; Fernández Quintela et al. 1997; Perez-Balibrea et al. 2008; Rochfort and Panozzo 2007; Yasmin et al. 2008). Indeed, isoflavonoids, an important class of flavonoids, are mainly produced by the subfamily *Papilionoideae* of the *Leguminosae* plants, and they have been found in soya grains, lupine, fava beans, chickpeas, and pulses (Aguiar et al. 2007; Eldridge 1982; Eldridge and Kwolek 1983; Ferrer and Barcelo 1994; Naim et al. 1974; Rochfort and Panozzo 2007; Wang and Murphy 1994a).

There are three main types of isoflavones in soy (*Glycine max*), daidzein, genistein, and glycitein (aglycone forms; Mebrahtu et al. 2004), and each type exists in four different chemical forms, including the aglycones and their glucoside conjugates: the β-glucosides, the 6″-*O*-acetyl-β-glucosides (6*O*AcGlc) and the 6″-*O*-malonyl-

β-glucosides (6*O*MalGlc), shown in Fig. 1 (Barnes et al. 1994; Jackson et al. 2002; Wang and Murphy 1994b).

Isoflavones have attracted a great deal of attention due to their properties as an antioxidant ingredient (Chung et al. 2008; Kao and Chen 2006; Richardson et al. 1947; Yue et al. 2008), but they also have anti-inflammatory (Garcia-Lafuente et al. 2009; Kao et al. 2007b; Kupeli et al. 2006; Park et al. 2007) and anti-allergic (Chang et al. 2000) functions. These compounds have shown to reduce the risk of cardiovascular disease (Jackman et al. 2007) and promote the inhibition of cancer cell growth (Davis et al. 2008; Kao et al. 2007a; Kohen et al. 2007; Sarkar and Li 2003). Furthermore, isoflavones play an important role in the prevention of several diseases, including osteoporosis, and menopausal symptoms (Coxam 2008; Dijsselbloem et al. 2004; Ma et al. 2008; Phrakonkham et al. 2007) since they act as anti-estrogens (Okamoto et al. 2006; Zhang et al. 2007a) and tyrosine protein kinase inhibitors (Papazisis et al. 2006). The effects of these flavonoid substances are strongly influenced by the chemical structure of the naturally occurring compound, for example, hydroxyl substitution of the B- and C-rings, but not the A-ring is essential for antioxidant activity (see Fig. 1; Lotito and Frei 2006).

The activity of isoflavonoids in the human body may be strongly influenced by absorption and bioavailability. Thus, relative disposition of isoflavones appears to be determined by their chemical nature in terms of solubility and susceptibility to degradation by gut microorganisms. For example, the aglycones are absorbed more easily than the glucosides conjugates because the lower molecular weight improves diffusion (Xu et al. 2000). Therefore, the hydrolysis of isoflavone glucosides to their aglycones is needed for absorption. On the other hand, hydrophobicity generally provides longer retention times in the human body; thus, genistein is expected to be more retained since it is capable of forming a hydrophobic structure because the OH groups interact by hydrogen bonds to create a hydrophilic core (Birt et al. 2001).

Isoflavones can be extracted and isolated from soybeans or soy-based foods by means of different techniques, such as microwaves, ultrasounds, solid-phase, pressurized liquid, or supercritical extraction (Rostagno et al. 2009). The content and profile of isoflavones is generally determined by means of high-performance liquid chromatography (Prabhakaran et al. 2005). The concentration and distribution of each isoflavone derivative in soybeans depends on the soy origin (Franke et al. 1999; Genovese et al. 2006; Genovese and Lajolo 2002; Hutabarat et al. 2001), agricultural conditions, such as soy variety and crop (Wang and Murphy 1994a), and soy maturity (Lee et al. 2007; Wang et al. 2000). Generally, soybeans have significantly higher levels of daidzein and genistein and their corresponding glucosides (Fukutake et al. 1996; Rochfort and Panozzo 2007) and little content of the glycitein derivates (Wang and Murphy 1994a).

Soybeans have been traditionally used to improve bread properties (Gelencser et al. 2008; López-Guel et al. 2009; Rosales-Juarez et al. 2008; Sabanis and Tzia 2009); furthermore, they are usually incorporated into a human diet either as fermented or as non-fermented foods (Umphress et al. 2005; Wang and Murphy 1996). The most commonly fermented soy-based foods include sufu, miso, natto, soy sauce, tempeh, and douchi, while the non-fermented food comprise fresh soybeans, soybean sprouts, soymilk, tofu, and protein-enriched foods such as soy protein flours and/or grits, textured soy protein, and soy protein concentrates or isolates. The industrial methods of soybean processing commonly result in significant changes

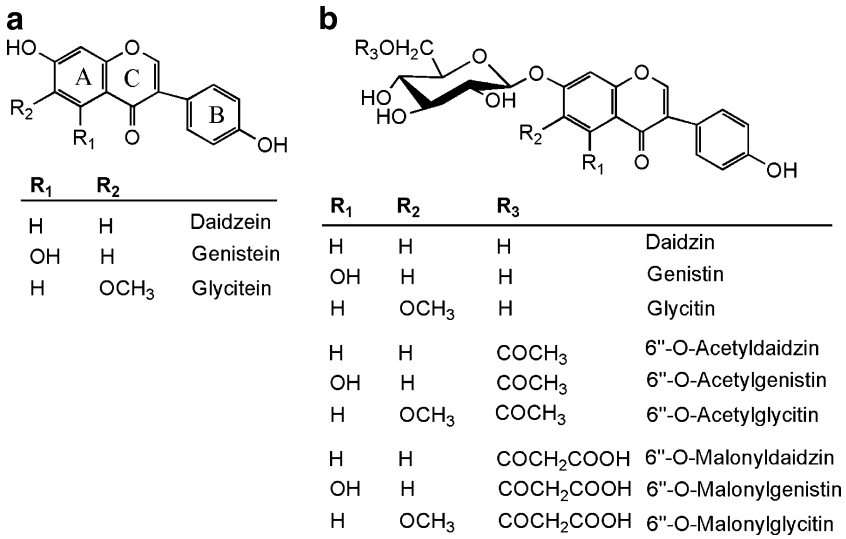


Fig. 1 Chemical structure of the main isoflavone conjugates: aglycones (**a**) and glycosides (**b**)

of the isoflavone content (Anderson and Wolf 1995; Golbitz 1995; Hui et al. 2001; Murphy et al. 1999; Umphress et al. 2005; Wiseman et al. 2002) in terms of glucoside conjugate concentrations, which may have significant effects on the bioavailability and pharmacokinetics of the isoflavones in human body (Allred et al. 2004, 2005; Jackson et al. 2002).

The purpose of this article was to identify and to discuss the effect of the different processing treatments employed to prepare the soy-derived foods in terms of the content and distribution of the three major isoflavone derivatives, daidzein, genistein, and glycitein, and their respective conjugates.

Soy-Based Food Products

Soymilk

Soymilk is a healthy food drink produced from raw beans, originated in Asia, being a common daily product in China and Japan and now widely extended and consumed around the world (Wang and Murphy 1996). Soymilk is made from soybeans which, after soaking in water for about 12 h at room temperature, are separated from the liquid phase, washed, and milled. The grinding of water-soaked beans results in the formation of a slurry, which is first cooked at temperature around 100 °C, then cooled at room temperature, and finally filtered to remove the soybean water-insoluble residue (okara) from the soy beverage (soymilk; Jackson et al. 2002; Wang and Murphy 1996).

Around 4–10% of the total isoflavones remain in water during the soaking process at room temperature. Regarding isoflavone distribution, at room temperature, both raw and soaked soybeans present comparable isoflavone profile to the raw soybeans (Jackson et al. 2002; Wang and Murphy 1996). However, Kao et al. (2004) observed that a prolonged soaking period of time (12 h) and a high temperature (45 °C) increased the aglycone concentration and decreased the glucoside content, the soaking temperature having a greater negative impact on the aglycone degradation than the soaking time. The higher levels of aglycones found when soaking at approximately 45 °C are probably due to the greater activity of the enzyme glucan endo-1,6-β-glucosidase (β-glucosidase; Pandjaitan et al. 2000), which produces the hydrolysis of the glucoside conjugates to form the corresponding aglycones (Araújo et al. 2007; Matsuura and Obata 1993; Xie et al. 2003).

The soaking process facilitates the dehulling of soybeans and reduces the energy required to mill beans in order to obtain the slurry. Depending on the treatment, the isoflavone composition and total content can be modified during grinding. At room temperature, the isoflavone profile is not significantly altered (Coward et al. 1998), while if the grinding temperature increases until 45 °C, β-glucosidase activity becomes maximum with the subsequent increase in the aglycone concentration (Prabhakaran and Perera 2006). When grinding is carried out in boiling water, the aglycone and the acetyl conjugates content increases due to the instability of the malonyl conjugates when exposed to heat (Jackson et al. 2002).

The cooking process results in variations of the isoflavone distribution: a decrease of the malonyl conjugates, mainly those of daidzin and genistin, and an increase of the three aglycones and the glucosides daidzin and genistin (Wang and Murphy 1996).

The filtration results in slight losses without changes in the isoflavone profile. Wang and Murphy (1996) reported an increase in the isoflavone recovery by pressing the okara. Regarding the profile, the soymilk contains a lower percentage of aglycones and 6″-*O*-acetyl-genistin than the okara, but due to the low solubility of aglycones in the aqueous media, the soymilk contains a higher concentration of glucosides and malonyl conjugates (Ishihara et al. 2007; Jackson et al. 2002).

Tofu

Tofu was initially consumed in ancient China and then introduced in Japan and East Asia (Nishio et al. 2007). There are different varieties of tofu depending on the moisture content: firm, soft, and silken tofu (Kao et al. 2003). Traditional tofu is produced by coagulating soymilk and pressing the resulting curds. Coagulation consists of protein precipitation by eliminating the electrical repulsion charges by means of a pH decrease (mild acid solutions). Thus, the coagulant creates bridges through which the soy proteins can aggregate by hydrophobic interactions (Obatolu 2008).

There are different coagulants employed in the preparation of tofu, such as inorganic salts, δ-gluconolactone, or organic acids. The nature and amount of coagulants may affect not only the quality of the final product but also the isoflavone yield. For example, the use of δ-gluconolactone (GDL) enables achieving the recovery of 67% of isoflavones with slight variations in the isoflavone profile regarding the original soymilk: only 5% of the malonyl glucosides are converted to glucosides due to the heating process (Jackson et al. 2002). Kao et al. (2004) compared the isoflavone recovery employing two inorganic coagulants, calcium sulfate and calcium chloride. Concerning the anion nature, $CaSO_4$ was found to be a better protein coagulant since the total isoflavone content in tofu increased with respect to the application of $CaCl_2$. DeMan et al. (1986) showed that the coagulation is slower with calcium sulfate than in calcium chloride, which explains the

higher isoflavone yield when employing $CaSO_4$. Although they are rarely used, calcium lactate and calcium acetate were studied as coagulants to prepare tofu (Prabhakaran et al. 2006b). Comparing both compounds, calcium lactate provided higher isoflavone retention, while calcium acetate produced more quantity of whey, and therefore, the resultant tofu was harder.

Prabhakaran et al. (2006b) also studied the influence of the coagulant cation. Besides the changes in the textural properties, hardness, and chewiness, they found higher isoflavone recoveries with calcium than with magnesium salts. This finding is probably due to the higher moisture in tofu derived from an incomplete precipitation of soy proteins with magnesium salts, which results in a less packed network encompassing many air gaps within it. Calcium chloride appears to be a stronger coagulant than magnesium sulfate since Ca^{2+} is an effective ion, which reacts with protein during soy milk coagulation (Liu and Chang 2004). For this reason, magnesium sulfate is commonly used after mixing with other coagulants such as magnesium chloride and calcium chloride (modified nigari; Prabhakaran et al. 2006b).

Organic acids can also be employed as a tofu coagulant. The acid treatment produces the protein aggregation by weakening the electric repulsive interactions among proteins when the hydration water is released. Prabhakaran et al. (2006b) demonstrated that the employment of mild acetic acid solutions yields similar isoflavone content in tofu than the use of calcium or magnesium chloride; however, tofu acquires a mild acidic taste and an increase in the redness and decrease in the yellowness.

Regarding coagulant quantity, Kao et al. (2004) found that the isoflavone recovery was higher for lower $CaSO_4$ concentrations (0.3%), which is consistent with the results of Prabhakaran et al. (2006b) for this coagulant.

Sufu

Sufu is a traditional and highly flavored fermented tofu. There are many types of sufu, although the most consumed is the red one. Sufu preparation consists of a former fermentation by inoculation of tofu with *Actinomucor elegans* and incubation for 48 h to produce pehtze (pizi). The later fermentation comprises the salting, until approximately 15% of salt is absorbed, and the maturation with a dressing mixture consisting of kojic red rice, an alcohol beverage, sugar, Chiang (wheat-based miso), and spices. After ripening for 2 months, red sufu is obtained (Li et al. 2004; Yin et al. 2005).

Fermentation is usually performed between 20°C and 35°C. In this context, (Han et al. 2003) reported that the optimal growth temperature for *A. elegans* is from 25°C and 30°C. Fermentation produces slight changes in isoflavone content, the major losses being produced during salting (8.1%). However, a redistribution of isoflavone isomers is observed: isoflavones are converted from the β-glucosides in tofu into the corresponding algycones under hydrolysis by the enzyme β-glucosidase. Yin et al. (2005) studied the influence of fermentation temperature on the isoflavone profile during sufu preparation. They studied two temperatures, 26°C and 32°C, and found lower contents of β-glucosides (93%) and acetyl derivatives (69%) when fermentation is carried out at 26°C. Furthermore, the malonyl derivatives are only detected when fermentation is performed at 32°C (Yin et al. 2005). The same authors found that the increase of aglycone content is produced in a greater extent during the former fermentation, the conversion being from the β-glucosides to the corresponding aglycones affected by the fermentation time. During the latter fermentation, much slower similar changes are observed (Yin et al. 2004). Finally, the ripening process does not produce significant losses of isoflavones and the profile is maintained. The final sufu contains high levels of aglycones (99.7%) and low concentrations of the β-glucosides (0.03%).

Protein-Enriched Soy-Based Foods and Food Ingredients

Since soybeans are a rich source of proteins; they are used as raw material to produce a number of soy-based products characterized by high-protein title: textured soy protein, soy protein concentrates (SPC), and soy protein isolates (SPI; Umphress et al. 2005).

Textured soy protein is mainly employed by food industry in products that resemble meat. It is granular, but after rehydrating, the texture and taste are similar to those of beef. Textured soy protein is obtained by pressing the soy flour (Mahungu et al. 1999). The extrusion process has a large influence on the isoflavone distribution, increasing the amount of acetylglucosides (Genovese et al. 2007), although the total isoflavone content does not change significantly. Generally, aglycone content is not significantly high in the extruded product since β-glucosidase is inactivated by the high temperatures of the extrusion process (100–150°C). Furthermore, heat, together with a limited aqueous environment during the extrusion process, produces a loss of the malonyl conjugates and an increase of the acetyl derivates.

SPC are usually composed of around 65% of protein and sometimes are added to wheat flour in order to increase the protein content (Sung et al. 2006). The most highly refined product is SPI, with protein content above 90%, which has many applications in the food industry, such as a constituent of health drinks and as an extender in many meat and dairy products. The processes for producing SPC and SPI include finely grinding and defatting of soybeans. Then, the flour is

extracted in alkaline conditions to produce the desired extract, which is then separated and acidified to precipitate proteins. Finally, they are washed and freeze-dried to obtain the isoelectric protein (Wang and Murphy 1996).

The soy flour defatting process is usually carried out with hexane. Isoflavones are not highly soluble in this solvent; therefore, content and distribution are not expected to change considerably (Coward et al. 1998; Eldridge and Kwolek 1983). Other solvents, such as petroleum ether or the chloroform/ethanol mixture, have been employed; however, isoflavone content is not reported (Khetarpaul et al. 2004). Barbosa et al. (2006) studied how the extraction conditions (pH and temperature) influenced the isoflavone content and distribution (Barbosa et al. 2006). Regarding the extraction temperature, they did not find significant changes in the isoflavone content; however, the profile varies. The percentage of aglycones from the total isoflavone amount increases with temperature from approximately 3% at 4 °C to 6% at 25 °C, and at 50 °C, they reach 20%. β-glucosides decreased proportionally with temperature. Heat produces a deactivation of the enzyme β-glucosidase, and in consequence, the aglycone content does not increase in such an extent. Between 4 °C and 50 °C, acetyl and malonyl-β-glucosides concentration did not change considerably (<2%).

The higher extraction of isoflavones from defatted soy flour is performed at basic pH. The pH influences malonyl conjugates content; thus, at pH 8, they comprise 50% of the total isoflavones, while at pH 10, they decrease to 36%. β-glucosides show the contrary tendency: they increase from 43% to 57% at high pH. Regarding acetyl derivatives, there is little change in content (<0.5%), and the aglycone concentration increases slightly from approximately 5% to 7% of total recovered isoflavones.

The following step in soy protein isolation is the precipitation of proteins. Barbosa et al. tested the pH values 4, 4.5, and 5 for isoflavone and protein yield. As the pH increases, the isoflavone recovery increases from 62% to 72%; however, the protein yield decreases. Therefore, from an industrial point of view, the precipitation is performed at pH 4.5 (isoflavone recovery of 67%), which results in the highest yield of proteins.

Finally, in order to remove undesirable compounds, the protein precipitate is washed with water or acidified solutions. As isoflavones are soluble in water, this step produces a loss of them (22%) within the washing water (Wang et al. 1998). In contrast, the application of acidified solutions (pH 4.5) causes a loss of 3–8% of isoflavones (Barbosa et al. 2006). The influence of temperature during precipitation and washing processes was studied by Lin et al. (2006). They tested several temperatures, 0 °C, 10 °C, 25 °C, and 50 °C, and postulated that isoflavone recovery was higher at lower temperatures. Regarding isoflavone distribution, aglycone content decreased for low temperatures. The protein yield did not change significantly from 40 °C to 10 °C, but at 0 °C, it decreases from approximately 90% to 85.6%.

Miso

Miso is a traditional Japanese fermented soy-based food rich in vitamins and minerals (Yamabe et al. 2007). Miso is commonly produced from koji. Soybean koji can be prepared by soaking soybeans in water and mixing with the conidia of *Aspergillus oryzae* or *Aspergillus sojae* and incubated at room temperature (Chiou et al. 1997). Steam-cooked and cooled soybeans are mixed with koji and ground into a paste. Then, the samples are pressed, screw-capped, and incubated at approximately 30 °C for fermentation (Chiou and Cheng 2001).

Soaking soybeans, as previously revised for the preparation of soymilk, produces a loss of total isoflavone content but very slight changes in profile. Soybeans are usually cooked in boiling water for 10 min to deactivate the enzyme β-glucosidase and subsequently soaked at room temperature during 4 h. After that, they are steam-cooked at 121 °C for 30 min, producing an increase in the β-glucosides daidzin and genistin (approximately 20% and 7%, respectively).

The artificial inoculation of cooled soybeans with *A. oryzae* at 28 °C for 3 days results in higher levels of aglycones (approximately 26%) but lower concentration of β-glucosides: daidzin decreases to half the content and genistin about 45%. Chiou et al. explain these changes by the activation of a β-glucosidase-like enzyme during inoculation which hydrolyzes daidzin and genistin, releasing daidzein and genistein, respectively.

Similarly to inoculation, fermentation increases aglycone concentration, while the glucoside levels decrease. The daidzein content increases approximately 60%, while genistein does approximately 30%. In the case of glucosides, daidzin decreases approximately 16% and genistin approximately 5% from the previous stage. As the time of incubation increases, the changes are more pronounced, but after 2 days, the variations in isoflavone concentrations do not change significantly (Chiou and Cheng 2001).

Natto

Natto is also a traditional Japanese food made from fermented soybeans. Natto preparation consists of splitting, soaking, and boiling of soybeans, followed by fermentation with *Bacillus subtilis*, called natto, at 37 °C for 48 h (Shimakage et al. 2006).

Soaked soybeans present less glucoside conjugates, but higher levels of aglycones, as described previously. The boiling process produces a decrease of malonyl isoflavones

due to the low stability of these derivatives. Fermentation with *B. subtilis* usually results in a decrease of the β-glucosides with an increase of the aglycones. Toda et al. (1999) postulated the formation of 6″-*O*-succinyl derivatives during fermentation. They are initially accumulated and then decrease, suggesting that enzymatic interconversion of the glucoside conjugates to the corresponding 6″-*O*-succinylated derivatives occurs in this media. Depending on the *B. subtilis* strain, the concentration of each conjugate can vary; for example, Wei et al. found that fermentation with *B. subtilis* BCRC 14718 produced higher yields of aglycones (68%) with respect to BCRC 14714, 14715, and 14716, which led to approximately 20% of aglycones. Furthermore, incubation with BCRC 14718 resulted in a slight increase of the total isoflavone content (approximately 1%), contrary to the reduction found for the other strains (5–20% of isoflavones lost). After 48 h of fermentation with *B. subtilis* BCRC 14718, daidzein and genistein had increased to 54% and 13%, respectively, of the total isoflavones (Wei et al. 2008).

Soy Sauce

Soy sauce, also named shoyu, is a fermented sauce made from soybeans, roasted grains, water, and salt (Fukushima 1979). It can be found as hydrolyzed sauce or as fermented soy sauce. The first one is not widely extended; it is produced by acid hydrolysis of raw soybeans and is only employed as an extender of fermented soy sauce. In contrast, Japanese fermented soy sauce is prepared by digesting wheat and soybeans (koji) with sodium chloride and fermenting the mash with molds or yeasts. After 6–8 months, the mixture is pressed to obtain a liquid, which is then pasteurized to produce the soy sauce (Kataoka 2005; Ling and Chou 1996).

Compared to other fermented soy-based foods, such as miso and natto, the isoflavone content of soy sauce is low. According to Genovese and Lajolo (2002), only genistin (22.6% of the total) and the aglycones daidzein and genistein are detected in soy sauce. The changes in isoflavone content and distribution are probably produced during fermentation. Chang et al. (2007) observed that some *Aspergillus* strains metabolized the isoflavones daidzein and genistein. The resulting products are 8-hydroxydaidzein and 8-hydroxygenistein, which were also found in other fermented soy foods with *A. oryzae*, such as douchi and miso. However, Maatooq and Rosazza (2005) found that some *Aspergillus* strains did not metabolize daidzein. Other authors have detected other isoflavone derivatives in soy sauce. Thus, Kinoshita et al. (1998) found the aglycone daidzein and three molecules derived from daidzein and genistein whose chemical structures are shown in Fig. 2. The aglycones are probably formed during the fermentation process, released by enzymatic action, and the daidzein and genistein derivatives may be formed by combination of the isoflavone conjugates with tartaric acid via ether linkage (Kinoshita et al. 1997).

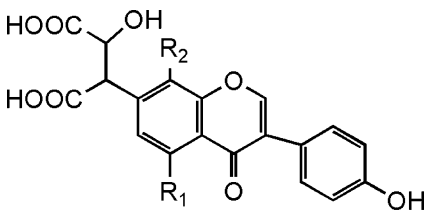
Tempeh

Tempeh is a fermented soy-based food original from Indonesia, although it is common in other parts of East Asia (Hachmeister and Fung 1993). Tempeh is produced by soaking soybeans at room temperature for 10–12 h and dehulling them by hand. They are then heated up to the boiling point and boiled for 20 min. Soybeans are immediately filtered to remove water. After cooling to 35–40 °C, an inoculum of *Rhizopus oligosporus* is added for incubation in the dark at 37 °C for 22 h (Wang and Murphy 1996).

The whole process of preparing tempeh leads to losses of 76% from the total content. Similarly to the fabrication of other soy-based foods, about 12% of the isoflavones are leached from soy during soaking and dehullling, and 49% are lost during the cooking step.

Fermentation does not generate great differences in isoflavone content (<30%) from cooked soybeans. Relating to the isoflavone profile, the concentration of aglycones is higher at long fermentation times. After 22 h, aglycone content of tempeh is more than 6.5 times higher with respect to cooked beans, and the corresponding glucosides decrease (57%). The formation of aglycones during tempeh fermentation is probably due to the hydrolytic action of β-glucosidase from the fungi. The acetyl and malonyl-β-glucosides do not change considerably with respect to cooked soybeans. The major variations are found in malonyl-β-glycitein, which decreases twofold in tempeh (Wang and Murphy 1996).

Nakajima et al. (2005) developed a method for increasing the isoflavone content in tempeh by combining the defatted soybean germ (hypocotyl) with the defatted soybean cotyledon at a ratio 20:80 (%). Isoflavone content increased three times regarding traditional tempeh fabrication, and the aglycones proportion was about 44% from the total isoflavones.



R_1	R_2
H	H
OH	H
OH	OH

Fig. 2 Chemical structure of the compounds found in soy sauce

Douchi

There are three main types of douchi depending on the microorganism employed for the fermentation, either incubated with *Mucor*, *Bacteria*, or *Aspergillus* strains, all of them originated in China. Douchi is prepared by soaking soybeans in water for 8 h at room temperature; after draining, soybeans are cooked (>100 °C) and then inoculated with *A. oryzae* at 30–35 °C (pre-fermentation). Afterwards, sodium chloride is added to select microorganisms responsible for flavor and taste and to inhibit the growth of pathogenic and spoilage microorganisms (post-fermentation; Wang et al. 2007b; Zhang et al. 2007b).

Fermentation to produce douchi implies a significant loss of isoflavones. Thus, during pre-fermentation, 43% of the total isoflavones are lost, and for the post-fermentation process, there is a loss of 18%. Generally, fermentation results in an increase of the aglycones (approximately ten

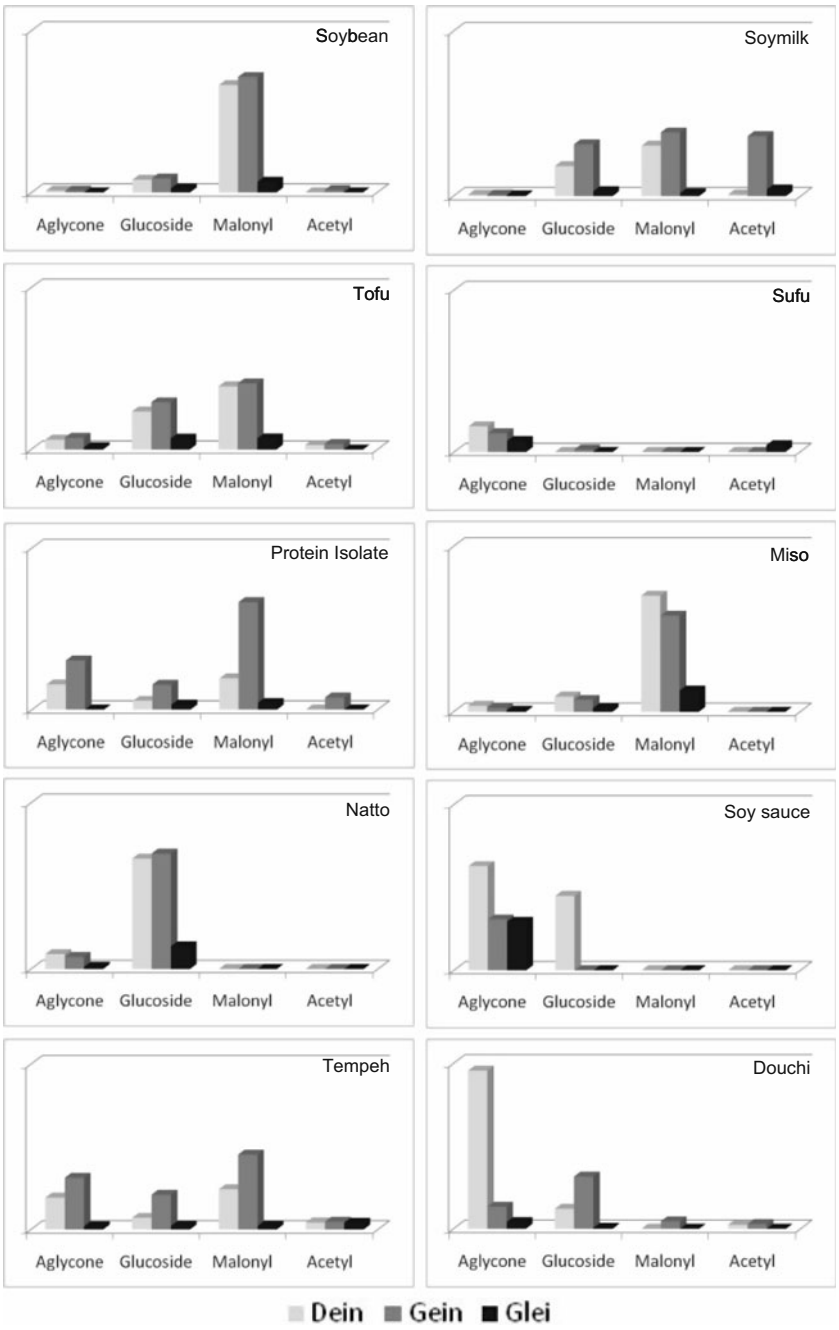


Fig. 3 Isoflavone conjugates distribution in representative soy-based foods and food ingredients. Data have been normalized from different bibliographic sources in the case of natto and soy sauce; the content of malonyl and acetyl derivatives was not found (*Dein* daizein, *Gein* genistein, *Glei* glycitein)

times higher in the final douchi) with the decrease of the β-glucosides and the malonyl and acetyl conjugates.

Following post-fermentation, aglycone content reaches 90% of the total isoflavones. The higher content of aglycones is due to the activation of the enzyme β-glucosidase during fermentation; thus, β-glucosides are hydrolyzed. β-glucosidase activity was found to be closely related to the sodium chloride content: for 5% of NaCl, the enzyme activity may remain high, but for greater concentrations (7.5%, 10%, and 12.5%), β-glucosidase activity is substantially inhibited (Wang et al. 2007). On the other hand, the loss of isoflavones during fermentation could be due to the transformation of aglycones into their hydroxylated derivatives. Esaki et al. (1999) postulated the formation of 8-hydroxydaidzein and 8-hydroxygenistein, similarly to soy sauce preparation, by microbial hydroxylation with *Aspergillus saitoi* from the corresponding aglycones daidzein and genistein, respectively (Esaki et al. 1999).

Discussion

Figure 3 shows the isoflavone distribution in the soy-based foods and food ingredients. Data have been obtained from different sources (Song et al. 1998; Wang et al. 1998, 2007; Yin et al. 2005) and have been normalized for the purpose of comparison. It must be noted that bibliography data show slight variations depending on the soy origin, variety, and processing conditions. In the case of natto and soy sauce, data for the acetyl and malonyl concentrations have not been found (Nakamura et al. 2000).

The different distributions of isoflavone compounds in soy-based foods can be explained in terms of their chemical structures. Thus, genistein is stabilized by the formation of intramolecular interactions between the hydroxyl group in ring A and the 4-oxo moiety in ring C, whereas daidzein, which only has one hydroxyl group, and glycitein, which does not have any hydroxyl group, seem to be less stable (Huang and Chou 2009; Ungar et al. 2003). Thus, glycitein and their derivatives are usually transformed into more stable compounds, while genistein and their conjugates are not so easily degraded despite the conditions. Generally, isoflavone content of processed soy-based foods is lower with respect to raw soybeans as a consequence of physical processes or chemical transformations. Furthermore, the distribution is modified due to the different stabilities of the conjugates.

In soymilk, aglycone content is generally low. The preparation at temperatures near the optimal β-glucosidase activity involves the hydrolysis of β-glucosides into their corresponding aglycones. On the other hand, the unstable malonyl conjugates are degraded by oxidative decarboxylation. When soymilk is coagulated to prepare tofu (solid–liquid separation), a loss of isoflavones is produced since they mainly remain in the liquid phase. If proteins are not totally coagulated and continue in the aqueous media, isoflavones would also remain in the whey, thanks to the intermolecular interactions they form with proteins (Rawel et al. 2004). If tofu is fermented to fabricate sufu, losses of isoflavones are observed and the levels of aglycones increase, while β-glucoside and malonyl content decreases by the hydrolysis performed by the β-glucosidase enzyme.

Table 1 Principal treatments performed during soybean transformation into soy-based foods and isoflavone changes

Process	Conditions	Isoflavone changes	Reference
Soaking	Room temperature	Loss	Kao et al. (2004)
	High temperature (100 °C)	Malonyl degradation	Jackson et al. (2002)
Grinding	Room temperature	No change	Kao et al. (2004)
	High temperature (100 °C)	Aglycone formation	Jackson et al. (2002)
Cooking	High temperature (100 °C)	Malonyl degradation Aglycone formation	
Coagulation	0.3% coagulant	Loss	Prabhakaran et al. (2006)
Defatting	Hexane	No change	Wang et al. (1998)
Extraction	High temperature Basic pH	Aglycone formation Malonyl degradation	Wang et al. (1998)
Precipitation	pH4.5	High isoflavone recovery	Wang et al. (1998)
Washing	Acid pH	Loss	Wang et al. (1998)
Fermentation	*Aspergillus*	Aglycone formation	Wang et al. (2007)
		Glucoside degradation	Kinoshita et al. (1998)
	Bacillus subtilis	Aglycone formation Glucoside degradation	Wei et al. (2008)
	Rhizopus oligosporus	Aglycone formation Glucoside degradation	Nakajima et al. (2005)

Regarding protein concentrates, the malonyl conjugates decrease when the extraction is carried out at basic pH. This fact is probably due to the ionization of the carboxylic group and, therefore, the solubilization into the aqueous phase. A loss of isoflavones is observed during precipitation (solid–liquid separation) as they may remain in the liquid phase or interact with proteins. Fermented soy-based foods generally contain less isoflavones than the corresponding non-fermented soy-based foods. Besides the process conditions, fermentation comprises the action of several enzymes that may transform the isoflavones. Consequently, fermentation results in degradations of the unstable isoflavones or chemical transformations into similar flavonoid compounds depending on the microorganism employed. Regarding the distribution, initially, the β-glucosides increase while the unstable malonyl derivatives decrease during cooking. Then, during fermentation, the level of aglycones increases and β-glucoside concentration is reduced due to the enzymatic hydrolysis. In miso, natto, and tempeh, isoflavone concentration tends to be low; however, malonyl proportion in miso and tempeh is significantly elevated with respect to other fermented soy-based foods. The higher levels of β-glucosides are found in natto, while in douchi, the aglycone daidzein is the major component.

In Table 1, a list of the processing treatments involved in the preparation of soy-based foods together with the isoflavone changes is shown.

Conclusions

Conditions for preparing high-quality soy-based foods and food ingredients maintaining a high yield of isoflavone recovery have been reviewed. In most of the cases, the preparation involves several steps, and the required properties of the final product (color, texture, etc.) may not yield the higher recovery of isoflavones. Consequently, the treatments commonly result in lower isoflavone content and changes in isoflavone profile during the different stages.

As a general rule, processes that involve an increase in temperature (such as cooking, extraction, etc.) produce the cleavage of the carboxylic group, and the malonyl derivatives are transformed into the acetyl or glucoside conjugates. Furthermore, if the temperature is near 45 °C, the activity of the enzyme β-glucosidase becomes optimal and the β-glucosides are transformed into the corresponding aglycones. During the solid–liquid extraction (soaking, defatting, or alkaline extraction), isoflavones are transferred onto the liquid phase, producing significant losses depending on the amount of liquid wetting the solid, isoflavone solubility, and other factors such as temperature. Coagulation processes (solid–liquid separation) commonly result in losses because isoflavones remain in the aqueous media after precipitation. Finally, fermentations involve chemical transformations and effect of temperature, which result in losses of isoflavones, transformations of the β–glucosides into aglycones, and, in some cases, the formation of isoflavone derivatives by the addition or substitution of functional groups in the chemical structure.

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References

Adhami, V. M., Syed, D. N., Khan, N., & Afaq, F. (2008). Phytochemicals for prevention of solar ultraviolet radiation-induced damages. *Photochemistry and Photobiology, 84*, 489–500.

Aguiar, C. L., Baptista, A. S., Alencar, S. M., Haddad, R., & Eberlin, M. N. (2007). Analysis of isoflavonoids from leguminous plant extracts by RPHPLC/DAD and electrospray ionization mass spectrometry. *International Journal of Food Sciences and Nutrition, 58*, 116–124.

Allred, C. D., Allred, K. F., Ju, Y. H., Goeppinger, T. S., Doerge, D. R., & Helferich, W. G. (2004). Soy processing influences growth of estrogen-dependent breast cancer tumors. *Carcinogenesis, 25*, 1649–1657.

Allred, C. D., Twaddle, N. C., Allred, K. F., Goeppinger, T. S., Churchwell, M. I., Ju, Y. H., et al. (2005). Soy processing affects metabolism and disposition of dietary isoflavones in ovariectomized balb/c mice. *Journal of Agricultural and Food Chemistry, 53*, 8542–8550.

Anderson, R. L., & Wolf, W. J. (1995). Compositional changes in trypsin-inhibitors, phytic acid, saponins and isoflavones related to soybean processing. *Journal of Nutrition, 125*, 581S–588S.

Araújo, J. M. A., Silva, M. V., & Chaves, J. B. P. (2007). Supercritical fluid extraction of daidzein and genistein isoflavones from soybean hypocotyl after hydrolysis with endogenous b-glucosidases. *Food Chemistry, 105*, 266–272.

Barbosa, A. C., Lajolo, F. M., & Genovese, M. I. (2006). Influence of temperature, pH and ionic strength on the production of isoflavone-rich soy protein isolates. *Food Chemistry, 98*, 757–766.

Barnes, S., Kirk, M., & Coward, L. (1994). Isoflavones and their conjugates in soy foods: Extraction conditions and analysis by HPLC–mass spectrometry. *Journal of Agricultural and Food Chemistry, 42*, 2466–2474.

Birt, D. F., Hendrich, S., & Wang, W. (2001). Dietary agents in cancer prevention: Flavonoids and isoflavonoids. *Pharmacology and Therapeutics, 90*, 157–177.

Cantoral, R., Fernández Quintela, A., Martinez, J. A., & Macarulla, M. T. (1995). Comparative study of the composition and nutritional value of the seeds and protein concentrations in legumes. *Archivos Latinoamericanos de Nutricion, 45*, 242–248.

Chang, C. Y., Huang, L. J., Wang, J. P., Teng, C. M., Chen, S. C., & Kuo, S. C. (2000). Synthesis and anti-platelet, anti-inflammatory and anti-allergic activities of methoxyisoflavanquinone and related compounds. *Chemical & Pharmaceutical Bulletin, 48*, 964–973.

Chang, T. S., Ding, H. Y., Tai, S. S. K., & Wu, C. Y. (2007). Metabolism of the soy isoflavones daidzein and genistein by

fungi used in the preparation of various fermented soybean foods. *Bioscience Biotechnology and Biochemistry, 71*(5), 1330–1333.

Charalampopoulos, D., Wang, R., Pandiella, S. S., & Webb, C. (2002). Application of cereals and cereal components in functional foods: A review. *International Journal of Food Microbiology, 79*, 131–141.

Chiou, R. Y.-Y., & Cheng, S.-L. (2001). Isoflavone transformation during soybean koji preparation and subsequent miso fermentation supplemented with ethanol and NaCl. *Journal of Agricultural and Food Chemistry, 49*, 3656–3660.

Chiou, R. Y.-Y., Ferng, S., & Chen, M. J. (1997). White particulate formation as a result of enzymatic proteolysis of protein substrates in brine-fermented foods. *Food Science and Technology, 30*, 241–245.

Chung, M. J., Sung, N.-J., Park, C.-S., Kweon, D.-K., Mantovani, A., Moon, T.-W., et al. (2008). Antioxidative and hypocholesterolemic activities of water-soluble puerarin glycosides in HepG2 cells and in C57 BL/6J mice. *European Journal of Pharmacology, 578*, 159–170.

Clavel, T., Fallani, M., Lepage, P., Levenez, F., Mathey, J., Rochet, V., et al. (2005). Isoflavones and functional foods alter the dominant intestinal microbiota in postmenopausal women. *Journal of Nutrition, 135*, 2786–2792.

Coward, L., Smith, M., Kirk, M., & Barnes, S. (1998). Chemical modification of isoflavones in soyfoods during cooking and processing. *American Journal of Clinical Nutrition, 68*, 1486S–1491S.

Coxam, V. (2008). Phyto-oestrogens and bone health. *Proceedings of the Nutrition Society, 67*, 184–195.

Davis, D. D., Díaz-Cruz, E. S., Landini, S., Kim, Y.-W., & Brueggemeier, R. W. (2008). Evaluation of synthetic isoflavones on cell proliferation, estrogen receptor binding affinity, and apoptosis in human breast cancer cells. *Journal of Steroid Biochemistry and Molecular Biology, 108*, 23–31.

DeMan, J. M., DeMan, L., & Gupta, S. (1986). Texture and microstructure of soybean curd (tofu) as affected by different coagulants. *Food Microstructure, 5*, 83–89.

Dijsselbloem, N., Berghe, W. V., Naeyer, A. D., & Haegeman, G. (2004). Soy isoflavone phyto-pharmaceuticals in interleukin-6 affections: Multi-purpose nutraceuticals at the crossroad of hormone replacement, anti-cancer and anti-inflammatory therapy. *Biochemical Pharmacology, 68*, 1171–1185.

Eldridge, A. C. (1982). Determination of isoflavones in soybean flours, protein concentrates, and isolates. *Journal of Agricultural and Food Chemistry, 30*, 353–355.

Eldridge, A. C., & Kwolek, W. F. (1983). Soybean isoflavones: Effect of environment and variety on composition. *Journal of Agricultural and Food Chemistry, 31*, 394–396.

Esaki, H., Watanabe, R., Onozaki, H., Kawakishi, S., & Osawa, T. (1999). Formation mechanism for potent antioxidative *o*-dihydroxyisoflavones in soybeans fermented with *Aspergillus saitoi*. *Bioscience Biotechnology and Biochemistry, 63*(5), 851–858.

Fernández Quintela, A., Macarulla, M. T., Del Barrio, A. S., & Martinez, J. A. (1997). Composition and functional properties of protein isolates obtained from commercial legumes grown in northern Spain. *Plant Foods for Human Nutrition, 51*, 331–342.

Ferrer, M. A., & Barcelo, A. R. (1994). Genistein as an endogenous natural substrate of acidic peroxidases in lupin hypocotyls. *Annals of Applied Biology, 125*, 173–178.

Franke, A. A., Hankin, J. H., Yu, M. C., Maskarinec, G., Low, S.-H., & Custer, L. J. (1999). Isoflavone levels in soy foods consumed by multiethnic populations in Singapore and Hawaii. *Journal of Agricultural and Food Chemistry, 47*, 977–986.

Fukushima, D. (1979). Fermented vegetable (soybean) protein and related foods of Japan and China. *Journal of the American Oil Chemists' Society, 56*(3), 357–362.

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, 34*, 457–461.

Garcia-Lafuente, A., Guillamon, E., Villares, A., Rostagno, M. A., & Martinez, J. A. (2009). Flavonoids as anti-inflammatory agents: Implications in cancer and cardiovascular disease. *Inflammation Research, 58*(9), 537–552.

Gelencser, T., Gal, V., Hodsagi, M., & Salgo, A. (2008). Evaluation of quality and digestibility characteristics of resistant starch-enriched pasta. *Food and Bioprocess Technology, 1*(2), 171–179.

Genovese, M. I., & Lajolo, F. M. (2002). Isoflavones in soy-based foods consumed in Brazil: Levels, distribution, and estimated intake. *Journal of Agricultural and Food Chemistry, 50*, 5987–5993.

Genovese, M. I., Davila, J., & Lajolo, F. M. (2006). Isoflavones in processed soybean products from Ecuador. *Brazilian Archives of Biology and Technology, 49*, 853–859.

Genovese, M. I., Barbosa, A. C., Pinto, M. S., & Lajolo, F. M. (2007). Commercial soy protein ingredients as isoflavone sources for functional foods. *Plant Foods for Human Nutrition, 62*(2), 53–58.

Golbitz, P. (1995). Traditional soyfoods—Processing and products. *Journal of Nutrition, 125*, 570S–572S.

Hachmeister, K. A., & Fung, D. Y. C. (1993). Tempeh—A mold-modified indigenous fermented food made from soybeans and or cereal-grains. *Critical Reviews in Microbiology, 19*(3), 137–188.

Han, B. Z., Rombouts, F. M., & Nout, M. J. R. (2003). Review: Sufu—A Chinese fermented soybean food. *International Journal of Food Science and Technology, 81*, 92–95.

Holzapfel, W. H., & Schillinger, U. (2002). Introduction to pre- and probiotics. *Food Research International, 35*, 109–116.

Huang, R. Y., & Chou, C. C. (2009). Stability of isoflavone isomers in steamed black soybeans and black soybean koji stored under different conditions. *Journal of Agricultural and Food Chemistry, 57*(5), 1927–1932.

Hui, E., Henning, S. M., Park, N., Heber, D., & Go, V. L. W. (2001). Genistein and daidzein/glycitein content in tofu. *Journal of Food Composition and Analysis, 14*, 199–206.

Hutabarat, L. S., Greenfield, H., & Mulholland, M. (2001). Isoflavones and coumestrol in soybeans and soybean products from Australia and Indonesia. *Journal of Food Composition and Analysis, 14*, 43–58.

Ishihara, M., Singh, H., Chung, G., & Tam, C. (2007). Content composition and antioxidant activity of isoflavones in commercial and homemade soymilk and tofu. *Journal of the Science of Food and Agriculture, 87*, 2844–2852.

Jackman, K. A., Woodman, O. L., & Sobey, C. G. (2007). Isoflavones, equol and cardiovascular disease: Pharmacological and therapeutic insights. *Current Medicinal Chemistry, 14*, 2824–2830.

Jackson, C.-J. C., Dini, J. P., Lavandier, C., Rupasinghe, H. P. V., Faulkner, H., Poysa, V., et al. (2002). Effects of processing on the content and composition of isoflavones during manufacturing of soy beverage and tofu. *Process Biochemistry, 37*, 1117–1123.

Kao, T. H., & Chen, B. H. (2006). Functional components in soybean cake and their effects on antioxidant activity. *Journal of Agricultural and Food Chemistry, 54*, 7544–7555.

Kao, F.-J., Su, N.-W., & Lee, M.-H. (2003). Effect of calcium sulfate concentration in soymilk on the microstructure of firm tofu and the protein constitutions in tofu whey. *Journal of Agricultural and Food Chemistry, 51*, 6211–6216.

Kao, T. H., Lu, Y. F., Hsieh, H. C., & Chen, B. H. (2004). Stability of isoflavone glucosides during processing of soymilk and tofu. *Food Research International, 37*, 891–900.

Kao, T. H., Huang, R.-F. S., & Chen, B. H. (2007). Antiproliferation of hepatoma cell and progression of cell cycle as affected by

isoflavone extracts from soybean cake. *International Journal of Molecular Sciences, 8*, 1095–1110.

Kao, T. H., Wu, W. M., Hung, C. F., Wu, W. B., & Chen, B. H. (2007). Anti-inflammatory effects of isoflavone powder produced from soybean cake. *Journal of Agricultural and Food Chemistry, 55*, 11068–11079.

Kataoka, S. (2005). Functional effects of Japanese style fermented soy sauce (Shoyu) and Itl components. *Journal of Bioscience and Bioengineering, 100*(3), 227–234.

Khetarpaul, N., Grewal, R. B., Goyal, R., & Garg, R. (2004). Development of partially defatted soy flour and *dhal*. *Food Chemistry, 87*, 355–359.

Kinoshita, E., Ozawa, Y., & Aishima, T. (1997). Novel tartaric acid isoflavone derivatives that play key roles in differentiating Japanese soy sauces. *Journal of Agricultural and Food Chemistry, 45*, 3753–3759.

Kinoshita, E., Sugimoto, T., Ozawa, Y., & Aishima, T. (1998). Differentiation of soy sauce produced from whole soybeans and defatted soybeans by pattern recognition analysis of HPLC profiles. *Journal of Agricultural and Food Chemistry, 46*, 877–883.

Kohen, F., Gayer, B., Kulik, T., Frydman, V., Nevo, N., Katzburg, S., et al. (2007). Synthesis and evaluation of the antiproliferative activities of derivatives of carboxyalkyl isoflavones linked to N-t-Boc-hexylenediamine. *Journal of Medicinal Chemistry, 50*, 6405–6410.

Kupeli, E., Orhan, I., Toker, G., & Yesilada, E. (2006). Anti-inflammatory and antinociceptive potential of *Maclura pomifera* (Rafin.) Schneider fruit extracts and its major isoflavonoids, scandenone and auriculasin. *Journal of Ethnopharmacology, 107*, 169–174.

Lee, S.-J., Ahn, J.-K., Khanh, T.-D., Chun, S.-C., Kim, S.-L., Ro, H.-M., et al. (2007). Comparison of isoflavone concentrations in soybean (*Glycine max* (L.) Merrill) sprouts grown under two different light conditions. *Journal of Agricultural and Food Chemistry, 55*, 9415–9421.

Li, L. T., Yin, L. J., & Saito, M. (2004). Function of traditional foods and food culture in China. *Japan Agricultural Research Quarterly, 38*(4), 213–220.

Lin, J., Krishnan, P. G., & Wang, C. (2006). Retention of isoflavones and saponins during the processing of soy protein isolates. *Journal of the American Oil Chemists' Society, 83*, 59–63.

Ling, M.-Y., & Chou, C.-C. (1996). Biochemical changes during the preparation of soy sauce koji with extruded and traditional raw materials. *International Journal of Food Science and Technology, 31*, 511–517.

Liu, Z. S., & Chang, S. K. C. (2004). Effect of soy milk characteristics and cooking conditions on coagulant requirements for making filled tofu. *Journal of Agricultural and Food Chemistry, 52*(11), 3405–3411.

Wang, L-j, Yin, L-j, Li, D., Zou, L., Saito, M., Tatsumi, E., et al. (2007). Influences of processing and NaCl supplementation on isoflavone contents and composition during douchi manufacturing. *Food Chemistry, 101*, 1247–1253.

López-Guel EC, Lozano-Bautista F, Mora-Escobedo R, Farrera-Rebollo R, Chanona-Perez J, Gutierrez-Lopez GF, & Calderon-Dominguez G (2009) Effect of soybean 7S protein fractions, obtained from germinated and nongerminated seeds, on dough rheological properties and bread quality. *Food and Bioprocess Technology.* doi:10.1007/s11947-009-0277-9.

Lotito, S. B., & Frei, B. (2006). Dietary flavonoids attenuate tumor necrosis factor α-induced adhesion molecule expression in human aortic endothelial cells. *Journal of Biological Chemistry, 281*(48), 37102–37110.

Ma, D. F., Qin, L. Q., Wang, P. Y., & Katoh, R. (2008). Soy isoflavone intake increases bone mineral density in the spine of menopausal women: Meta-analysis of randomized controlled trials. *Clinical Nutrition, 27*(1), 57–64.

Maatooq, G. T., & Rosazza, J. P. N. (2005). Metabolism of daidzein by *Nocardia* species NRRL 5646 and *Mortierella isabellina* ATCC 38063. *Phytochemistry, 66*(9), 1007–1011.

Mahungu, S. M., Diaz-Mercado, S., Li, J., Schwenk, M., Singletary, K., & Faller, J. (1999). Stability of isoflavones during extrusion processing of corn/soy mixture. *Journal of Agricultural and Food Chemistry, 47*, 279–284.

Matsuura, M., & Obata, A. (1993). Beta-glucosidases from soybeans hydrolize daidzin and genistin. *Journal of Food Science, 58*, 144–147.

Mebrahtu, T., Mohamed, A., Wang, C. Y., & Andebrhan, T. (2004). Analysis of isoflavone contents in vegetable soybeans. *Plant Foods for Human Nutrition, 59*, 55–61.

Murphy, P. A., Song, T., Buseman, G., Barua, K., Beecher, G. R., Trainer, D., et al. (1999). Isoflavones in retail and institutional soy foods. *Journal of Agricultural and Food Chemistry, 47*, 2697–2704.

Naim, M., Gestetner, B., Zilkah, S., Birk, Y., & Bondi, A. (1974). Soybean isoflavones. Characterization, determination, and antifungal activity. *Journal of Agricultural and Food Chemistry, 22*, 806–810.

Nakajima, N., Nozaki, N., Ishihara, K., Ishikawa, A., & Tsuji, H. (2005). Analysis of Isoflavone content in tempeh, a fermented soybean, and preparation of a new isoflavone-enriched tempeh. *Journal of Bioscience and Bioengineering, 100*, 685–687.

Nakamura, Y., Tsuji, S., & Tonogai, Y. (2000). Determination of the levels of isoflavonoids in soybeans and soy-derived foods and estimation of isoflavonoids in the Japanese daily intake. *Journal of AOAC International, 83*(3), 635–650.

Nishio, K., Niwa, Y., Toyoshima, H., Tamakoshi, K., Kondo, T., Yatsuya, H., et al. (2007). Consumption of soy foods and the risk of breast cancer: Findings from the Japan Collaborative Cohort (JACC) Study. *Cancer Causes and Control, 18*, 801–808.

Obatolu, V. A. (2008). Effect of different coagulants on yield and quality of tofu from soymilk. *European Food Research and Technology, 226*(3), 467–472.

Okamoto, Y., Suzuki, A., Ueda, K., Ito, C., Itoigawa, M., Furukawa, H., et al. (2006). Anti-estrogenic activity of prenylated isoflavones from *millettia pachycarpa*: Implications for pharmacophores and unique mechanisms. *Journal of Health Science, 52*, 186–191.

Pandjaitan, N., Hettiarachchy, N. S., & Ju, Z. Y. (2000). *Journal of Food Science, 65*, 403–407.

Papazisis, K. T., Kalemi, T. G., Zambouli, D., Geromichalos, G. D., Lambropoulos, A. F., Kotsis, A., et al. (2006). Synergistic effects of protein tyrosine kinase inhibitor genistein with camptothecins against three cell lines in vitro. *Cancer Letters, 233*, 255–264.

Park, J.-S., Woo, M.-S., Kim, D.-H., Hyun, J.-W., Kim, W.-K., Lee, J.-C., et al. (2007). Anti-inflammatory mechanisms of isoflavone metabolites in lipopolysaccharide-stimulated microglial cells. *Journal of Pharmacology and Experimental Therapeutics, 320*, 1237–1245.

Perez-Balibrea, S., Moreno, D. A., & García-Viguera, C. (2008). Influence of light on health-promoting phytochemicals of broccoli sprouts. *Journal of the Science of Food and Agriculture, 88*, 904–910.

Phrakonkham, P., Chevalier, J., Desmetz, C., Pinnert, M.-F., Bergès, R., Jover, E., et al. (2007). Isoflavonoid-based bone-sparing treatments exert a low activity on reproductive organs and on hepatic metabolism of estradiol in ovariectomized rats. *Toxicology and Applied Pharmacology, 224*, 105–115.

Prabhakaran, M. P., & Perera, C. O. (2006). Effect of extraction methods and UHT treatment conditions on the level of

isoflavones during soymilk manufacture. *Food Chemistry, 99*, 231–237.

Prabhakaran, M. P., Perera, C. O., & Valiyaveettil, S. (2005). Quantification of isoflavones in soymilk and tofu from South East Asia. *International Journal of Food Properties, 8*(1), 113–123.

Prabhakaran, M. P., Perera, C. O., & Valiyaveettil, S. (2006). Effect of different coagulants on the isoflavone levels and physical properties of prepared firm tofu. *Food Chemistry, 99*, 492–499.

Ranalli, A., Marchegiani, D., Pardi, D., Contento, S., Girardi, F., & Kotti, F. (2007). Evaluation of functional phytochemicals in destoned virgin olive oil. *Food and Bioprocess Technology, 2*(3), 322–327.

Rawel, H. M., Ranters, H., Rohn, S., & Kroll, J. (2004). Assessment of the reactivity of selected lsoflavones against proteins in comparison to quercetin. *Journal of Agricultural and Food Chemistry, 52*(16), 5263–5271.

Richardson, G. A., El-Rafey, M. S., & Long, M. L. (1947). Flavones and flavone derivatives as antioxidants. *Journal of Dairy Science, 30*, 397–413.

Roberfroid, M. B. (2000). Prebiotics and probiotics: Are they functional foods? *American Journal of Clinical Nutrition, 71*, S1682–S1687.

Rochfort, S., & Panozzo, J. (2007). Phytochemicals for health, the role of pulses. *Journal of Agricultural and Food Chemistry, 55*, 7981–7994.

Rosales-Juarez, M., Gonzalez-Mendoza, B., Lopez-Guel, E. C., Lozano-Bautista, F., Chanona-Perez, J., Gutierrez-Lopez, G., et al. (2008). Changes on dough rheological characteristics and bread quality as a result of the addition of germinated and non-germinated soybean flour. *Food and Bioprocess Technology, 1*(2), 152–160.

Rostagno, M. A., Villares, A., Guillamon, E., Garcia-Lafuente, A., & Martinez, J. A. (2009). Sample preparation for the analysis of isoflavones from soybeans and soy foods. *Journal of Chromatography, 1216*(1), 2–29.

Sabanis, D., & Tzia, C. (2009). Effect of rice, corn and soy flour addition on characteristics of bread produced from different wheat cultivars. *Food and Bioprocess Technology, 2*(1), 68–79.

Sarkar, F. H., & Li, Y. (2003). Soy isoflavones and cancer prevention. *Cancer Investigation, 21*(5), 744–757.

Shimakage, A., Shinbo, M., Yamada, S., & Ito, H. (2006). Changes in isoflavone content in soybeans during the manufacturing processes of tubu–natto and hikiwari–natto. *Journal of the Japanese Society for Food Science and Technology-Nippon Shokuhin Kagaku Kogaku Kaishi, 53*, 185–188.

Song, T., Barua, K., Buseman, G., & Murphy, P. A. (1998). Soy isoflavone analysis: Quality control and a new internal standard. *American Journal of Clinical Nutrition, 68*(6), 1474S–1479S.

Sung, M. J., Park, Y. S., & Chang, H. G. (2006). Quality characteristics of sponge cake supplemented with soy protein concentrate. *Food Science and Biotechnology, 15*, 860–865.

Toda, T., Uesugi, T., Hirai, K., Nukaya, H., Tsuji, K., & Ishida, H. (1999). New 6-*O*-acyl isoflavone glycosides from soybeans fermented with *Bacillus subtilis* (natto). I. 6-*O*-succinylated isoflavone glycosides and their preventive effects on bone loss in ovariectomized rats fed a calcium-deficient diet. *Biological and Pharmaceutical Bulletin, 22*, 1193–1201.

Umphress, S. T., Murphy, S. P., Franke, A. A., Custer, L. J., & Blitz, C. L. (2005). Isoflavone content of foods with soy additives. *Journal of Food Composition and Analysis, 18*, 533–550.

Ungar, Y., Osundahunsi, O. F., & Shimoni, E. (2003). Thermal stability of genistein and daidzein and its effect on their antioxidant activity. *Journal of Agricultural and Food Chemistry, 51*(15), 4394–4399.

Wang, H.-J., & Murphy, P. A. (1994a). Isoflavone composition of American and Japanese soybeans in Iowa: Effects of variety, crop year, and location. *Journal of Agricultural and Food Chemistry, 42*, 1674–1677.

Wang, H.-J., & Murphy, P. A. (1994b). Isoflavone content in commercial soybean foods. *Journal of Agricultural and Food Chemistry, 42*, 1666–1673.

Wang, H.-J., & Murphy, P. A. (1996). Mass balance study of isoflavones during soybean processing. *Journal of Agricultural and Food Chemistry, 44*, 2377–2383.

Wang, C., Ma, Q., Pagadala, S., Sherrard, M., & Krishnan, P. G. (1998). Changes of isoflavones during processing of soy protein isolates. *Journal of the American Oil Chemists' Society, 75*, 337–341.

Wang, C., Sherrard, M., Pagadala, S., Wixon, R., & Scott, R. A. (2000). Isoflavone content among maturity group 0 to II soybeans. *Journal of the American Oil Chemists' Society, 77*, 483–487.

Wang, L. J., Li, D., Zou, L., Chen, X. D., Cheng, Y. Q., Yamaki, K., et al. (2007). Antioxidative activity of douchi (a Chinese traditional salt-fermented soybean food) extracts during its processing. *International Journal of Food Properties, 10*(2), 385–396.

Wei, Q. K., Chen, T. R., & Chen, J. T. (2008). Use of *Bacillus subtilis* to enrich isoflavone aglycones in fermented natto. *Journal of the Science of Food and Agriculture, 88*(6), 1007–1011.

Wiseman, H., Casey, K., Clarke, D. B., Barnes, K. A., & Bowey, E. (2002). Isoflavone aglycon and glucoconjugate content of high- and low-soy U.K. foods used in nutritional studies. *Journal of Agricultural and Food Chemistry, 50*, 1404–1410.

Xie, L., Hettiarachchy, N. S., Cai, R., Tsuruhami, K., & Koikeda, S. (2003). Conversion of isoflavone glycosides to aglycones in SoyLife and soymeal using beta-glycosidase. *Journal of Food Science, 68*, 427–430.

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, 130*(4), 798–801.

Yamabe, S., Kobayashi-Hattori, K., Kaneko, K., Endo, H., & Takita, T. (2007). Effect of soybean varieties on the content and composition of isoflavone in rice–koji miso. *Food Chemistry, 100*, 369–374.

Yasmin, A., Zeb, A., Khalil, A. W., Paracha, G. M., & Khattak, A. B. (2008). Effect of processing on anti-nutritional factors of red kidney bean (*Phaseolus vulgaris*) grains. *Food and Bioprocess Technology, 1*(4), 415–419.

Yin, L. J., Li, L. T., Li, Z. G., Tatsumi, E., & Saito, M. (2004). Changes in isoflavone contents and composition of sufu (fermented tofu) during manufacturing. *Food Chemistry, 87*(4), 587–592.

Yin, L. J., Li, L. T., Liu, H., Saito, M., & Tatsumi, E. (2005). Effects of fermentation temperature on the content and composition of isoflavones and beta-glucosidase activity in sufu. *Bioscience Biotechnology and Biochemistry, 69*(2), 267–272.

Yue, X., Xu, Z., Prinyawiwatkul, W., Losso, J. N., King, J. M., & Godber, J. S. (2008). Comparison of soybean oils, gum, and defatted soy flour extract in stabilizing Menhaden oil during heating. *Journal of Food Science, 73*, C19–C23.

Zhang, E. J., Ng, K. M., & Luo, K. Q. (2007). Extraction and purification of isoflavones from soybeans and characterization of their estrogenic activities. *Journal of Agricultural and Food Chemistry, 55*(17), 6940–6950.

Zhang, J. H., Tatsumi, E., Fan, J. F., & Li, L. T. (2007). Chemical components of *Aspergillus*-type Douchi, a Chinese traditional fermented soybean product, change during the fermentation process. *International Journal of Food Science and Technology, 42*(3), 263–268.