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# Soybean and Isoflavones – From Farm to Fork

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Additional information is available at the end of the chapter

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## 1. Introduction

### 1.1. General

Soybean (*Glycine max* L. Merrill) were first grown as a crop in China about 5000 years ago [1] and have been widely consumed as folk medicines in China, India, Japan and Korea for hundreds of years [2]. Today is a major source of plant protein (70%) and oil (30%) and become a globally important crop [3,4]. Its nutrients become basic for humans consumption, beyond its by-products, that offer great diversities of products to the food industry. Soybean oil is highly consumed world-wide and soy milk is often used as a milk substitute to people who have lactose intolerance [5]. In addition soybean has phytoestrogens which can be used in replacement to women hormone [6]. Usage oil can be reused in several forms, including as a fuel source [7,8].

There are many kinds of soybean cultivars with different biological composition and economic values. According to the consensus recommendations of the Organization for Economic Cooperation and Development, soybean nutrients (such as amino acids, fatty acids, isoflavones) and antinutrients (such as phytic acid, raffinose and stachyose) are important markers in assessment the nutritional quality of soybean varieties [9].

Soybean production has expanded to most of continents and 90% of the world's soybean production is concentrated in tropical and semi-arid tropical regions which are characterized by high temperatures and low or erratic rainfall. In tropics, most of the crops are near their maximum temperature tolerance [10].

USA is the largest soybean producer in the world, following by Brazil and Argentina. World soybean harvest production reached 264.25 million metric tons in 2010/2011. USA, Brazil and Argentina were responsible to about 92.75% of all production [11].

Soybeans have been appreciated by consumers as a health-promoting food [12]. Soybeans and soy products are wide consumed by Asian populations and are encouraged for western diets because of its nutritional benefits. Soy products are abundant in traditional Asian diets which daily intake is from 7–8 g/day in Hong-Kong and China, up to 20–30 g/day in Korea and Japan. Most of Europeans and North Americans, however, consume less than 1 g/day [13].

The soybean consumption will depend on grains characteristics, for example large seed sizes with high sucrose content are desirable for the production of vegetable soybean, which is harvested at immature stage, also called edamame. On the other hand, cultivars with small seed size and low calcium contents are desirable for natto, a traditional fermented soy food in Japan with a firmer texture. For soymilk and tofu production, soybeans with light hilum color, large seed size, high water absorption, high protein and sucrose contents and low oligosaccharides contents are desirable [14].

## 1.2. Composition

Soybean's unique chemical composition place this food products as one of the most economical and valuable agricultural commodity. Among cereal and other legume species, it has the highest protein content (around 40%) and the second highest (20%) oil content among all food legumes, after peanuts. Other valuable components found in soybeans include phospholipids, vitamins and minerals. Furthermore, soybeans contain many minor substances known to be biologically active including oligosaccharides, phytosterols, saponins and isoflavones. The actual composition of the whole soybean and its structural parts depends on many factors, including varieties, growing season, geographic location and environmental stress [12,15].

### 1.2.1. Macronutrients

On the average, oil and protein together constitute about 60% of dry soybeans. The remaining dry matter is composed of mainly carbohydrates (about 35%) and ash (5%) [15].

Soybeans store their lipids in an organelle known as oil bodies or lipid-containing vesicles. Their lipids are mainly in the form of triglycerides or triacylglycerols, with varying fatty acids as part of their structure. Triglycerides are neutral lipids, each consisting of three fatty acids and one glycerol that bound to three acids. Both saturated and unsaturated can occur in the glycerides of soybean oil, however the fatty acids of soybean oil are primarily unsaturated [15,16]. The highest percentage of fatty acid in soybean oil is linoleic acid, following in

decreasing order by oleic, palmitic, linolenic and stearic acid. Soybean oil contains some minor fatty acids, including arachidic, behenic, palmitoleic and myristic acid [16].

Protein content varies between 36% and 46% depending on the variety [17-19]. This component is present in the greatest amount in soybeans. Seed proteins are usually classified based on biological function in plants (metabolic proteins and storage proteins) and on solubility patterns. According to their functionality, metabolic proteins (such as enzymatic and structural) are concerned in normal cellular activities, including even the synthesis of storage proteins. Storage proteins, together with reserve of oils, are synthesized during soybean seed development and provide a source of nitrogen and carbon skeletons for the development seedling. In soybeans, most of proteins are storage type. A solubility pattern divides proteins into those soluble in water (albumins) and in salt solution (globulins). Globulins are further divided in legumins and vicilins. Under this classification system, most of soy protein is globulin. Certain soy proteins have their trivial names, as glycinin (legumins) and conglycinin (vicilins). Others, particularly those with enzymatic function, are based on the biological function of proteins themselves. Examples include hemagglutinin, trypsin inhibitors and lipoxygenases. A solubility classification can pose problems because an association with other proteins can change their solubility profile. Thus, a more precise means of identifying proteins has been based on approximate sedimentation coefficient using ultracentrifugation to separate seed proteins. Under appropriate buffer conditions, soy proteins exhibit four fractions after centrifugation. These fractions are designed as 2, 7, 11 and 15S. The major portion of the protein component is formed by storage proteins such as 7S globulin ( $\beta$ -conglycinin) and 11S globulin (glycinin), which represent about 80% of the total protein content [17]. Other proteins or peptides present in lower amounts include enzymes such as lipoxygenase, chalcone synthase and catalase.

On average, moisture-free soybeans contain about 35% of carbohydrates and defatted dehulled soy grits and flour contain about 17% soluble and 21% insoluble carbohydrates. Therefore, they are the second largest component in soybeans. However, the economical value of soy carbohydrates is considered much less important than soy protein and oil. A limited use of soybeans in human diet is due the flatulence produced by soluble carbohydrates such as raffinose and stachyose. Humans lack the enzymes to hydrolyze the galactosidic linkages of raffinose and stachyose to simpler sugars, so the compounds enter the lower intestinal tract intact, where they are metabolized by bacteria to produce flatus [16]. As a result, relatively fewer efforts have been made to study soy carbohydrates and their potential utilization. The principal use of soybean carbohydrate has been in animal feeds (primarily ruminant because they can digest the compound better than monogastric animals) where it contributes calories to the diet. Food processing can alter the carbohydrates composition making them more digestible to human organism. Although the presence of these oligosaccharides is generally considered undesirable because of their flatus activity, some studies shown some beneficial effects of dietary oligosaccharides in humans, mainly due to a increasing population of indigenous bifidobacteria in colon, such as: suppressing the activity of putrefactive bacteria by antagonist effect; preventing pathogenic and autogenous diarrhea; anti-constipation due to the production of high levels of short-chain fatty acids; pro-

ducing nutrients such as vitamins. Other positive effects are: toxic metabolites and detrimental enzymes reduction; protecting liver function due to reduction of toxic metabolites; blood pressure reduction; anticancer effects [15,16].

### 1.2.2. Micronutrients

Soybean also contains a wide range of micronutrients and phytochemicals including minerals, vitamins, phytic acid (1.0–2.2%), sterols (0.23–0.46%) and saponins (0.17–6.16%) [20].

The primary inorganic compounds of the soybeans are minerals. Potassium is found in the highest concentration, followed by phosphorus, magnesium, sulfur, calcium, chloride and sodium, which vary in concentration to the variety, growing location and season. Minor minerals include silicon, iron, zinc, manganese, copper and other [15,16].

Both water-soluble and oil-soluble vitamins are present in soybeans. Water-soluble vitamins in soybeans include thiamin, riboflavin, niacin, pantothenic acid and folic acid. They are not substantially lost during oil extraction and subsequently toasting of flakes. The oil-soluble vitamins present in soybeans are vitamins A and E. Vitamin E is especially unstable during soybean processing. During solvent extraction of soybeans, vitamin E goes with oil [15,16].

Phytate is the calcium-magnesium-potassium salt of inositol hexaphosphoric acid commonly known as phytic acid. As in the most seeds, phytate is the principal source of phosphorus in soybeans. Its content depends on not only variety, but also growing conditions and assay methodology [15,16].

One important group of minor compounds present in soybean that has received considerable attention is a class of phytoestrogen called the isoflavones. Phytoestrogens are non-steroidal compounds that bind to and activate estrogen receptors (ERs)  $\alpha$  and  $\beta$ , due to the fact that they mimic the conformational structure of estradiol. Phytoestrogens are naturally occurring plant compounds found in numerous fruits and vegetables, and are categorized into three classes: the isoflavones, lignans and coumestans [21,22]. Isoflavone compounds have been considered as nonnutrients, because they neither yield any energy nor function as vitamins. However, they play significant roles in the prevention of several diseases, so they may be considered health-promoting substances.

Isoflavones belong to a group of compounds that share a basic structure consisting of two benzyl rings joined by a three-carbon bridge, which may or may not be closed in a pyran ring. This group of compounds is known as flavonoids, which include by far the largest and most diverse range of plant phenolics [15,16].

Isoflavones are present in just a few botanical families, because of the limited distribution of the enzyme chalcone isomerase, which converts 2(R)-naringenin, a flavone precursor, into 2-hydroxydaidzein. The soybean is unique in that it contains the highest amount of isoflavones, being up to 3 mg.g<sup>-1</sup> dry weight [15,23].

The isoflavones in soybeans and soy products are of three types, with each type being present in four chemical forms. Therefore there are twelve isomers of isoflavones.

Isoflavones in soybean are mainly found as aglycones (daidzein, genistein, glycitein) (Figure 1),  $\beta$ -glucosides (daidzin, genistin, glycitin), malonyl- $\beta$ -glucosides (6''-O-malonyldaidzin, 6''-O-malonylgenistin, 6''-O-malonylglycitin) and acetyl- $\beta$ -glucosides (6''-O-acetyldaidzin, 6''-O-acetylgenistin, 6''-O-acetylglycitin) (Figure 2) [24].

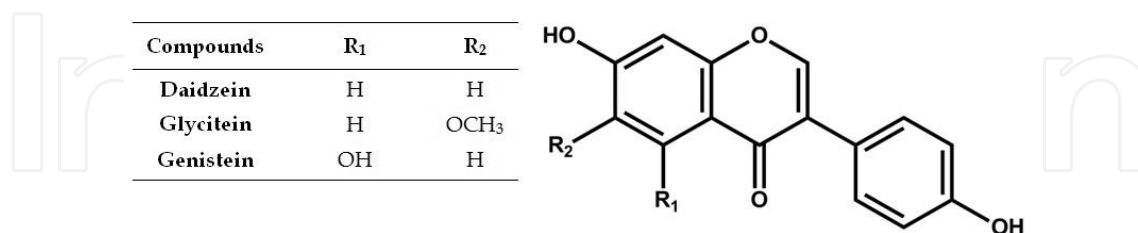


Figure 1. Chemical structure of soy aglycones.

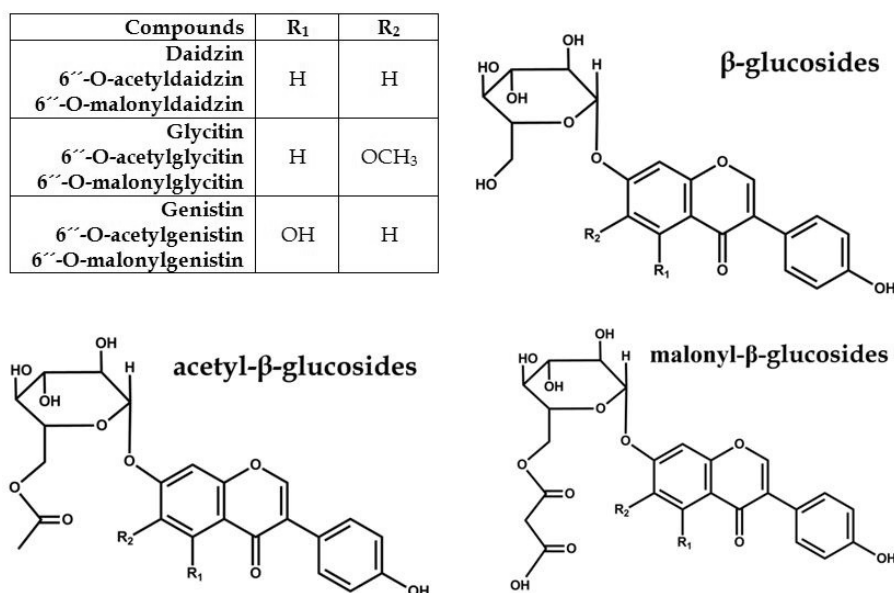


Figure 2. Chemical structure of  $\beta$ -glucosides soybeans isoflavones.

Aglycones are flavonoid molecules without any attached sugars or other modifiers. Among the different forms of isoflavones, aglycones are especially important because they are readily bioavailable to humans [24]. Flavonoid  $\beta$ -glucosides also may carry additional small molecular modifiers, such as malonyl and acetyl groups. Sugar-linked flavonoids are called glycosides. The term glucoside only applies to flavonoids linked to glucose [25]. The malonyl- $\beta$ -glucosides are the predominant form in conventional raw soybean [24].

Isoflavones concentration and composition vary greatly with structural parts within soybean seed. The concentration of total isoflavones in soybean hypocotyl is 5.5-6 times higher than that in cotyledons. Glycitein and its three derivatives occur extensively in the hypocotyl. Isoflavones are almost absent in seed coats [15]. The isoflavone content varies

among soybean varieties, but most varieties contain approximately 100–400 mg per 100 g dry basis [26].

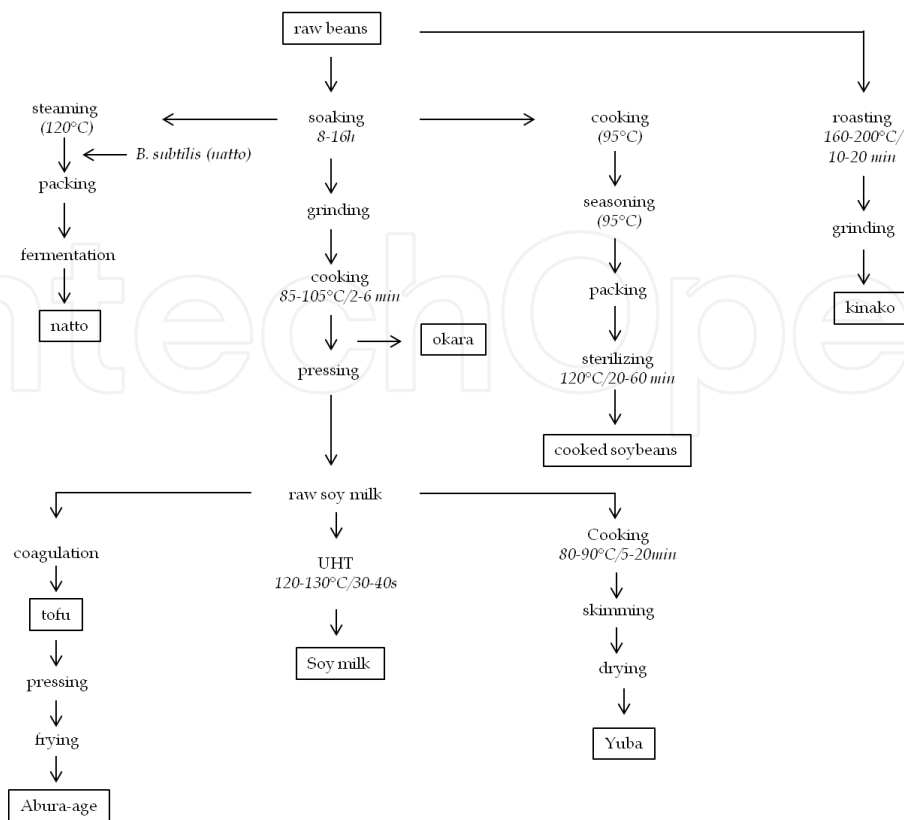
### 1.3. Isoflavones importance in human health

Isoflavones have received much attention because of their weak estrogenic property and other beneficial functions [24]. A large number of researchers have reported the positive aspects of isoflavones on human health, such as the ability to reduce the risk of cardiovascular, atherosclerotic and haemolytic diseases; alleviation of osteoporosis, menopausal and blood-cholesterol related symptoms; inhibition of the growth of hormone-related human breast cancer and prostate cancer cell lines in culture; and increased antioxidant effect in human subjects [27-30]. Isoflavones are also known for having anticancer activity and an effect on cell cycle and growth control [31-33].

Isoflavones in glycosides forms are poorly absorbed in the small intestine, due to their higher molecular weight and hydrophilicity. However bacteria in the intestine wall can biologically activate by action of  $\beta$ -glucosidase to their corresponding bioactive aglycone forms. Once hydrolyzed, aglycone forms are absorbed in the upper small intestine by passive diffusion. Nevertheless, pharmacokinetic studies confirm that healthy adults absorb isoflavones rapidly and efficiently. The average time to ingested aglycones reach peak plasma concentrations is about 4–7 h, which is delayed to 8–11 h for the corresponding  $\beta$ -glycosides. Despite the fast absorption, isoflavones or their metabolites are also rapidly excreted [13].

### 1.4. Food processing effect in isoflavones

Several investigations have been performed during the last years on soybeans consumption and their benefits, however the effects of seed processing and soybean processing into foods on the distribution of soy isoflavones are sparse. Processing significantly affects the retention and distribution of isoflavone isomers in soyfoods. The conversion and loss of isoflavones during processing significantly affect the nutraceutical values of soybean. Post-harvest changes in isoflavones in soybeans are influenced by processing methods however genotype has an effect on isoflavones profiles during seed development [34] and the environment has a greater effect than the genotype [26,35-37]. Distribution of isoflavones in soybean can be altered during various processing steps including fermentation, cooking, frying, roasting, drying and storage [24]. These effects in isoflavones can be even accelerated by heat, acid, alkaline and enzymes [38-40] (Figure 3). The effects of several food processing techniques on soybeans isoflavones will be reviewed and discussed in the following section.



**Figure 3.** Flow diagram illustrating the processing of soybeans to commercial soybeans products. Adapted from Toda *et al.* [41].

#### 1.4.1. Harvesting

Harvesting soybean seeds after their development and maturation is a critical step in profitable soybean production. Although most soybeans are harvested at the dry mature stage, a very small portion is harvested at the immature stage in certain regions. Immature soybeans refer to soybeans harvested at 80% maturity. The immature seed is used as a vegetable or as ingredient recipes. Soybeans are considered dry mature when seed moisture reduces to less than 14% in the field [15,42]. At this stage, seeds are ready for harvesting. The exact harvesting date depends on the variety, growing regions, planting date and local weather conditions [15].

#### 1.4.2. Drying

Drying is an important process to extend the shelf life or to prepare food, including soybean, for subsequent production [43]. After harvest, if moisture content is more than 14%, soybeans need to be dried immediately in order to the following reasons: meet the quality standard of soybean trading; retain maximum quality of the grain; reach a level of moisture that does not allow the growth of bacteria and fungi and; prevent germination of seeds. Drying could be done naturally or artificially. Sun drying, or natural drying, soybeans are spread on the threshing for 2-3 days with frequently turning between top to bottom layers.



Once dried, seeds are transferred to storage facilities. Sun drying is not suitable for large quantities of soybeans or under humid and cloudy weather conditions. Artificially drying is carried out with various mechanical driers, including low temperature driers, on-the-floor driers, in-bin driers, medium temperature driers, tray driers, multiduct ventilated driers, countercurrent open-flame grain driers and solar driers. Regardless of which driers are used, caution is required so as to avoid too rapid drying; rapid drying hardens outlayers of seeds and seals moisture within the inner layer. Although the temperature of soybeans must be raised sufficiently to achieve the desired moisture content during drying process, excess heating (not exceed 76%) should be avoided to protect beans from discoloration and beans proteins from denaturation [15].

It is well known that drying significantly affects the quality and nutrients of dried food products. Drying temperatures affect the activity and stability due to chemical and enzymatic degradation which can for example alter significantly the isomeric distribution of the 3 aglycones [44]. All forms of isoflavones are generally lost due to thermal degradation and oxidation reactions during processing [45]. Conventional thermal treatment decreases malonyl derivatives into  $\beta$ -glucosides via intra-conversion while aglycones have higher heat resistance. Dry heat treatment such as frying, toasting, or baking process increases the formation of acetyl derivatives of isoflavones through decarboxylation from malonyl derivatives [24,41].

A comparison between freeze-drying and drum-drying of germ flour demonstrated that the former contained higher isoflavone aglycones than the latter. However, isoflavone glucoside contents in freeze-dried germ flour were lower than those of drum-dried germ flour. The content of isoflavone glucosides was significantly lower in processed (drum- and freeze-dried) germ flours compared with that of unprocessed germ flour because of conversion of isoflavone glucosides to isoflavone aglycones. Total isoflavone contents of drum-dried and freeze-dried germ flours were comparable but more than that of unprocessed germ flour [46].

Different drying methods (hot-air fluidized bed drying, HAFBD; superheated-steam fluidized bed drying, SSFBD; gas-fired infrared combined with hot air vibrating drying, GFIR-HAVD) were compared at various drying temperatures (50, 70, 130 and 150 °C). Higher drying temperatures led to higher drying rates and higher levels of  $\beta$ -glucosides and antioxidant activity, but to lower levels of malonyl- $\beta$ -glucosides, acetyl- $\beta$ -glucosides and total isoflavones. Comparing different drying methods to each other, at the same drying temperature GFIR-HAVD resulted in the highest drying rates and the highest levels of  $\beta$ -glucosides, aglycones and total isoflavones, antioxidant activity as well as  $\alpha$ -glucosidase inhibitory activity of dried soybean. A drying temperature of 130 °C gave the highest levels of aglycones and  $\alpha$ -glucosidase inhibitory activity in all cases [47].

Dehydration also helps to achieve longer shelf life and easier transportation and storage, enabling wider distribution of a product. Fermentation of soymilk with microorganisms with  $\beta$ -glucosidase activity promotes the biotransformation of isoflavone glucosides into bioactive aglycones, brings down the contents of aldehydes and alcohols responsible for the beany flavor in soy milk [48] and reduces the content of indigestible oligosaccharides. A spray-drying technology has been also applied to fermented soymilk. Daidzein and genis-

tein retention were of about 87.6% and 85.3%, respectively. Retention was better at the lower inlet air temperatures. Coarser droplets formed at higher feed rates helped in higher retention of isoflavones because it reduced the incidence of direct exposure of isoflavones to higher temperature [49].

#### 1.4.3. Storage

Soybeans are stored at farms, elevators and processing plants in various types of storage structures (steel tanks or concrete silos) before being channeled to next destination, and finally to processing. Loss in quality of soybeans during storage results from the biological activities of seeds themselves, microbiological activities and attacks of insects, mites and rodents. Quality loss is characterized by reduced seed viability and germination rate, coloration, reduced water absorption, compositional changes and ultimately reduced quality of protein and oil. Heat damage is a major cause of quality loss. Characterized by darkening of seed color coat, it results mainly from the improper control of temperature and moisture during storage and transportation. The excess presence of foreign matter can also cause heat buildup. Thus, cleaning soybeans before drying minimizes heat damage. Although minor losses are inevitable, major losses can be prevented by carefully control of storage temperature and humidity. Any biological activity requires a certain level of moisture present. Higher moisture content (or high moisture humidity) not only promotes bacteria and mold infection but also speeds up biological activity of seed themselves. Excessive moisture may also lead to seed germination. Generally, moisture content of 13.5% or below is considered to ensure storage stability of soybeans over reasonably long periods. However, this is true only when temperatures are kept below certain levels [15].

Storage conditions have also an important effect in the composition of soybeans, including isoflavone, anthocyanin, protein, oil and fatty acid.

Variation in isoflavone contents of different soybean cultivars were evaluated under different location and storage duration. Total isoflavone contents of soybeans stored for 1 year were only slightly higher than those of soybeans stored for 2 or 3 years. However, the concentrations of individual isoflavones, especially 6''-O-malonyldaidzin and 6''-O-malonylgenistin, decreased markedly in soybeans stored for 2 or 3 years, probably due to high temperatures during storage and oxidative reactions which transformed malonylated type to glycoside and aglycone groups, increasing their amounts with longer storage. They also pointed that the effect of crop year seemed to have a much greater influence on the variation in isoflavone content than did the location because of weather differences from one year to another. Differences among cultivars have been already expected [35]. Similar results were found comparing cropping year and storage for 3 years of soybeans seeds. Malonyldaidzin and malonylgenistin concentrations also decreased and the concentration of glucosides increased slightly over the 3 years [50]. Storage effect in soybeans isoflavones have been exhaustively studied, and a markedly decreased in isoflavones content is proportional to storage periods, whereas protein, oil, and fatty acid of black soybeans showed a slight decrease over storage at room temperature [51].

A combined effect of storage temperature and water activity was evaluated on the content and profile of isoflavones of soy protein isolates and defatted soy flours. Storage for up to 1 year of soy products, at temperatures from -18 to 42 °C, had no effect on the total content of isoflavones, but the profile changed drastically at 42 °C, with a significant decrease of the percentage of malonyl glucosides with a proportional increase of  $\beta$ -glucosides. A similar effect was observed for soy protein isolates stored at  $a_w = 0.87$  for 1 month. For defatted soy flours, however, there was observed a great increase in aglycons (from 10 to 79%), probably due to the action of endogenous  $\beta$ -glucosidases [52].

#### 1.4.4. Fermentation

Processing and fermentation of soybean has been reported to influence the forms isoflavones take. Studies have shown that the fermentation process of soybean promotes changes in the phytochemical compounds, causing changes in the isoflavone forms, hydrolysing the proteins and reducing the antinutritional factors, by reducing trypsin inhibitor content [53-55]. Fermentation process of soy leads to manufacturing different soy fermented foods, such as tempeh, soy extract, miso and natto.

Differences on isoflavones content between non-fermented and fermented soybean products have been extensively describe in literature in the last years. Isoflavone glucosides were the major components in soybean and non-fermented products, while isoflavone aglycones were abundant in sufu and partially in miso of soybean fermented products [56].

Tempeh is a traditional fermented soybean food product from Indonesia. It is normally consumed fried, boiled, steamed or roasted. A way to processing soybeans into tempeh occurs by fungus mediated fermentation. Several authors have already studied the effect of fermentation on isoflavones content. It was reported an increase of aglycones amount with fermentation time of tempeh, approximately two-fold higher after 24 h fermentation [57]. Later on, similar results were found by Haron *et al.* [58] who reported higher values of aglycone forms in raw tempeh. In addition, these researchers showed that fried tempeh had its total isoflavone content reduced in almost 50% during frying processing (reduction from 205 to 113 mg in 100 g of fried tempeh). A combined process of fermentation and refrigeration was evaluated by Ferreira *et al.* [25]. They quantified isoflavones content of two different soybean cultivars (low isoflavone content versus high isoflavone content cultivar) during processing of tempeh combined to refrigeration at 4 °C for 6, 12, 18, and 24 hours. After 24 hours fermentation, isoflavone glucosides were 50% reduced, and the aglycone forms in the tempeh from both cultivars was increased. The malonyl forms reduced 83% after cooking. Refrigeration process up to 24 hours did not affect the isoflavone profile of tempeh from either cultivar. The fermentation process improves the nutritional value of tempeh by increasing the availability of isoflavone aglycones. Fermented soy foods, which are usually prepared by mixing soy with other components such as barley, rice and wheat, contained isoflavones at lower concentrations. In addition these fermented soy foods contain predominantly isoflavone aglycones [15]. Fermentation with microorganisms or natural products containing high  $\beta$ -glucosidase activity converts  $\beta$ -glucosides into corresponding aglycones by breaking the carbohydrate bond [59,60]. These aglycones exist in smaller amounts in other nonfermented

soy products such as tofu and soymilk [37,61]. Other soybean products or by-products showed a similar behavior. Soy pulp is generated as a by-product during tofu or soymilk production and is sometimes used as animal food but is mainly burnt as waste. Fermentation increased isoflavone aglycone contents in black soybean pulp. Genistein concentrations in black soybean pulp were 6.8 and 7.2 fold higher than controls respectively after 12 h and 24 h of fermentation with *L. acidophilus*. Fermentation with *B. subtilis* showed a similar genistein concentration increase [62]. The effect of fermentation of whole soybean flour was investigated and also showed a conversion of isoflavone glycosides to the aglycone form [63].

Sufu, a fermented tofu product, showed ambiguous changes in isoflavone contents during manufacturing. Sufu manufacturing procedure promoted a significant loss of isoflavone content mainly attributed to the preparation of tofu and salting of pehtze. The isoflavone composition was altered during sufu processing. The initial fermentation corresponded to the fastest period of isoflavone conversion. Aglycones levels increased while the corresponding levels of glucosides decreased. The changes in the isoflavone composition were significantly related to the activity of  $\beta$ -glucosidase during sufu fermentation, which was inhibited by the NaCl content [64]. These influence of processing and NaCl supplementation on isoflavone contents was also investigated during douchi manufacturing. Douchi is a popular Chinese fermented soybean food. These results indicated that 61% of the isoflavones in raw soybeans were lost when NaCl content was 10%. Indeed, changes in isoflavone isomer distribution were found to be related to  $\beta$ -glucosidase activity during fermentation, which was affected by NaCl supplementation [65].

Other fermented soybean products are miso and soy sauce. Their production involves the application of pressurized steaming following to fermentation by bacteria for a lengthy period. During the fermentation of miso and soy sauce,  $\beta$ -glucosides are also reported to be hydrolyzed to aglycons by  $\beta$ -glucosidase of bacteria [41].

Fermentation shows the same pattern on isoflavones transformation, no matter the fermented soybean product. The fungi-fermented black soybeans (koji) also contained a higher content of aglycone, the bioactive isoflavone, than did the unfermented black soybeans [66]. However, it was later realized that the contents of various isoflavone isomers in black soybean koji may reduce during after 120 days of storage. Although the retention of isoflavone varied with storage temperature, packaging condition enabled black soybean koji to retain the highest residual of isoflavone [67].

#### 1.4.5. Non-fermentation processing

Tofu is a popular nonfermented soy food. Processing of tofu involves soaking and heating procedures as well as the addition of protein coagulants such as calcium sulfate to soymilk to coagulate to make tofu. This soybean product has been also target of several studies during its manufacturing. Results of the stability of isoflavone during processing of tofu showed that the concentrations of the three aglycones increased with increasing soaking temperature and time, while a reversed trend was found for the other nine isoflavones. Tofu produced with 0.3% calcium sulfate was found to contain the highest total isoflavones yield (2272.3  $\mu\text{g/g}$ ) whereas a higher level (0.7%) of calcium sulfate resulted in a lower yield

(1956.6  $\mu\text{g/g}$ ) of total isoflavones in tofu. In the same study, authors showed that during processing of soymilk, an increase of concentration for  $\beta$ -glucosides and acetyl genistin, whereas malonyl glucosides exhibited a decreased tendency and the aglycones did not show significant change [68]. Previous reports have already demonstrated that during soaking of soybean malonyl glucosides can be converted to acetyl glucosides, which can be further converted to glycosides or aglycones depending on soaking temperature and time [34,69].

Regarding soymilk isoflavones, a research was done on the transformation of isoflavones and the  $\beta$ -glucosidase activity in soymilk during fermentation. Regardless of employing a lactic acid bacteria or a bifidobacteria as starter organism, fermentation causes a major reduction in the contents of glucoside, malonyl glucoside and acetyl glucoside isoflavones along with a significant increase of aglycone isoflavones content. Indeed, the increase of aglycones and decrease of glucoside isoflavones during fermentation coincided with the increase of  $\beta$ -glucosidase activity observed in fermented soymilk [70].

#### 1.4.6. Heating processing

Distribution of isoflavones in soybeans and soybeans products are significantly affected by the method, temperatures and duration of heating.

Toasted soy flour and isolated soy protein had moderate amounts of each of the isoflavone conjugates. Pananum *et al.* [44] evaluated the effect of longer toasting of defatted soy flakes at 150 °C. They stated that toasting led to higher aglycone concentration, which increased the total phenolic recovery. Apparently, malonyl glucoside conjugates are thermally unstable and are converted to their corresponding isoflavone glycosides at high temperatures [15]. The chemical modification of isoflavones in soy foods during cooking and processing studies showed interesting results on isoflavones stability. Baking or frying of textured vegetable protein at 190 °C and baking of soy flour in cookies did not alter total isoflavone content. However, there was a steady increase in  $\beta$ -glucoside conjugates at the expense of 6''-O-malonylglucosides conjugates [71]. The de-esterifying reaction was presumably a result of transesterification of the ester linkage between the malonate or acetate carboxyl group and the 6''-O-hydroxyl group of the glucose moiety, yielding methyl malonate or methyl acetate and the isoflavone glucoside [15].

Roasting has been used to deactivate anti-nutritional components in soybeans and to give characteristic flavour and brown color to final products [43]. Kinako is a soybean product produced by roasting raw soybeans around 200 °C for 10-20 min, following by grinding. Roasting also promotes changes in isoflavones contents. Soybeans roasted upon at 200 °C without prior soaking in water showed a change in isoflavones profile. It was found that at the first 10 min of roasting caused an increase in 6''-O-acetyl- $\beta$ -glucosides while 6''-O-malonyl- $\beta$ -glucosides decreased drastically. Continued roasting showed a slightly decreased proportion of the 6''-O-acetyl- $\beta$ -glucosides. These authors proposed that most 6''-O-malonyl- $\beta$ -glucosides were decarboxylated and changed to 6''-O-acetyl- $\beta$ -glucosides when roasted at a high temperature. Besides,  $\beta$ -glucosides and aglycons also increased gradually over time [41]. A similar trend was found roasting soybeans at 200 °C for 7, 14 and 21 min. Ma-

lonyl derivatives decreased drastically and acetyl- $\beta$ -glucosides and  $\beta$ -glucosides increased significantly [24].

Similar to the roasting process, explosive puffing caused a significant decrease in malonyl derivatives and significant increase in acetyl derivatives and  $\beta$ -glucosides through 686 kPa explosive puffing treatment. Otherwise, aglycones did not increase during the explosive puffing process. This fact was suggested due that temperature of explosive puffing may not be high enough to cleave glycosidic linkage between  $\beta$ -glucopyranose and aglycones [24].

Some soy products are prepared by frying process. Abura-age is one of them being produced by frying tofu in oil. As pointed by Toda *et al.* [41], In comparison with tofu, heating in oil at a high temperature to produce abura-age resulted in a smaller proportion of 6''-O-malonyl- $\beta$ -glucosides and a higher proportion of 6''-O-acetyl- $\beta$ -glucosides.

Simonne *et al.* [34] evaluated the retention and changes of soy isoflavones in immature soybeans seeds during processing and found total isoflavone retention percentages means of 46% after boiling, 53% after freezing and 40% after freeze-drying. They assumed that probably the loss of isoflavones could be due to their leaching into the cooking water, however these authors did not analyze isoflavones in the cooking water. In the same study, they noted that boiling process also caused a substantial increase in daidzin, genistin, and genistein. In a previous work on soy milk production and cooking of dry soy products, it was proposed that hydrolysis of the malonyl and acetyl glucosides during boiling probably contributes to the conversion of isoflavone forms [71]. Changes in isoflavone compositions of different soybean foods during cooking process were performed later and supported this proposition [41]. Concerning to a possible leaching effect of isoflavones during blanching or boiling, Wang *et al.* [72] reported about 26% retention of isoflavones during the production of soy protein isolate. This soybean product is made by extracting soy flour under slightly alkaline pH, followed by precipitation, washing, and drying. Soy protein isolate showed a different isoflavone profile in comparison to soy flour. The former contained much more aglycones (genistein and daidzein), while the latter had almost none [12,72]. The high content of aglycones in soy protein isolate was probably due to the hydrolysis of glycosides. The percentages of total isoflavones lost during extraction, precipitation, and washing were 19, 14, and 22%, respectively. Washing was the step where most isoflavones were lost [72]. This statement was supported by another study on thermal processing of tofu. It was demonstrated a significant total isoflavone content decrease, most likely due to leaching of isoflavones into the water [69].

An approach on the stability of isoflavones in soy milk stored at temperatures ranging from 15 to 90 °C showed that genistin in soy milk is labile to degradation during storage. Although the loss rate was low at ambient temperatures, authors highlighted the potential loss of genistin when estimating shelf life of soy milk products [73].

Influence of thermal processing such as boiling, regular steaming and pressure steaming were also investigated in yellow and black soybeans. Again, all thermal processing caused significant increases in aglycones and  $\beta$ -glucosides of isoflavones, but caused significant decreases in malonyl glucosides of isoflavones for both kinds of soybeans. The malonyl

glucosides decreased dramatically with an increase in  $\beta$ -glucosides and aglycones after thermal processing [74].

#### 1.4.7. Irradiation

Food irradiation is a process in which food is exposed to ionizing radiations such as high energy electrons and X-rays produced by machine sources or gamma rays emitted from the radioisotopes  $^{60}\text{Co}$  and  $^{137}\text{Cs}$ . Depending on the absorbed radiation dose, various effects can be achieved resulting in reduced storage losses, extended shelf life and/or improved microbiological and parasitological safety of foods [75,76]. Food irradiation is one of the most effective means to disinfect dry food ingredients. Disinfestation is aimed at preventing losses caused by insects in stored grains, pulses, flour, cereals, coffee beans, dried fruits, dried nuts, and other food products. The dosage required for insect control is fairly low, of the order of 1 kGy or less [77,78].

Soybeans have been processed by ionizing radiation in order to improve their properties. An important improvement in microbiological properties, such as insect disinfestation and microbial contamination, can be achieved by radiation treatment. Physical properties are also enhanced, such as reduction of soaking and cooking time. Indeed, higher radiation doses may break glycosidic linkages in soybean oligosaccharides to produce more sucrose and decrease the content of flatulence causing oligosaccharides [79].

Gamma irradiation at 2.5-10.0 kGy caused the reduction of soaking time in soybeans by 2-5 hours and the reduction of cooking time by 30-60% compared to non-irradiated control samples. The irradiation efficacy on physical quality improvement was also recognized in stored soybeans for one year at room temperature [80,81].

Influence of radiation processing on isoflavones content has been also studied in the last years. Gamma-radiated (0.5-5.0 kGy) soybeans showed a radiation-induced enhancement of antioxidant contents. Interestingly, a decrease in content of glycosidic conjugates and an increase in aglycons were noted with increasing radiation doses. These results suggested a radiation-induced breakdown of glycosides resulting in release of free isoflavones. Whereas the content of genistein increased with radiation dose, that of daidzein showed an initial increase at a dose of 0.5 kGy and then decreased at higher doses. Degradation of daidzein beyond 0.5 kGy could thus be assumed. Glycitein appears to be the least stable among the three aglycons as its content decreased at all of the doses studied [82]. Gamma irradiation also induced an enhancement in isoflavones content of varying seed coat colored soybean up to a radiation dose of 0.5 kGy. However, the genotypes showed decrease in total isoflavone content at a higher radiation dose of 2.0 and 5.0 kGy [83].

Such as gamma-irradiation, an enhanced effect on soy germ isoflavones was found after electron beam processing. Interestingly, this study showed that applied radiation doses ranging from 1.0 to 20.0 kGy showed an increase in the amount of both glucosides and aglycones simultaneously [84].

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