Plant Foods for Human Nutrition **62:** 53–58, 2007.

DOI: 10.1007/s11130-007-0041-0

Commercial Soy Protein Ingredients as Isoflavone Sources for Functional Foods

MARIA INÉS GENOVESE,* ANA CRISTINA LOPES BARBOSA, MÁRCIA DA SILVA PINTO & FRANCO M. LAJOLO

*Departamento de Alimentos e Nutrição Experimental, Universidade de São Paulo, Av. Prof. Lineu Prestes, 580, Bloco 14, Cep 05508-900, São Paulo, SP Brazil (*author for correspondence; e-mail: genovese@usp.br)*

Published online: 28 February 2007

Abstract. The knowledge of the contents and profile of isoflavones present in soy protein ingredients, as well as the effect of industrial processing, is important for the development of functional foods rich in these compounds. The results obtained here showed that the total isoflavone content varied significantly among products. For defatted and whole soy flours the total isoflavone content ranged from 120 to 340 mg/100 g, for soy protein isolates from 88 to 164 mg/100 g, and for commercial textured soy proteins, from 66 to 183 mg/100 g (wet basis, expressed as aglycones). The highest isoflavone content was found for soy hypocotyl flours, from 542 to 851 mg/100 g. Compared to hypocotyl and whole and defatted flours, soy ingredients presented a decrease of malonylglycosides and deesterified β-glycosides with a significant increase in the percentage of aglycones, mainly for soy fibers (65–76%). While defatting was shown to cause isoflavone concentration without altering conjugation, extrusion process caused destruction of isoflavones and a significant increase in the amount of acetylglycosides, but this effect was less intense for the concentrates. From the results obtained it can be concluded that differences in isoflavone concentration and profile may be related to oscillations in the isoflavone content present in the raw material and to the type of processing.

Key words: isoflavones, soy protein ingredients, processing, functional foods

Introduction

Isoflavones represent the most common group of phytoestrogens and have been associated to beneficial effects in humans, such as prevention of cancer, cardiovascular diseases, osteoporosis and menopausal symptoms [1]. These bioactive substances are found in particularly high levels in soybeans as glycosilated conjugates (malonylglycosides and underivatized β-glycosides), which, as a result of processing, form aglycones and acetylglycosides [2]. The biological significance of the presence of these different forms in soybeans is not clear yet, but differences in their antioxidant activities and bioavailabilities were reported [3–5].

The levels of the soy isoflavones, daidzein, genistein and glycitein, are affected by genetic and environmental factors which, depending on processing conditions, determine the concentration and profile of these compounds in soy based products and soy protein ingredients [6–9]. The levels of isoflavones in soy based foods consumed in Brazil was determined as varying from 2 (soy based enteral/oral diets) to 100 (textured soy proteins used as meat substitutes) mg/100 g [8]. As in Brazil the consumption of soybeans is not as widespread as that of beans, there is an increasing interest of the food industry on the development of functional foods rich in isoflavones, based on the utilization of soy protein ingredients.

Soy protein ingredients such as defatted soy flours, protein isolates, concentrates and textured proteins are already largely used in the food industry because of their functional properties such as geleification, water absorbing and emulsifying capacities [10].

The major isoflavones in unprocessed soybean-malonylgenistin, genistin, malonyldaidzin and daidzin- can be lost and/or transformed into other forms of isoflavones during processing [11]. Important losses are observed during soaking, heating, filtration, and alkaline extraction, used in soy ingredients production [11–14]. Storage was also found to alter isoflavone profile of soy protein ingredients [15].

The development of food products rich in isoflavones depends on the knowledge of the content present in the various soy ingredients and the effect of processing on these compounds. Some studies in lab or pilot-plant scale have already been conducted [16, 17]. The objectives of this work were to determine the isoflavone content and distribution in soy ingredients available in the Brazilian market and to evaluate the effect of industrial processing.

Materials and Methods

Materials

Soy ingredients were obtained at the local market and also supplied by Exin International Ltda (São Paulo, SP). The samples analyzed consisted of: whole soy flours (WF) and defatted flours (DSF), soy protein isolates (SPI), soy protein concentrates (SPC), textured proteins (textured defatted flours—TDF- and textured protein concentrates—TPC), soy hypocotyl flours (SH), textured soy hypocotyl flours (TSH), and soy dietary fibers (SF). All chemicals and solvents were reagent or HPLC grade.

Isoflavone Extraction

Powdered samples (1 g) were extracted with 80% aqueous methanol (20:1 v/w) under agitation for 2 h at 4°C, except for the textured proteins, for which the extraction was performed for 3 h, same conditions and ratio, according to Genovese and Lajolo [7]. The homogenate was filtered through filter paper Whatman no 06 and concentrated until methanol elimination on a rotatory evaporator (Rotavapor® RE 120, Büchi, Flawil, Sweden) at ≤40°C. The volume of the extracts was adjusted to 5 mL with HPLC grade methanol, and aliquots were filtered through a 0.22 μm PTFE (polytetrafluoroethylene) filter unit (Millipore Ltd., Bedford, MA) and analyzed by HPLC. The extractions were run in triplicate.

HPLC Quantitation of Isoflavones

Isoflavone quantitation was performed according to Song et al. [18] with a C18 NovaPak (30 cm × 4.6 mm i.d.) column (Waters, Milford, MA) and a Hewlett-Packard 1100 system with autosampler and quaternary pump coupled to a diode array detector (Palo Alto, CA), based on external calibration. Standards of daidzein and genistein were from Sigma Chemicals Co. (St. Louis, MO), daidzin and genistin were from Apin Chemicals Ltd. (Abingdon, UK), glycitin and glycitein were from Nacalai-Tesque Inc. (Kyoto, Japan) and acetylglycosides and malonylglycosides were from LC Laboratories (Woburn, MA). Total isoflavone contents were expressed as mg of aglycone/100 g of sample fresh weight (FW), after normalization of individual isoflavones to account for differences in molecular weight between glycoside derivatives. The mass of each isoflavone form (β-glycoside, malonylglycoside and acetylglycoside) was multiplied by the ratio of its aglycone molecular weight to the molecular weight of the individual form before summing.

Statistical Analysis

Statistical analysis was done by using the Statistic software package version 5.0 (StatSoft, Inc., Tulsa, OK). All the analysis were performed in triplicate and results expressed as mean ± standard deviation. Differences between means were first analyzed by ANOVA test and then least significant difference (LSD) test ($p < 0.05$).

Results and Discussion

Isoflavone Contents of Soy Ingredients

Soybean flours are normally produced by a process consisting of cleaning, heating, cracking, dehulling, grinding and, for defatted flours, removing the oil with hexane. This is the starting material for most commercial soybean products such as protein isolates and concentrates [19]. The isoflavone content of soy flours depends directly on that of the seeds used in their production, which was previously shown to be related to the variety and the growing conditions [20–22]. The isoflavone content reported for Brazilian soybean varieties was in the range of 57–188 mg per 100 g [9]. As shown in Fig. 1, whole flours analyzed here presented 128–190 mg of isoflavones per 100 g FW, which is in the range reported for Brazilian soybeans. Defatted flours, on the other side, presented higher contents, from 120 to 340 mg of isoflavones per 100 g FW (results expressed as aglycones), mostly due to concentration caused by oil removing.

Umphress et al. [23] reported isoflavone contents ranging from 46 to 100 mg/100 g for defatted soy flours, much lower than those found here. This variation can be considered normal since different varieties present different contents of isoflavones and even the same variety can present differences depending on climate conditions and growth place [24–26]. An isoflavone content from 118 to 175 mg/100 g was reported for the same crop of an American variety grown in three different locations [27].

In both whole and defatted flours the main isoflavone forms present were malonylglycosides and deesterified β-glycosides, corresponding to 86–98% of total isoflavones, similar to the results of Coward et al. [28].

Soy protein isolates contain over 90% protein and are made from defatted flours/flakes by solubilizing protein at pH 6.8–10 and separating the extract, by centrifugation or filtration, from insoluble fibrous residues. The resulting supernatant is acidified (pH 4.5), to precipitate protein as a curd, and separated from soluble oligosaccharides by centrifugation. Then the protein is directly spray-dried or it can be previously neutralized to pH 6.5–7 [19]. Commercial soy protein isolates analyzed here presented isoflavone contents from 88 to 164 mg/100 g (Fig. 1). These differences could be due to oscillations in the isoflavone content present in the raw material (defatted flour) and/or type of processing [14].

The defatted soy flour residue obtained after protein extraction and centrifugation or filtration is rich in fibers, which is known commercially as "dietetic soy fiber." The samples analyzed here presented isoflavone contents of 48 and 60 mg/100 g (Fig. 1). These results show that the aqueous alkaline extraction is efficient for the co-extraction of isoflavones but a reasonable amount still remains in the residue. This process probably leads to hydrolysis of glycosides to their respective aglycones, which were found in high percentages in both kind of products (22–54% for SPI and 65–76% of the total for SF).

In the soy protein concentrate manufacture a water (pH 4–5) or alcohol wash of the defatted flour can be used to remove soluble carbohydrates and improve its functionality. The proteins that are not soluble in these conditions are concentrated in the residue (60–70% protein) after carbohydrate extraction. Alcohol washing results in the loss of

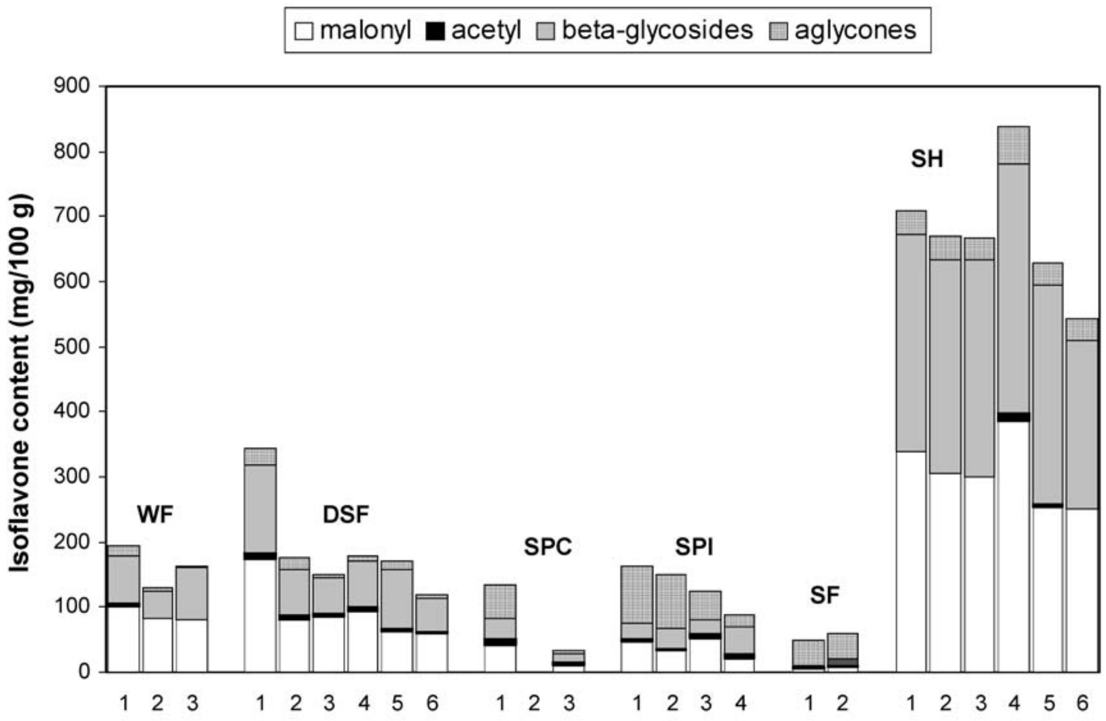

Figure 1. Total isoflavone content (mg/100 g FW) of commercial soy products. WF, whole soy flours; DSF, defatted soy flours; SPC, soy protein concentrates; SPI, soy protein isolates; SF, soy dietary fibers; and SH, soy hypocotyl flours. Numbers (1, 2, 3 etc.) indicate different samples.

most of the isoflavones, whereas a substantial amount of them are retained after water washing [2]. Commercial soy protein concentrates from three different suppliers were analyzed here: SPC 1 and 3 presented isoflavone contents of 33 and 135 mg/100 g (Fig. 1). However, no isoflavones were detected in one sample (SPC 2), indicating the utilization of alcohol washing in its production. The isoflavone profile varied significantly among the samples, SPC 1 presenting 40% of aglycones and SPC 3 only 11%.

Soy hypocotyls, which represent 2% of the seed weight [24], became a very popular product for utilization in supplements of isoflavones due to their elevated isoflavone content. As can be seen in Fig. 1, values ranging from 542 to 842 mg/100 g FW were observed in the samples analyzed. Similar to the flours, the isoflavone profile was mainly composed of malonylglycosides and deesterified β-glycosides, representing between 92 and 95% of the total forms.

Extrusion cooking is a heating process at high temperatures during a short time in which soy flour or protein concentrate change into texturized soy [19]. Figure 2 presents the isoflavone content of commercial textured defatted flours, textured concentrates and textured hypocotyl flours. The presence of acetylglycosides was detected in all the extruded products, in amounts ranging from 6 (TPC II) to 33% (TSH I) of the total. Aglycones represented 44 and 59% of the total isoflavones in the two extruded TPC analyzed. All the other products showed a predominance of β-glycosides (49–57% of the total). The decrease of malonylglycosides and the increase of acetylglycosides in samples of textured products indicate that decarboxilation reactions may have occurred. The differences found in the isoflavone profiles are probably associated to the several degrees of exposition to heat during the processing and drying processes.

The average distribution of the total forms of isoflavones (daidzein, genistein and glycitein) in all soy products is presented in Table 1. The main forms of isoflavones found in all soy products were similar, with genistein present in the highest proportion followed by daidzein and glycitein. These results showed that the distribution of isoflavones was the same of that in soybeans, indicating that only the profile of isoflavones changed during the processing (Table 1). The only exception was the prevalence of glycitein and daidzein in soy hypocotyls, which is in accordance to previous report by Eldridge and Kwolek [24]. Barbosa et al. [14] also reported that SPI presented the three main isoflavones in proportions similar to those found in the flour, with prevalence of genistein (~62%), similar to our results.

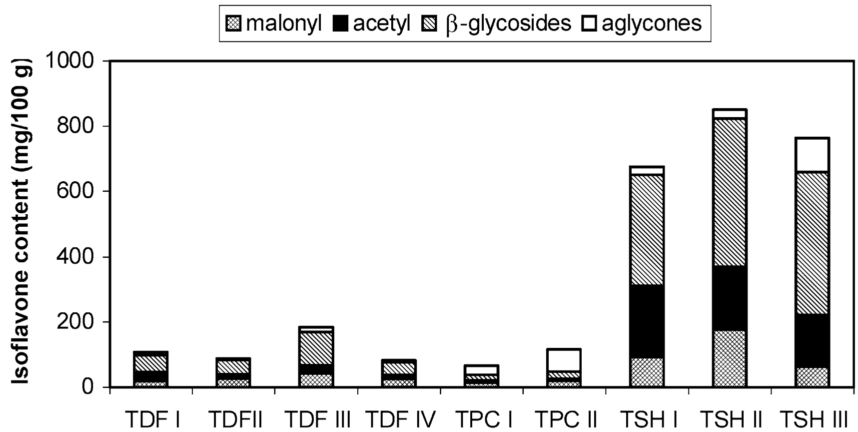

Figure 2. Total isoflavone content (mg/100 g FW) of textured soy products. TDF, textured defatted soy flours; TPC, textured soy protein concentrates; and TSH, textured soy hypocotyl flours. Numbers (I, II, III etc.) indicate different samples.

Isoflavone Contents in the Starting Materials and Derived Soy Ingredients

The great variation found in the total content of isoflavones for soy products does not allow an adequate evaluation of the effect of processing, since one of the main causes could be differences in the raw material used in their production. In this way, analysis of the raw material and final product were made to determine precisely the effects of processing. Figure 3 shows the total content and profile of isoflavones in different raw materials and their respective derived products.

The defatted soy flour showed a higher concentration of isoflavones (343 mg/100 g) than the starting material, the whole flour (193 mg/100 g), consistent with the concentration caused by oil removing, since hexane does not extract isoflavones. Setchell [29] attributed the traces of isoflavones found in soy oil to the highly polar nature of the glycosidic conjugates and their inability to partition into the lipophilic oil during soy oil extraction. Also, the defatted flour presented almost the same isoflavone profile than the starting material, showing that oil removing does not affect isoflavone forms distribution. Malonylglycosides were the main forms present in both whole flour and defatted soy flour, whereas the aglycones were relatively minor constituents, consistent with the profile of the seeds previously reported [9].

The defatted flour presented an isoflavone content (156 mg/100 g) 17.3% higher than that of the protein isolate produced from it (129 mg/100 g). The isoflavone conjugation was significantly altered, mainly in relation to the aglycone content, which increased from 4.5 to 31%, with a decrease of malonylglycosides and β-glycosides from 67 to 51% and 26 to 14% of the total, respectively. The increase in aglycones could be attributed to the action of endogenous β-glycosidases as it was

Table 1. Total content of the main isoflavones (%) present in commercial soy products

Soy products	Daidzein	Glycitein	Genistein
Whole flours	38 ± 3a	13 ± 5a	49 ± 7a
Defatted flours	36 ± 3ab	6 ± 2ab	59 ± 5ab
Protein isolates	30 ± 2c	5 ± 1b	66 ± 3b
Protein concentrates	32 ± 1b	5 ± 2b	62 ± 3b
Fibers	32 ± 1b	4 ± 1b	64 ± 1b
Hypocotyls	50 ± 3d	34 ± 3c	16 ± 3c

Note. The total percentage of each aglycone represents the sum of the free and conjugated forms, expressed as aglycones, in relation to the total. Results are the mean ± standard deviation. Means in the same column with common letters are not significantly different ($p < 0.05$).

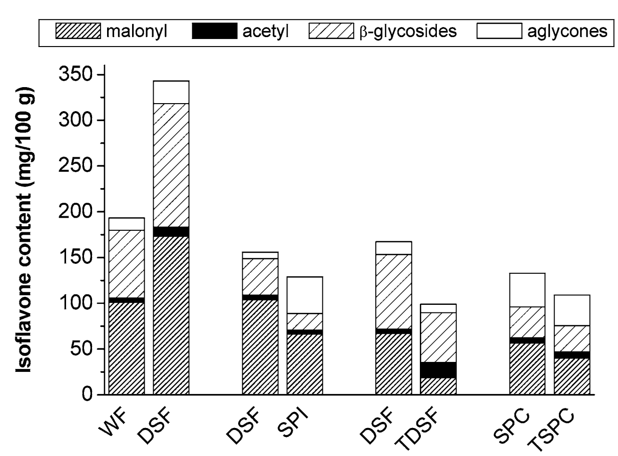

Figure 3. Total isoflavone content (mg/100 g FW) of the starting materials and the respective derived products. WF, whole soy flour; DSF, defatted soy flour; SPC, soy protein concentrate; TDSF, textured defatted soy flour; TSPC, textured soy protein concentrate SPI, soy protein isolate.

previously shown that temperatures below 50°C during aqueous protein extraction caused hydrolysis of β-glycosides with increase of aglycones, by soy β-glycosidase activity [14].

Extrusion caused a great decrease, of more than 40%, in the content of isoflavones of defatted flour, indicating that there was a significant degradation of these compounds during the process. Similarly, Mahungu et al. [30] reported a lower content of isoflavones for extruded samples in relation to unextruded ones, with a decrease of 38% for extruded corn/soy mixture. On the other side, in the extrusion processing of the soy protein concentrate there was a loss of only 18% of the total content of isoflavones (Fig. 3), indicating that the conditions used could had been less drastic or the isoflavone more protected due to the different matrix. The alteration in isoflavone conjugation seems to corroborate this. While the main effect on textured protein concentrate was a decrease of malonylglycosides from 42.5 to 36.7% of the total, for the textured defatted flour these forms dropped from 40 to 19%, and acetylglycosides increased from 2.8 to 16.8% of the total. This increase indicated the occurrence of heat-induced decarboxylation of the malonylglycosides to acetylglycosides.

In conclusion, different protein derivatives and different lots of the same product can present a great variation in the content and profile of isoflavones, related to variations in the raw material and processing conditions. For the development of functional foods, soy hypocotyls were shown to represent the richest sources of isoflavones, followed by defatted flours. However, the kind and conjugation of isoflavones differed deeply among products, whose biological significance remains to be determined. Products derived from hypocotyls were shown to be richer in daidzein and glycitein conjugates. Malonylglycosides appeared to be the most sensitive forms to processing, being subjected to both decarboxilation and deesterification reactions, forming the respective acetylglycosides and β-glycosides. Among all the commercial soy products analyzed in this study, soy fibers presented the greatest percentage of isoflavones in the aglycone form, which could be faster and more extensively absorbed by humans [31]. In addition, it is known that the consumption of dietary fibers could promote beneficial physiological effects including lowering of blood cholesterol and reduced risk of coronary heart diseases [32], which could also make these products interesting for inclusion in functional foods.

Acknowledgements

To FAPESP and CNPq, for the financial support to this work, CNPQ and CAPES for the grants to Márcia da Silva Pinto and Ana Cristina Lopes Barbosa.

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