Changes of Isoflavones During Processing of Soy Protein Isolates

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ABSTRACT: Soy protein isolate (SPI) is a widely used food ingredient and is made by extracting soy flour (SF) under slightly alkaline pH, followed by precipitation, washing, and drying. Soy foods and foods containing soy protein ingredients have great potential in the prevention of cardiovascular diseases and cancers. These health benefits have been attributed to isoflavones in soy protein ingredients. However, the current processing techniques were developed many years ago without this knowledge. The objective of this study was to investigate the mass balance of different isoflavones during manufacturing of SPI and to provide basic information to assist further development efforts leading to preservation of soy isoflavones in soy protein ingredients. The study revealed that only about 26% of the total isoflavones in SF remained in SPI. 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. The isoflavone profile of the SPI was different from that of SF. The former contained much more aglucones (genistein and daidzein), while the latter had almost none. The high content of aglucones in SPI was probably due to the hydrolysis of glycosides.

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KEY WORDS: Daidzein, genistein, isoflavones, processing, soy protein isolate.

Soybeans have been a valuable resource for humankind by providing excellent proteins and other nutrients. Dietary soy proteins have been shown to reduce risks of heart disease (1–3). There is also increasing evidence that consumption of soy foods can prevent various cancers (4,5). Researchers have credited phytochemicals in soybeans, especially isoflavones, for these beneficial effects. Soy processing technologies currently employed in the industries were developed without this knowledge. Therefore, no effort has been made to preserve these health-promoting phytochemicals during processing. There is an urgent need for these processes to be evaluated in terms of their impact on these phytochemicals.

Isoflavones are a class of flavonoids (Fig. 1). They include daidzein, glycitein, and genistein. They exist in soybeans either as glucosides or in free form (aglucones). The glucosides of daidzein, glycitein, and genistein are called daidzin, glycitin, and genistin, respectively. Six derivatives of the glucosides also exist in soybeans: 6″-*O*-acetyl-daidzin, -glycitin, -genistin; and 6″-*O*-malonyl-daidzin, -glycitin, -genistin (6). All of these 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 heart diseases and cancers, so they may become the vitamins of the twenty-first century (7).

The concentration of isoflavones in soy foods and soy ingredients varies dramatically. First, the genetics of soybean cultivars and the environment where soybeans are grown have a significant impact on the composition and the amount of isoflavones in soybeans (6,8). Second, processing techniques also may affect the concentration of isoflavones (6,9,10). Systematic studies are needed to investigate the effects of processing conditions on the retention of isoflavones. New processing technology or modifications of existing processes are needed to minimize the loss of isoflavones during processing.

Soybeans are generally processed into soybean oil products and soy protein products (11). Soy flour (SF), soy protein concentrate (SPC), and soy protein isolate (SPI) are three major soy protein products. They have different protein contents and are used in different applications. SPI are the most highly refined soy protein products, with a protein content of more than 90%. SPI is made by alkali extraction of protein, to remove insoluble fiber, and subsequent acid precipitation, to remove soluble sugars. SPI have many applications in the food industry, including as the major ingredients in health drinks and as extenders in many meat and dairy products. The isoflavone content of SPI varies with different suppliers (12). Little was known about the retention of isoflavones during each processing step. Therefore, the objective of this study was to investigate the mass balance of different isoflavones during manufacturing of SPI.

MATERIALS AND METHODS

Materials. Defatted soy flour was purchased from the Archer Daniels Midland Company (Decatur, IL). Three isoflavone standards (genistein, daidzein, and genistin) were obtained from Sigma Chemical Company (St. Louis, MO).

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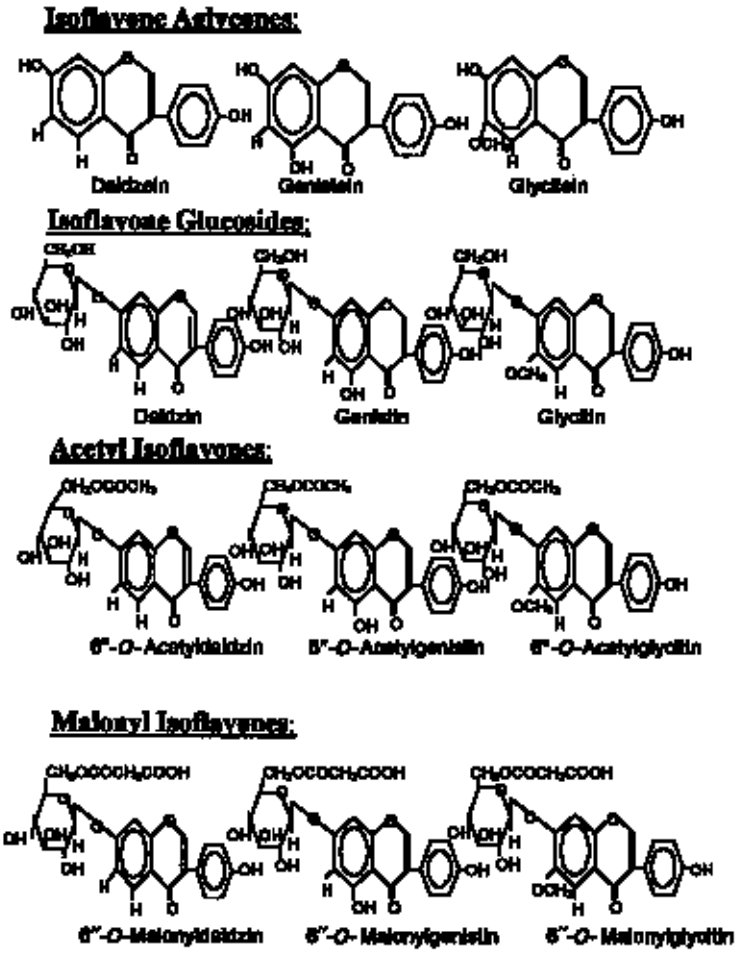

FIG. 1. Chemical structures of soy isoflavones.

Sample preparation. As shown in Figure 2, preparation of SPI included four steps: extraction, precipitation, washing, and drying. First, 16 g of soy flour was extracted at pH 8 for 1 h, and the slurry was centrifuged for 10 min at 1000 × *g*. Second, the pH of the supernatant was adjusted to 4.5, the slurry was centrifuged at the same force for the same length of time, and the precipitates were collected. Third, an equal amount of fresh distilled water was added to the precipitate, and the slurry was centrifuged under the same condition. Finally, the precipitate was collected on a drying plate and dried in a freeze-drier. Solid waste, whey, and wash water were also collected, freeze-dried, and weighed.

Isoflavone extraction. A freeze-dried sample (1 g), taken from different processing steps, was mixed with 12 mL of 80% methanol, stirred for 30 min at room temperature, and filtered through Whatman No. 42 filter paper (Maidstone, England). Two 10-mL portions of 80% methanol were used to wash the filter and the residue. The filtrate was then transferred to a 50-mL flask, and 80% methanol was used to dilute the filtrate to the mark. The solution then was filtered again through a 25-mm 0.4-µm Nylon syringe filter (Scientific Resources, Inc., Eatontown, NJ) before high-performance liquid chromatography (HPLC) analysis.

HPLC analysis. HPLC was used for the quantitative analysis of isoflavones. This system consisted of a Waters 510 HPLC pump (Milford, MA), a Waters automated gradient controller, a Spark Holland Basic-Marathon autosampler (Emmen, The Netherlands), a YMC-Pack ODS-AQ 303 column (5 µm, 25 × 4.6 mm i.d.) (Wilmington, NC), a Waters 486 tunable absorbance detector, and a Hewlett-Packard 3390A integrator (Avondale, PA). A modified gradient program (13) was used to achieve the best separation. Solvent A (0.1% glacial acetic acid in H_2O) was decreased from 85 to 69% over 45 min, while solvent B (0.1% glacial acetic acid in acetonitrile) was increased from 15 to 31%. A flow rate of 1.7 mL/min was used. The detecting wavelength was 254 nm. Concentrations of the isoflavones that have purified standards were calculated from the corresponding standard curves. The rest of the isoflavones were calculated based on the standard curve of the corresponding aglucones. All calculations were conducted on the normalized bases, i.e., all concentrations and mass balances were calculated with the molecular

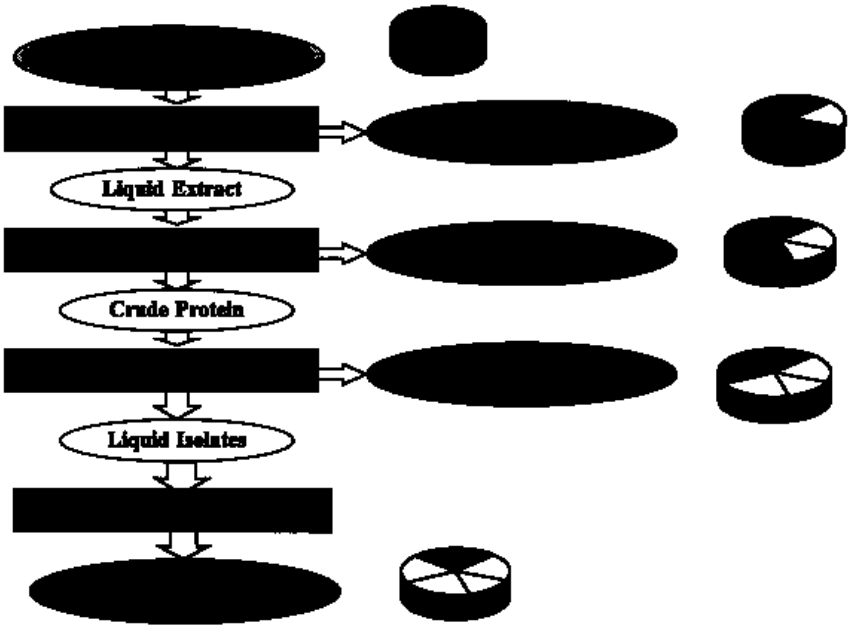

FIG. 2. Processing steps of soy protein isolate and mass balance of total isoflavones.

weights of the aglucones, without taking the conjugates into consideration.

Mass balance calculation. Isoflavone concentrations from all processing streams were expressed as µg isoflavones per g of dry sample. The actual amount of isoflavones is calculated by timing the concentration with the total dry weight of the processing stream collected. The percentages of distribution were also calculated for the total isoflavones.

RESULTS AND DISCUSSION

The isoflavone concentrations of SF, SPI, and other processing streams are shown in Table 1. All concentrations are calculated on a dry basis (db). Nine of twelve known isoflavones (6) were detected and quantified. They were genistein, daidzein, genistin, daidzin, glycitin, acetyl genistin, malonyl genistin, malonyl daidzin, and malonyl glycitin. Glycitein, acetyl daidzin, and acetyl glycitin were not detected. The total isoflavone content of the SPI was 1352 µg/g, while that of SF was 1512 µg/g. The two were not significantly different. The total isoflavone content (db) of the byproduct streams, solid waste, whey, and wash water were 958, 1865, and 28,684 µg/g, respectively. On a db, the wash water fraction had the highest concentrations of all individual and total isoflavones. Concentrations of genistin, daidzin, glycitin, acetyl genistin, malonyl genistin, malonyl daidzin, and malonyl glycitin in SPI were comparable with those of SF. However, concentrations of daidzein and genistein, the aglucones, were much higher than that of SF. This may be due to the hydrolysis of genistin and daidzin during processing.

The isoflavone profiles, comprising the individual isoflavone percentages of the total isoflavone, of SF and SPI are illustrated in Figure 3. As shown in the pie charts, the isoflavone profile of SPI was different from its starting material SF. The percentage of genistein in SPI was about 18.2%, while only 1.6% of the total isoflavone in SF is genistein. The percent-

TABLE 1
Isoflavone Concentrations of Processing Streams of Soy Protein Isolate (SPI) (µg/g)[a]

	Soy flour	Solid waste	Whey	Wash water	SPI
Daidzin	121.65^{b}	46.81^{b}	84.45^{b}	1061.13^{a}	44.16^{b}
Glycitin	43.88^{b}	23.89^{b}	41.35^{b}	433.59^{a}	24.26^{b}
Genistin	309.07^{b}	75.54^{b}	135.17^{b}	2949.54^{a}	123.94^{b}
Malonyl daidzin	285.77^{b,c}	129.30^{c}	515.67^{b}	7728.87^{a}	155.33^{c}
Malonyl glycitin	55.03^{b,c}	29.86^{b}	90.53^{b}	1243.14^{a}	33.59^{c}
Malonyl genistin	672.18^{b}	289.11^{b}	797.12^{c}	13247.77^{a}	538.26^{b}
Daidzein	0^{d}	106.50^{b,c}	76.54^{c}	893.11^{a}	125.39^{b}
Acetyl genistin	0^{c}	54.91^{b}	49.50^{b}	401.16^{a}	61.39^{b}
Genistein	24.17^{c}	202.68^{b}	75.37^{c}	726.02^{a}	246.08^{b}
Total isoflavones	1511.74^{b}	958.59^{b}	1865.70^{b}	28684.33^{a}	1352.40^{b}

[a]Within a row, numbers with the same letter in their superscripts are not significantly different.

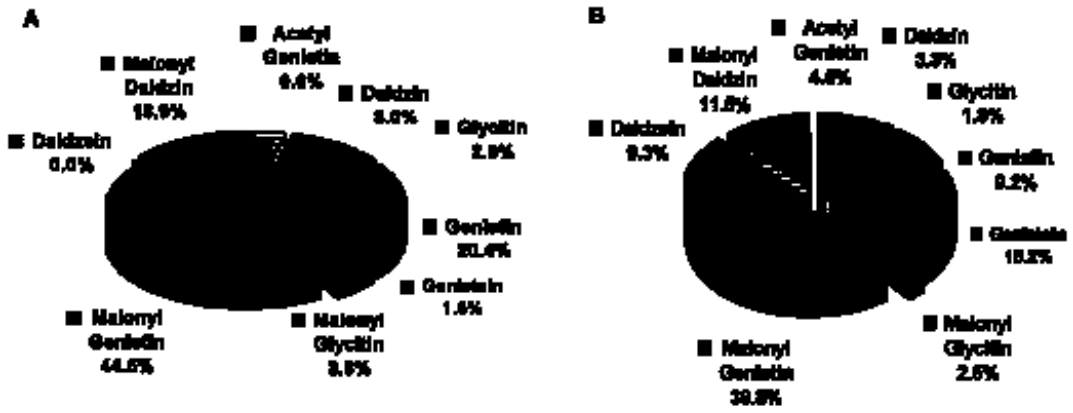

FIG. 3. Comparison of soy isoflavone profiles of (A) soy flour and (B) soy protein isolate.

TABLE 2
Distribution of Total Isoflavones Among Processing Streams

	Dry weight (g)	Total isoflavones concentration (μg/g)	Amount of total isoflavones (μg)	Percentage[a]
Soy flour	16.70	1511.74	25246.06	100[a]
Solid waste	5.00	958.59	4792.95	18.98[c]
Whey	1.93	1865.70	3600.80	14.26[d]
Wash water	0.19	28684.33	5450.02	21.59[c]
SPI	4.82	1352.40	6518.57	25.82[b]

[a]Values with the same superscripts are not significantly different ($P < 0.05$). See Table 1 for abbreviation.

ages of daidzein and acetyl genistin in SPI were also much higher than that in SF. However, the percentages of genistin, daidzin, and glycitin in SPI were significantly lower than those of SF. The increases of genistein and daidzein are apparently due to hydrolysis of the glucosidic forms of isoflavones during processing. The increase of acetyl genistin is probably due to conversion of malonyl genistin into acetyl genistin in the presence of heat, as shown by Wang and Murphy (6). The percentages of malonyl genistin and malonyl glycitin were slightly higher in SF than in SPI. However, the percentages of malonyl daidzin decrease significantly in SPI compared with that of SF. This is an indication that malonyl daidzin was more unstable than malonyl genistin and malonyl glycitin under the processing conditions.

Table 2 shows the mass balance of the total isoflavone among different fractions. The total isoflavone distributions among different processing streams are also illustrated in Figure 4. Only about 26% of the total isoflavones in SF remained in SPI, while 74% was lost during processing. About 19, 14, and 22% of that went to solid waste, wash water, and whey, respectively. About 19% was not recovered in any fraction. This unrecovered fraction could be attributed to the following. First, minor amounts of isoflavone could be lost to the equipment during the experiment. Second, there could be degradation of isoflavones during processing. However, degradation was more likely to be microbiological than thermal-chemical because a separate experiment has shown that the isoflavone aglucones were extremely stable to thermal degradation. The conversion from malonyl forms to acetyl forms or the conversion from the glucosides to aglucones was not a factor in the unrecovered fraction because normalized calculations were conducted.

In conclusion, isoflavones were lost in each processing step: extraction, precipitation, and washing. However, the loss during washing was the most severe. Almost 22% of the total isoflavones in SF was lost during this step. Therefore, the washing step should be the focus in preserving isoflavones.

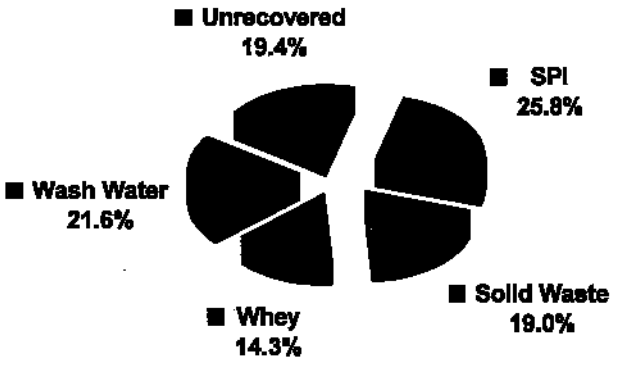

FIG. 4. Total isoflavone distribution among processing streams of soy protein isolate (SPI).

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REFERENCES

1. Carrol, K.K., Review of Clinical Studies on Cholesterol-Lowering Response to Soy Protein, *J. Am. Diet. Assoc. 91*:820–827 (1991).
2. Slavin, J., Nutritional Benefits of Soy Fiber, *Ibid. 91*:816–819 (1991).
3. Jacues, H., D. Laurin, S. Moorjani, F.H. Steinke, C. Gagne, D. Brun, and P.J. Lupien, Influence of Diets Containing Cow's or Soy Protein Beverage on Plasma Lipid in Children with Familial Hypercholesterolemia, *J. Am. Coll. Nutr. 11*:69S–73S (1992).
4. Adlercreutz, H., H. Honjo, A. Higashi, T. Fotsis, E. Hamalainen, T. Hasegawa, and H. Okada, Urinary Excretion of Lignans and Isoflavonoid Phytoestrogens in Japanese Men and Women Consuming a Traditional Japanese Diet, *Am. J. Clin. Nutr. 54*:1093–1100 (1991).
5. Lee, H.P., L. Gourley, S.W. Duffy, J. Esteve, J. Lee, and N.E. Day, Dietary Effects on Breast-Cancer Risk in Singapore, *Lancet 337*:1197–1200 (1991).
6. Wang, H.J., and P.A. Murphy, Isoflavone Content of Commercial Soybean Foods, *J. Agric. Food Chem 42*:1666–1673 (1994).
7. Messina, M.J., V. Persky, K.D.R. Setchell, and S. Barnes, Soy Intake and Cancer Risk: A Review of the *In Vitro* and *In Vivo* Data, *Nutr. Cancer 21*:113 (1994).
8. Eldridge, A.C., and W.F. Kwolek, Soybeans Isoflavones: Effect of Environment and Variety on Composition, *J. Agric. Food Chem. 31*:394–396 (1983).
9. Anderson, R.L., and W. Wolf, Compositional Changes in Trypsin Inhibitors, Phytic Acid, Saponins and Isoflavones Related to Soybean Processing, *J. Nutr. 125*:581S–588S (1995).
10. Barnes, S., and M. Messina, Soy and Its Role in Cancer Prevention, *The Soy Connection. 1*:1–3 (1993).
11. Smith, A.K., and S.J. Circle, in *Soybeans: Chemistry and Technology,* edited by A.K. Smith and S.J. Circle, AVI Publishing Co., Westport, 1976, p. 339.
12. Eldridge, A.C., Determination of Isoflavones in Soybean Flours, Protein Concentrates, and Isolates, *J. Agric. Food Chem. 30* :353 (1982).
13. Wang, H.J., and P.A. Murphy, Isoflavone Composition of American and Japanese Soybeans in Iowa: Effects of Variety, Crop Year, and Location, *J. Agric. Food Chem 42*:1674–1677 (1994).

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