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Isoflavones in Soy-Based Foods Consumed in Brazil: Levels, Distribution, and Estimated Intake

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The isoflavone content and profile in processed soy-based products consumed in Brazil were determined by high-performance liquid chromatography and photodiode array detection of the intact isoflavones (naturally occurring aglycons, malonyl, acetyl, and β-glycosides derivatives). Total isoflavone content varied significantly among products, from 2 to 100 mg/100 g (wet basis, expressed as aglycons), with the lowest content being found for soy-based enteral/oral diets and the highest found for textured soy proteins. For soy beverages isoflavone content varied from 12 to 83 mg/L. Soy sauce, miso, and tofu had isoflavone contents of 5.7 mg/L, 20 mg/100 g, and 7 mg/100 g, respectively. The β-glycosides were the predominant form of the isoflavones in the products analyzed, except for miso, shoyu, and "Diet Shake" in which the aglycons were present in the highest proportions. On the basis of these data, the daily intake of isoflavone from soy products was estimated: the highest values were found for infants fed soy-based formulas, from 1.6 to 6.6 mg/kg of body weight.

KEYWORDS: Isoflavones; soy products; daily intake; Brazilian diet

INTRODUCTION

Isoflavones represent the most common group of phytoestrogens and have been associated with 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, whose consumption in Brazil is not as significant as in Asian countries. On the other hand, soy protein products are largely used as ingredients in meat products, breads, beverages, soups, and other foods. There is also an increasing interest of the food industry on the development of functional foods rich in isoflavones, and supplements of isoflavones are being indicated to women to attenuate menopausal symptoms.

Soy isoflavones, daidzein, genistein, and glycitein, are present in seeds as glycosilated conjugates (malonylglycosides and underivatized β-glycosides), and, as a result of processing, aglycons and acetylglycosides are formed. There is little information about the bioavailability of these different forms. It has recently been shown that the absorption of aglycons in humans was faster and more extensive than that of the glycosides (*2*). In contrast to this, Setchell et al. (*3*) reported a higher bioavailability of glycosides compared to aglycons, and Xu et al. (*4*) observed no differences in bioavailability associated with isoflavone form.

Isoflavone content in soybean seeds is affected by genetic and environmental factors (*5*) which, together with processing conditions, determine the concentration and profile of these compounds in soy-based products and foods containing soy-protein ingredients. Although the role of soybeans in the Brazilian diet is by far superseded by that of dry beans, there are special groups such as vegetarians, lactose intolerants, or those allergic to milk proteins, who have a high intake of soy-based products. As the effects of isoflavones probably depend on the age, dose, sex, and health state, it is uncertain what could be expected if significant amounts were present in the diet of male or female children at an early stage of development or for patients fed exclusively soy-based enteral/oral diets. Besides these groups, there are some products which are largely consumed by people in general, such as soy sauces and soy-based beverages, as the latter are becoming increasingly popular. There are no data about the isoflavone content and profile of these soy-based products consumed in Brazil. With these results an estimate of isoflavone daily intake could be possible.

MATERIALS AND METHODS

Materials. All processed soy-based products were purchased locally in São Paulo. The samples of soy-based infant formulas and oral/enteral diets were purchased from local drugstores, and all were in the form of dry instant powders. Textured soy proteins, soy sauce (shoyu), miso, soy beverages, and instant soy beverages powders were purchased from local supermarkets. Commercial brands of powdered soy-based infant formulas analyzed were the following: Nestlé's Alsoy and Nestogeno, Wyeth's Nursoy, Mead Johnson's Prosobee, and those imported and distributed by Support, Kasdorf's Aptamil 1 (Holland), Nutricia's Aptamil 2 (Argentina), and Milupa's Pregomin (Germany). The enteral/oral diets were Abbott's Ensure and those imported and distributed by Support, Kasdorf's Soyac and Soya Diet (Argentina). The diet shake for weight control "Diet Shake Bioscience" (Nutrilatina, Brazil) was also purchased from the supermarket. Textured soy proteins were "Proteína de soja fina Pró-Vida" (Pró-Vida Alimentos Ltda., Brazil),

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"Proteína texturizada de soja Mãe-Terra" (Mãe-Terra Produtos Naturais Ltda., Brazil), and "Proteína texturizada de soja MaisVita" (Yoki Alimentos S. A., Brazil). The commercial soy sauce was Shoyu Premium Miyako and miso (made of rice and soybeans) Miso (Sakura-Nakaya Alimentos Ltda., Brazil). Tofu was Kinugoshi tofu extra soft, coagulated with calcium sulfate (Agro Nippo S/A, Brazil). Soy beverages were from three commercial brands (Refinações de Milho Brasil Ltda., Agro Nippo, and Yakult S/A, Brazil), soy beverage powders were from Olvebra Industrial S/A (Brazil), and "Ensure Plus milkshake style" from Abbott. All chemicals and solvents were reagent or HPLC grade.

Isoflavone Extraction. Best conditions for isoflavone extraction were previously determined in our laboratory (*6*). Powdered samples (1 g) were extracted with 80% aqueous methanol (1:20 w/v) under agitation for 2 h at 4 °C. Recovery of isoflavones (99−103% for daidzin and 96−103% for genistin) was determined by spiking with standards at levels similar to those found in the samples. Textured soy proteins were previously ground in a mill until the sample passed through a 0.25-mm sieve and the extraction time increased to 3 h (*6*). Tofu was previously freeze-dried. The extracts were filtered through Whatman n*o*. 6 filter paper and concentrated until methanol elimination on a rotatory evaporator (Rotavapor RE 120, Büchi, Flawil, Sweden) at ≤40 °C. Volume of the extracts was adjusted to 10 mL with distilled water for posterior application to SPE columns. The extractions were made in triplicate.

Solid-Phase Extraction. Differently from soy protein products (isolates, concentrates, and flours), for the determination of isoflavones in foods in which these are used as ingredients, it was found necessary to cleanup the extracts and concentrate isoflavones by means of solid-phase extraction, in conditions previously optimized in our laboratory (*6*), described as follows. The extracts obtained above were passed through polyamide (CC 6, Macherey-Nagel, Germany) columns (1 g/6 mL) previously conditioned with 20 mL of methanol and 60 mL of distilled water. Impurities were washed out with 20 mL of distilled water and retained isoflavones were eluted with 50 mL of 99.5:0.5 methanol/ammonia. Aliquots (5, 10, or 20 mL) of soy beverages and soy sauce were applied directly to polyamide columns, except for those samples showing suspended particles which were previously centrifuged (10.000 g/20 min). The flow rate through the columns was controlled by means of a vacuum manifold Visiprep 24 DL (Supelco, Bellefonte, PA). The effluents were evaporated on a rotatory evaporator until the volume of 0.2−0.4 mL, and then the volume was adjusted to 2 mL with HPLC grade methanol. Aliquots of the samples were filtered through a 0.22-μm PTFE filter unit [poly(tetrafluoroethylene), Millipore Ltd., Bedford, MA] and analyzed by HPLC.

HPLC Quantitation of Isoflavones. Isoflavone quantitation was performed according to Song et al. (*7*) with a C18 NovaPak (30 cm × 4.6 mm i.d.) column (Waters, Milford, MA) and a Hewlett-Packard 1100 system with a diode array detector (Palo Alto, CA), based on external calibration. Standards of daidzein and genistein were obtained from Sigma Chemicals Co. (St. Louis, MO), daidzin and genistin were from Apin Chemicals Ltd. (Abingdon, UK), and glycitin and glycitein were from Nacalai-Tesque Inc. (Kyoto, Japan). Concentrations of malonyl and acetylisoflavones were calculated using standard curves for the respective β-glycosides, adjusting for differences in molecular weight. Total isoflavone contents were expressed as mg of aglycon/100 g of sample, 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 aglycon molecular weight to the molecular weight of the individual form before summing, as recommended by Song et al. (*7*).

Protein Content. The protein content (N × 6.25) of samples which had not this value declared was determined in triplicate by the micro-Kjeldahl method (*8*).

Statistical Analysis. All analyses were run in triplicate. Isoflavone concentrations were expressed as mean ± standard deviation (SD). Statistical analysis was done by using the Statistica software package version 5.0 (StatSoft, Inc., Tulsa, OK). Differences between means were first analyzed by ANOVA test and then least significant difference (LSD) test ($P < 0.05$).

Table 1. Total Isoflavone Content (mg/100 g powder) of Soy-Based Infant Formulas, Expressed as Aglycons[a]

formula	protein (%)	total isoflavone (mg/100 g)
Alsoy	14.0	7.4 ± 0.3a
Aptamil soja 1	14.2	14.3 ± 0.4b
Aptamil soja 2	15.4	13.7 ± 0.5b
Nursoy	14.0	11.8 ± 0.4c
Pregomin	13.3	7.4 ± 0.1a
Prosobee	15.6	20.3 ± 0.5d
Nestogeno	16.6	24.0 ± 1.1e

[a] Results are expressed as mean ± standard deviation ($n = 3$). Means with common letters are not significantly different ($P < 0.05$).

RESULTS AND DISCUSSION

Isoflavones in Soy-Based Infant Formulas. Soy-based infant formulas are lactose-free preparations indicated in case of galactosemia and intolerance to lactose, representing also an alternative in cases of allergy to cow milk. Their protein content is about 15% (corresponding exclusively to isolated soy proteins), the fat content (24−28%) is a mixture of vegetable oils, and carbohydrates (51−57%) are composed of maltodextrins and, in some cases, sucrose. The formulas are supplemented with vitamins, minerals, methionine, and carnitine (*9*).

Soy-protein isolates are the most-refined soy-protein ingredients and are produced by alkaline extraction of the proteins present in the defatted flour, followed by acid precipitation. After centrifugation, the precipitated proteins are washed and spray-dried. The final product has a protein content of more than 90% and a variable isoflavone content; for those produced in Brazil values from 137 to 180 mg/100 g were reported (*6*).

Soy-based infant formulas commercialized in Brazil were found to have total isoflavone contents ranging from 7.4 to 20.3 mg per 100 g of powder, expressed as aglycons (**Table 1**). As they show a similar protein content, this variation would be the result of different isoflavone contents between soy-protein isolates used as ingredients in the formulas, except for Pregomin, which also has collagen in its composition. The product Nestle's Nestogeno with soy surprisingly showed the highest isoflavone content (24 mg/100 g). Nestogeno is an infant formula for 6-month and older infants which, besides soy protein isolate, has defatted cow milk in its composition. Considering that the presence of isoflavones is correlated with the amount of soy proteins, the highest isoflavone content would be expected for those formulas having exclusively soy protein as the protein source. A possible explanation would be a high ratio between soy and milk proteins, a soy-protein isolate with a high content of isoflavones, and the higher protein content of this formula.

The isoflavone content of soy-protein isolates depends on the isoflavone content of soybeans used in their production, which in turn is related to variety and environmental factors (*5*). The total isoflavone content found for six soy-based infant formulas commercialized in the United States showed a lower variation and higher values: from 21 to 29 mg per 100 g of powder (*10*). In another report, for two powdered formulas the isoflavone content found was 31 and 32 mg per 100 g (*11*).

The distribution of isoflavones also varied significantly among products (**Table 2**). The percentage of malonylglycosides varied between 11.6 (Pregomin) and 42.7 (Nestogeno), and aglycons percentages varied between 2.4 (Nestogeno) and 29.7% (Pregomin). The low content of malonylglycosides and the high content of aglycons found in Pregomin indicated more drastic processing conditions, and in the case of Nestogeno, for which the contrary was observed, less severe conditions are indicated.

Table 2. Isoflavone Distribution (%) in Soy-Based Infant Formulas[a]

isoflavone	Alsoy	Aptamil 1	Aptamil 2	Nursoy	Pregomin	Prosobee	Nestogeno
β-glycosides	56.2ae	62.0bd	71.7c	67.2cd	58.7ab	56.5ae	53.0e
malonylglycosides	29.2a	26.8b	18.3c	25.3b	11.6d	25.5b	42.7e
acetylglycosides	1.4a	2.4b	n.d.	n.d.	n.d.	9.6c	1.9d
aglycons	13.2a	8.8b	10.0c	7.5d	29.7e	8.4b	2.4e
total daidzein	23.9a	30.0b	30.5b	30.9b	29.6b	31.7b	42.2c
total glycitein	16.3a	14.8b	16.2a	11.7c	8.4d	9.0d	6.3e
total genistein	59.8ae	55.2ab	53.3b	57.4abe	62.0e	59.3ae	51.5b

[a] n.d., Not detected. CV of the values <5%. Means in the same row with common letters are not significantly different ($P < 0.05$).

Table 3. Daily Intake of Isoflavones (mg) of Infants Consuming Soy-Based Formulas, by Age and Weight

intake	Alsoy	Aptamil 1	Aptamil 2	Nursoy	Prosobee	Nestogeno
0–2 Weeks (∼3 kg)						
formula (g/day)[a]	80	76.5	-	72	77.4–90.3	
isoflavones (mg/day)	5.9	10.9	-	8.5	15.7–18.3	
2–8 Weeks (∼4 kg)						
formula (g/day)[a]	89–111	85–106	-	108	103–129	
isoflavones (mg/day)	6.6–8.2	12.2–15.2	-	12.7	21.0–26.2	
2–3 Months (∼5 kg)						
formula (g/day)[a]	111–133	106–128	-	108	129	
isoflavones (mg/day)	8.2–9.8	15.2–18.3	-	12.7	26.2	
3–6 Months (∼6.5 kg)						
formula (g/day)[a]	124–133	119–128	-	135	138–172	
isoflavones (mg/day)	9.2–9.8	17.0–18.3	-	15.9	27.9–34.9	
> 6 Months						
formula (g/day)[a]	93	-	86–101	144	138–172	101–134
isoflavones (mg/day)	6.9	-	11.8–13.8	17.0	27.9–34.9	24.2–32.2

[a] The amount of formula consumed per day was calculated from manufacturer's directions.

Except for Prosobee, the acetylglycosides were not detected or were detected in low levels. These compounds are more commonly formed during toasting of soy flour or extrusion to produce textured soy protein (*12*). These results are quite different from those reported by Setchell et al. (*11*) who found a similar composition for liquid and powdered soy-based formulas, characterized by a very high content of β-glycosides (near 80%) and a small proportion of aglycons (no more than 6%). In relation to the total percentage of daidzein and its conjugates (24−42%), glycitein and its conjugates (6−16%), and genistein and its conjugates (52−62%), the results were similar for all the formulas, which showed a higher proportion of genistein and its conjugates than of daidzein and glycitein and their conjugates. The same was reported for the six soy-based infant formulas commercialized in the U.S., with isoflavone levels of approximately 29% of daidzein, 59% of genistein, and 12% of glycitein (*10*).

From the total isoflavone content of the soy-based infant formulas presented in **Table 1**, and according to the manufacturer's directions, the daily intake of isoflavones for each age was calculated (**Table 3**). The product Pregomin was not included as it did not provide the daily recommendations for consumption. However, daily intakes of isoflavones similar to those obtained for Alsoy could be expected, as they present similar levels of isoflavones.

Table 3 shows that the daily intake of isoflavones of infants consuming soy formulas can vary between 5.9 and 35 mg per day according to the product and age of the infant. Considering the average body weights,the isoflavone daily intake would be as follows: for infants 0 to 2 weeks old, 2.0−6.1 mg/kg; for those 2−8 weeks old, 1.7−6.6 mg/kg; for those 2−3 months old, 1.6−5.2 mg/kg; and for those 3−6 months old, 1.4−5.4 mg/kg. These doses correspond to a daily intake between 98 and 462 mg of isoflavones for a 70-kg adult. For comparison, the Japanese population, which consumes a high amount of soybeans and soybean-based processed foods, has a daily intake of isoflavones estimated at 28 mg/day (*13*), corresponding to 0.4 mg/day/kg body weight for a 70-kg adult. As a result, the daily exposure to isoflavones of infants consuming soy-based formulas can be considered high, as these compounds have been shown to be efficiently absorbed by them, and resulting circulating concentrations seem sufficient to exert biological effects (*11*). Although there are no reports of deleterious effects associated with consumption of soy-based formulas, long-term studies to assess possible chronic effects of high ingestion levels are still necessary, and these formulas should be avoided for infants showing hormonal disruption.

Table 4. Total Isoflavone Content (mg/100 g Powder) of Oral/Enteral Diets (Ensure, Soyac, and Soya Diet) and the Diet for Weight Control "Diet Shake", Expressed as Aglycons[a]

	protein (%)	total isoflavone (mg/100 g)
Ensure	14.2	2.5 ± 0.1a
Soyac	17.0	9.1 ± 0.3b
Soya Diet	15.9	5.8 ± 0.2c
Diet Shake	21.3	2.6 ± 0.1a

[a] Results are expressed as mean ± standard deviation ($n = 3$). Means with common letters are not significantly different ($P < 0.05$).

Isoflavones in Oral and Enteral Diets and Diet for Weight Control. Among powdered diets destined for oral or enteral use, Soyac presented the highest content of isoflavones, near 9 mg/100 g (**Table 4**), slightly superior to that of the soy infant formulas Alsoy and Pregomin (**Table 1**). The products Soyac and Soya Diet are from the same manufacturer, and thus

Table 5. Isoflavone Distribution (%) in Oral/Enteral Diets (Ensure, Soyac, and Soya Diet) and the Diet for Weight Control "Diet Shake"[a]

isoflavone	Soyac	Soya Diet	Ensure	Diet Shake
β-glycosides	71.5a	78.2b	78.5b	15.3c
malonylglycosides	8.6a	3.2b	13.2c	17.7d
acetylglycosides	1.1	n.d.	n.d.	n.d.
aglycons	18.7a	18.6a	8.3b	67.0c
total daidzein	23.4a	24.7a	29.4b	21.2c
total glycitein	4.0a	5.9b	14.1c	n.d.
total genistein	72.6ac	69.4a	56.6b	78.8c

[a] n.d., Not detected. CV of the values <5%. Means in the same row with common letters are not significantly different ($P < 0.05$).

probably have in their composition soy-protein isolates produced by the same supplier, allowing the direct comparison between them. In this way, the higher isoflavone content of Soyac would be the result of a higher protein content corresponding exclusively to soy protein, whereas in Soya Diet it corresponds to 70% soy protein and 30% caseinate, according to the manufacturer. The low content of isoflavones found for the product Ensure, near 2.5 mg/100 g, is probably related to a lower protein content and to the fact that besides soy protein, calcium and sodium caseinates are also present, though in an unknown proportion. For the powdered product indicated as a diet for weight control a low isoflavone content was also found, similar to that of Ensure. Despite a high protein content (21.3%), Diet Shake is composed of a mixture of hydrolyzed gelatin, soy concentrate, and dehydrated egg white. Besides this, soy concentrates may have very low levels of isoflavones if produced by aqueous alcohol extraction of defatted soy flakes, as isoflavones are also extracted in this process.

The distribution of isoflavones into aglycons, β-glycosides, malonyl-β-glycosides, and acetyl-β-glycosides is shown in **Table 5**. For the oral/enteral diets, the greatest amount of isoflavones, between 70 and 80%, corresponded to β-glycosides. The product Diet Shake showed a high concentration of aglycons (67%), which could be explained by the fact of having soy concentrate in its composition instead of soy isolate, as in the case of soy-based formulas and oral/enteral diets. Different processing conditions used to obtain these two soy ingredients probably result in a different isoflavone distribution.

The content of malonyl-β-glycosides varied between 3% for Soya Diet and 18% for Diet Shake, and acetyl-β-glycosides were detected only in Soyac, at a low level of approximately 1%. The product Ensure showed a higher content of malonyl-β-glycosides and a lower content of aglycons when compared to those of Soyac and Soya Diet, which presented a composition more similar to each other. Genistein and its conjugates were also present in a higher proportion than of daidzein and glycitein and their conjugates in the four products.

The daily intake of isoflavones resulting from consumption of three meals prepared with the oral/enteral diets is 3.5 mg for Ensure, 7.9 mg for Soya Diet, and 12.3 mg for Soyac. The product Diet Shake is recommended in an amount of 35 g of the powder to 300 mL of skimmed milk, which corresponds to 0.91 mg of isoflavones per beverage ready to consume. As the indication is to substitute up to two meals per day, this would result in a daily intake of 1.82 mg of isoflavones. On the basis of these data can be estimated an intake between 0.03 and 0.18 mg of isoflavones per kg of body weight for a 70-kg adult, which can be considered very low compared to the daily intake of the Japanese of 0.4 mg/kg for a 70-kg adult (*13*), and probably without biological significance, mainly considering that these products are normally consumed for a short period of time.

Table 6. Total Isoflavone Content of Textured Soy Proteins, Expressed as Aglycons[a]

	protein (%)	total isoflavone (mg/100 g)
Mais Vita	49.7	86.9 ± 2.1a
Pro-Vida	49.5	99.9 ± 4.0b
Mãe-Terra	48.0	88.0 ± 2.9a

[a] Results are expressed as mean ± standard deviation ($n = 3$). Means with common letters are not significantly different ($P < 0.05$).

Table 7. Isoflavone Distribution (%) in Textured Soy Proteins[a]

isoflavone	Mais Vita	Pro Vida	Mãe Terra
β-glycosides	46.4a	42.8b	47.5a
malonylglycosides	20.4a	32.3b	24.0c
acetylglycosides	23.1a	17.2b	19.6c
aglycons	10.2a	7.6b	8.9c
total daidzein	39.1a	40.4a	39.2a
total glycitein	9.3a	10.9b	9.6a
total genistein	51.6a	48.7a	51.2a

[a] CV of the values <5%. Means in the same row with common letters are not significantly different ($P < 0.05$).

Isoflavones in Textured Soy Proteins. Textured soy proteins are largely used by vegetarians as meat substitutes, besides being used as ingredients in food systems. They are produced by thermoplastic extrusion of defatted soy flour, soy isolate, or soy concentrate to impart a fibrous texture. The three products analyzed are commercialized in supermarkets and showed a protein content of almost 50% (**Table 6**), indicating that they are produced from defatted soy flour instead of concentrates or isolates, whose protein contents are about 70% and 90%, respectively. They also presented a high content of isoflavones (**Table 6**), (87−100 mg/100 g, slightly inferior to the values found previously by us for industrial samples (*6*). A mean value of 149 mg/100 g can be found in the USDA database on the isoflavone content of foods (*14*), with a significant range of variation, from 4 to 296 mg/100 g.

The isoflavone distribution found for the three products was quite similar (**Table 7**), characterized by a high amount of acetylglycosides (17−23%) whose formation is associated with the process of extrusion (*12*). The β-glycosides were the compounds present in the highest levels (43−48% of the total).

In Brazil, the use of textured soy protein as a food ingredient in meat products is allowed until a maximum of 11.2% wet weight, and as a result the ingestion of 100 g of the final product would correspond to an ingestion of 9.7−11.2 mg of isoflavones. These results showed that, depending on the quantity and frequency of consumption of textured soy proteins, they can represent an important source of isoflavones in the diet.

Isoflavones in Soy Beverages. Soy beverages are becoming increasingly popular in Brazil and normally constitute soymilk mixed with fruit juices and/or containing flavoring ingredients. Soymilk is obtained by water extraction of raw soybeans which were previously soaked in water, washed, and ground. The slurry is cooked and filtered to separate soymilk from the water-insoluble residue (okara). The samples analyzed (Tonyu, Mupy, and Ades) showed protein contents ranging from 0.6 (Ades fruits) to 2.5 g/100 mL (Ades natural) (**Table 8**), and the protein is derived almost exclusively from soy in all of them. The product Ensure Plus Milkshake showed a higher protein content (6.3 g/100 mL) but has, besides soy protein isolate, sodium caseinate in its composition, and as a result the isoflavone

Table 8. Total Isoflavone Content (mg/L) of Soy-Based Beverages, Expressed as Aglycons[a]

beverage	protein (g/100 mL)	total isoflavone (mg/L)
Ades peach	0.6	22.2 ± 1.1ac
Ades apple	0.6	17.8 ± 0.9b
Ades orange	0.6	20.6 ± 0.6a
Ades natural	2.5	82.9 ± 3.6d
Tonyu apple	1.4	30.8 ± 0.9f
Tonyu pineapple	1.4	32.6 ± 0.9f
Mupy pineapple	0.7	23.4 ± 0.5cg
Mupy apple	0.7	24.8 ± 0.9g
Ensure Plus milkshake chocolate	6.3	12.1 ± 0.1h

[a] Results are expressed as mean ± standard deviation ($n = 3$). Means with common letters are not significantly different ($P < 0.05$).

concentration cannot be correlated to the protein content. Total content of isoflavones in soy beverages varied significantly, ranging from 17.8 to 82.9 mg/L. The beverages called Mupy and Ades containing fruit juices presented similar amounts of isoflavones, between 20 and 25 mg/L. However, the beverage Ades without fruit juice (natural) presented an isoflavone content four times higher, probably resulting from its higher protein content. In fact, when the isoflavone content is calculated on a protein basis all the beverages presented values ranging from 2 to 4 mg of isoflavones per g of protein. The isoflavone content of both Tonyu beverages analyzed was superior to the others containing fruit juices (around 32 mg/L) which could be also related to a higher protein content. The product Ensure Plus Milkshake showed the lowest concentration of isoflavones, but differently from the other beverages it is not a soymilk derived product. Although it has soy protein isolate in its composition it also contains milk proteins, probably in a higher proportion.

The isoflavone distribution presented a great variation between beverages (**Table 9**). The percentage of malonylglycosides ranged between 1.6 (Ades apple and orange) and 38.9 (Mupy apple), and they were not detected in Ensure Plus Milkshake. The percentage of aglycons was low, varying from 0.3 (Mupy apple) to 5.6 (Ades peach). Acetylglycosides were practically absent. The most abundant compounds in all the beverages were the β-glycosides, representing around 90% of the total in Tonyu and from 90 to 98% in Ades fruit. For the products Mupy (60%) and Ades natural (75%) a lower level was found, associated to a higher percentage of malonylglycosides, indicating a less severe heat treatment. Genistein conjugates (43 to 68%) represented the main isoflavones of the products analyzed, followed by daidzein conjugates (29 to 42%), similar to what is observed in soy protein products.

According to the results obtained, the consumption of just a glass of 250 mL of Ades natural would result in an intake of around 20 mg of isoflavones, or 0.29 mg/kg for a 70-kg adult, almost equivalent to the Korean daily intake (21 mg/day) (*15*). This means that soy beverages may represent important sources of isoflavones in our diet. However, the amount of isoflavones that humans would need to consume to provide an anticarcinogenic dose was estimated as 1.5−2.0 mg/kg of body weight per day (*16*), which means that having soy beverages as the sole isoflavone source would require the consumption of at least 5 glasses. On the other hand, a daily intake of around 40−50 mg seems to be enough to have benefits in preventing/treating osteoporosis (*17*).

Isoflavones in Instant Soy Beverages Powders. Instant soy beverages consist basically of soymilk powder with flavoring ingredients. Those containing exclusively soymilk proteins (Soymilke) showed high isoflavone contents (**Table 10**) of 39 and 48 mg/100 g. Conversely, the product Novo Milke, which consists of soymilk powder and whey proteins, presented a much lower isoflavone content of 9 mg/100 g. Much higher values, around 100 mg/100 g, were reported by Wang and Murphy (*18*) for four commercial soymilk powders. However, lower isoflavone contents, of approximately 9 and 17 mg/100 g, were found by Barnes et al. (*19*) in two soymilks analyzed. A mean value of 110 mg/100 g (ranging from 100 to 125) is reported in the USDA database on the isoflavone content of foods (*14*). These differences are probably the result of differences in the isoflavone contents of soybeans used for soymilk production and the amount of other ingredients added to instant soy beverages.

The isoflavone distribution in instant soy beverages powders was quite different from that found for ready-to-consume soy beverages, characterized by a higher proportion of aglycons, from 16 to 25%. Except for Novo Milke, there was predominance of β-glycosides, around 50% of the total (**Table 11**). The higher amount of aglycons indicated more favorable conditions for the action of endogenous β-glycosidases during soy milk production. It has been shown that during soaking of soybeans maximum production of aglycons occurred at 45 °C and pH 5.5, with almost complete inactivation of β-glycosidases at 60 °C and pH below 4.3 or above 7 (*20*).

According to the manufacturer's directions, for a glass of 200 mL it is recommended the utilization of 35 g of Soymilke banana, 30 g of Soymilke natural, and 26 g of Novo Milke. These amounts correspond to approximately 17, 12, and 2 mg of isoflavones, respectively, showing that instant soy beverages containing exclusively soy proteins also may represent important sources of isoflavones.

Isoflavones in Soy Sauce (Shoyu), Miso, and Tofu. Although they are traditional Asian soy foods, shoyu, tofu, and miso are frequently used in Brazilian dishes, and are normally found at the local commerce. Shoyu is a soy sauce produced through fermentation of a paste of soybeans, rice, and other cereals. Tofu is produced by precipitation of a curd from soymilk with calcium salts and removal of fluid by pressing. According to our results (**Table 12**), soy sauce showed a low isoflavone content, and, besides genistin (22.6% of the total), only the aglycons daidzein and genistein were detected. A mean content of 1.6 mg/100 g is reported in the USDA database on the isoflavone content of foods for shoyu (*14*). Miso (made of fermented rice and soybeans) showed a significant amount of isoflavones (58.8 mg/100 g dry weight (20 mg/100 g fw)), and also predominance of aglycons (56.6% of the total), although the three β-glycosides (36.1% of the total) and malonylgenistin (7.3% of the total) were also present. Wang and Murphy (*18*) reported a total isoflavone content of 29 mg/100 g dw for miso made of rice and soybeans, with 48% as aglycons. Franke et al. (*21*) reported a mean content of 23 mg/100 g fw with 55% in the form of aglycons. A range between 23 and 89 mg/100 g fw is reported in the USDA database on the isoflavone content of foods (*14*). The high amount of aglycons in these products would be the result of the action of exogenous β-glycosidases during fermentation. The sample of tofu analyzed here showed a low isoflavone content of 7 mg/100 g fw, probably because it was of the extra soft kind and had a high moisture content (87.8%). An isoflavone content ranging from 20 to 35 mg/100 g fw was previously reported for 12 different samples of tofu, from regular to extra firm kinds (*22*). The isoflavone profile of tofu was characterized by a predominance of β-glycosides (49%) and malonylglycosides (38%), followed by aglycons (10%).

Table 9. Isoflavone Distribution (%) in Soy-Based Beverages[a]

isoflavone	Tonyu apple	Tonyu pineapple	Mupy apple	Mupy pineapple	Ensure	Peach	Ades apple	Orange	Natural
β-glycosides	90.9ac	88.5a	60.4b	63.5b	98.4c	90.9ac	97.5c	95.6ac	74.8d
malonylglycosides	4.9a	6.5b	38.9c	35.8c	n.d.	3.5d	1.6e	1.6e	22.5f
acetylglycosides	0.9a	1.0b	0.4c	n.d.	n.d.	n.d.	0.1d	0.8e	0.2f
aglycons	3.3a	3.9b	0.3c	0.7d	1.6e	5.6f	0.8g	2.0h	2.5i
total daidzein	35.8a	33.9ac	41.9b	41.7b	32.0cd	29.6de	33.6ac	28.8e	39.3b
total genistein	43.1a	49.8b	56.2c	57.3d	68.0b	50.4b	47.9b	47.2b	50.2b
total glycitein	21.1a	16.3b	1.9c	1.0d	n.d.	20.0a	18.5e	24.0f	10.5g

[a] n.d., Not detected. CV of the values <5%. Means in the same row with common letters are not significantly different ($P < 0.05$).

Table 10. Total Isoflavone Content (mg/100 g powder) of Instant Soy Beverages Powders, Expressed as Aglycons[a]

	protein (%)	total isoflavone (mg/100 g)
Soymilke banana	17.5	48.2 ± 0.5a
Soymilke natural	24.5	39.3 ± 0.0b
Novo milke banana	7.8	9.0 ± 0.5c

[a] Results are expressed as mean ± standard deviation ($n = 3$). Means with common letters are not significantly different ($P < 0.05$).

Table 11. Isoflavone Distribution (%) in Instant Soy Beverages Powders

isoflavone	Soymilke Natural	Soymilke Banana	Novo Milke
β-glycosides	50.5a	49.3a	40.4b
malonylglycosides	21.7a	21.9a	43.7b
acetylglycosides	6.2a	4.2b	n.d.
aglycons	21.7a	24.6b	15.9c
total daidzein	37.6ab	35.9b	39.8a
total glycitein	17.1a	14.4b	20.4c
total genistein	45.2a	49.7a	39.8b

[a] n.d., Not detected. CV of the values <5%. Means in the same row with common letters are not significantly different ($P < 0.05$).

Table 12. Total Isoflavone Content of Traditional Soy Foods As Consumed, Expressed as Aglycons

food	total isoflavones
soy sauce (shoyu)	5.7 ± 0.0 mg/L
miso	20.0 ± 0.6 mg/100 g
tofu	6.8 ± 0.3 mg/100 g

Taking into consideration that soy sauce is consumed in very low amounts, its contribution to the intake of isoflavones would be insignificant. Miso, though having a higher isoflavone content, is also used in low amounts, usually as a seasoning, and as a consequence would also not have a significant role as an isoflavone source in our diet. Although the daily intake of tofu can be quite high − 40 g per day for Japanese people (*13*) − the low content of isoflavone results in a poor contribution to the total intake of these compounds.

Among the products analyzed, the isoflavone content of soy-based infant formulas can be considered high, taking into account their elevated daily intake as mg/kg of body weight. In the absence of clinical studies demonstrating that the potential beneficial effects observed for adults could be extended to children, it would be advisable to reconsider their utilization, especially in the presence of hormonal disruption. The levels of isoflavones in soy-based oral/enteral diets are low, and considering that their use is temporary no significant biological effects are expected. For those individuals who are willing to increase isoflavone intake, the consumption of soy seeds or other soy-based foods is recommended. Among the products analyzed, textured soy proteins and some of the soy-based beverages presented the highest isoflavones contents, representing potential sources of isoflavones in our diet. In almost all the products analyzed, the β-glycosides were the predominant form of the isoflavones, with the exception of the products of fermentation, miso and shoyu, in which the aglycons were present in the highest proportions.

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LITERATURE CITED

(1) Adlercreutz, H.; Mazur, W. Phyto-oestrogens and Western diseases. *Ann. Med.* **1997**, *29*, 95−120.
(2) Izumi, T.; Piskula, M. K.; Osawa, S.; Obata, A.; Tobe, K.; Saito, M.; Kataoka, S.; Kubota, Y.; Kikuchi, M. Soy isoflavone aglycones are absorbed faster and in higher amounts than their glucosides in humans. *J. Nutr.* **2000**, *130*, 1695−1699.
(3) Setchell, K. D. R.; Brown, N. M.; Desai, P.; Zimmer-Nechemias, L.; Wolfe, B. E.; Brashear, W. T.; Kirschner, A. S.; Cassidy, A. Bioavailability of pure isoflavones in healthy humans and analysis of commercial soy isoflavone supplements. *J. Nutr.* **2001**, *131*, 1362−1375.
(4) Xu, X.; Wang, H. J.; Murphy, P. A.; Hendrich, S. Neither background diet nor type of soy food affects short-term isoflavone bioavailability in women. *J. Nutr.* **2000**, *130*, 798−801.
(5) Eldridge, A. C.; Kwolek, W. F. Soybean isoflavones: effects of environment and variety on composition. *J. Agric. Food Chem.* **1983**, *31*, 394−396.
(6) Genovese, M. I.; Lajolo, F. M. Determinação de isoflavonas em derivados de soja. *Ciênc. Tecnol. Aliment.* **2001**, *21*, 86−93.
(7) Song, T.; Barua, K.; Buseman, G.; Murphy, P. A. Soy isoflavones analysis: quality control and a new internal standard. *Am. J. Clin. Nutr.* **1998**, *68*, 1474S−1479S.
(8) AOAC. *Official Methods of Analysis*, 16th ed.; Association of Official Analytical Chemists: Washington, DC, 1995.
(9) Maldonado, J.; Gil, A.; Narbona, E.; Molina, J. A. Special formulas in infant nutrition: a review. *Early Hum. Dev.* **1998**, *53*, S23−S32.
(10) Murphy, P. A.; Song, T.; Buseman, G.; Barua, K. Isoflavones in soy-based infant formulas. *J. Agric. Food Chem.* **1997**, *45*, 4635−4638.
(11) Setchell, K. D. R.; Zimmer-Nechemias, L.; Cai, J.; Heubi, J. E. Exposure of infants to phyto-oestrogens from soy-based infant formula. *Lancet* **1997**, *350*, 23−27.
(12) Coward, L.; Smith, M.; Kirk, M.; Barnes, S. Chemical modification of isoflavones in soyfoods during cooking and processing. *Am. J. Clin. Nutr.* **1998**, *68*, 1486S−1491S.

(13) Nakamura, Y.; Tsuji, S.; Tonogai, Y. Determination of the levels of isoflavonoids in soybeans and soy-derived foods and estimation of isoflavonoids in the Japanese daily intake. *J. AOAC Int.* **2000**, *83*, 635−650.

(14) USDA−Iowa State University Isoflavones Database. http://www.nal.usda.gov/fnic/foodcomp/Data/isoflav.

(15) Choi, Y.-S.; Lee, B.-H.; Kim, J.-H.; Kim, N.-S. Concentration of phytoestrogens in soybeans and soybean products in Korea. *J. Sci. Food Agric.* **2000**, *80*, 1709−1712.

(16) Hendrich, S.; Lee, K.-W.; Xu, X.; Wang, H.-J.; Murphy, P. A. Defining food components as new nutrients. *J. Nutr.* **1994**, *124*, 1789S−1792S.

(17) Anderson, J. J. B.; Garner, S. C. The effects of phytoestrogens on bone. *Nutr. Res.* **1997**, *17*, 1617−1632.

(18) Wang, H.; Murphy, P. A. Isoflavone content in commercial soybean foods. *J. Agric. Food Chem.* **1994**, *42*, 1666−1673.

(19) Barnes, S.; Kirk, M.; Coward, L. Isoflavones and their conjugates in soy foods: extraction conditions and analysis by HPLC−mass spectrometry. *J. Agric. Food Chem.* **1994**, *42*, 2466−2474.

(20) Matsuura, M.; Obata, A. β-Glucosidases from soybeans hydrolyze daidzin and genistin. *J. Food Sci.* **1993**, *58*, 144−147.

(21) Franke, A. A.; Hankin, J. H.; Yu, M. C.; Maskarinec, G.; Low, S. H.; Custer, L. J. Isoflavone levels in soy foods consumed by multiethnic populations in Singapore and Hawaii. *J. Agric. Food Chem.* **1999**, *47*, 977−986.

(22) Murphy, P. A.; Song, T.; Buseman, G.; Barua, K.; Beecher, G. R.; Trainer, D.; Holden, J. Isoflavones in retail and institutional soy foods. *J. Agric. Food Chem.* **1999**, *47*, 2697−2704.

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