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Nutritional Aspects of Second Generation Soy Foods

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ABSTRACT: Samples of 15 second generation soy-based products ($n = 3$), commercially available, were analyzed for their protein and isoflavone contents and in vitro antioxidant activity, by means of the Folin−Ciocalteu reducing ability, DPPH radical scavenging capacity, and oxygen radical absorbance capacity. Isoflavone identification and quantification were performed by high-performance liquid chromatography. Products containing soy and/or soy-based ingredients represent important sources of protein in addition to the low fat amounts. However, a large variation in isoflavone content and in vitro antioxidant capacity was observed. The isoflavone content varied from 2.4 to 18.1 mg/100 g (FW), and soy kibe and soy sausage presented the highest amounts. Chocolate had the highest antioxidant capacity, but this fact was probably associated with the addition of cocoa liquor, a well-known source of polyphenolics. This study showed that the soy-based foods do not present a significant content of isoflavones when compared with the grain, and their in vitro antioxidant capacity is not related with these compounds but rather to the presence of other phenolics and synthetic antioxidants, such as sodium erythorbate. However, they may represent alternative sources and provide soy protein, isoflavones, and vegetable fat for those who are not ready to eat traditional soy foods.

KEYWORDS: Soy-based products, protein, nutritional composition, antioxidant capacity, isoflavones

INTRODUCTION

Soybean is a functional food with a large number of positive human physiological effects,[1] due to the high protein content, fat composition (rich in unsaturated, especially polyunsaturated linoleic acid), and isoflavones. Nowadays, there is an increasing interest in incorporating soybeans or soy protein ingredients into provide functional foods,[2] which could contribute to a more balanced diet. This interest of the food industry is associated with a health claim authorized by the FDA in 1999, due to the relationship between soy protein and a reduced risk of coronary heart disease.[3] Soy has been used in Japanese and Chinese diets for a long time, and recently, its consumption has been extending to the West.[4] This legume is an excellent source of protein (30−45%) and shows a high content of unsaturated fatty acids, besides presenting a suitable profile of essential minerals.[1]

In western countries, the soybean extract ("soy milk") has been used as an important alternative for people who have lactose intolerance or cow's milk protein allergy. Scientific evidence of the beneficial effects associated with soy consumption also has contributed to increases in soybean extract and soy protein intake.[5]

Traditional soy foods include tofu, miso, tempeh, and soy sauces.[6] However, in Brazil, the consumption of soybeans is not as widespread as that of kidney beans. Accordingly, in recent years, there has been an increasing interest for food manufacturers to incorporate soy protein ingredients in a variety of products known as "functional foods".[2] Many industrialized foods enriched with soy, such as beverages, breads, soups, and vegetarian meat, have been highlighted in the national market.[5] These products have been adapted to occidental taste and represent a new class known as "second generation" soy foods.[7] The status of soy as a healthy food has encouraged the development of new possibilities for incorporating soy or soy-based products in Brazilian dietary habits. This allows our population to obtain the positive effects attributed to soybeans.[6]

Epidemiological research has demonstrated a reduced incidence of certain cancers (breast, colon, prostate, and uterine) in Asian populations, where the consumption of soy is about 20−50 times higher than in the traditional western diet.[1] Both the protein and the isoflavones may contribute to these beneficial effects on health, mainly due but not limited to their antioxidant properties. Anderson et al. performed a meta-analysis of 38 clinical trials for the relationship among soy protein consumption, total cholesterol, low-density lipid cholesterol (LDL), and triglyceride levels. All three biomarker values decreased significantly with the consumption of soy protein-containing products: total cholesterol by 9%, LDL cholesterol by 13%, and triglycerides by 11%. Cassidy et al.[9] performed a critical review regarding the health effects of soybean phytoestrogens in postmenopausal women. The consumption of whole-soybean foods and soy protein had some beneficial effects on lipid markers of cardiovascular risk. However, the consumption of isolated isoflavones did not affect blood lipid levels or blood pressure, although it may improve endothelial function.

The levels of the soy isoflavones daidzein, genistein, and glycitein in soybeans are affected by genetic and environmental factors, such as differences among the soybean cultivars, temperature, and rain during the seed growth,[4] as well as storage period, localization, and planting dates.[10] The processing conditions also are important factors that interfere with the concentration and profile of these compounds in products containing soy.[5]

It is very usual to find researchers using univariate approaches, such as one-way analysis of variance (ANOVA), to display the results of a large set of samples. Besides being attractive, this

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approach fails to show differences among samples and response variables simultaneously. To overcome this limitation, principal component analysis (PCA) can be employed. PCA represents one of the most frequently used chemometric tools and allows the visualization of the original arrangement of soy products in an *n*-dimensional space, by identifying the directions in which most of the information is retained. It is therefore possible to explain differences among the various soy products by means of the factors obtained from the generalized correlation matrix of the data sets and at the same time to determine which variables contribute most to such differentiation.[11]

Although soy foods have been consumed for more than 3000 years in earstern countries, only in recent years a new variety of products have been introduced into western cultures and diets. For this reason, the objective of this work was to investigate the second generation of soy foods in relation to the amounts of protein and isoflavones, as well as the antioxidant activity, and correlate these parameters using a chemometric approach.

MATERIALS AND METHODS

Materials and Chemicals. A survey of all varieties and brands of soy products available in the Brazilian markets was performed in March 2007 through September 2009. Fifteen different soy-based products were selected and purchased from local supermarkets in São Paulo in the period of 2007−2009, from three different batches. The analyzed samples (three samples from each batch) consisted of soy condensed milk (from soy extract), whole bread containing soy grain, cereal bar containing soy protein isolate and soy flakes, chocolate based on soybean extract, cookies containing soy protein isolate (A brand), cookies based on soy flour (B brand), soy steak based on textured protein, soy hamburger based on textured protein containing soy protein isolate and concentrate, lasagna containing textured soy protein, nuggets based on textured soy protein and containing soy protein concentrate (A brand), nuggets (B brand), soy pasta, soy kibe, soy sausage, and soy stroganoff. The last five products were made of textured soy protein. Folin−Ciocalteu reagent, AAPH (azo-initiator 2,2′-azobis 2-methylpropionamedine dihydrochloride), trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl), fluorescein (3′,6′-dihydroxyspiro[isobenzofuran-1[3*H*],9′[9*H*]-xanthen]-3-one), catechin, and gallic acid were obtained from Sigma (St. Louis, MO). The aqueous solutions were prepared by using ultrapure Milli-Q water. All other reagents used in the experiments were of analytical grade.

Moisture Content. The moisture content of samples was determined in triplicate after freeze drying (−70 °C/96 h) in a Dura-Top MP, Bulk Tray Dryer, FST Systems.

Protein Content. The protein content ($N \times 6.25$) of samples was determined in triplicate by the micro-Kjeldahl method.[12]

Isoflavone Analysis. *Solid-Phase Extraction.* The extracts were obtained by extraction (1:20, w/v) of powdered samples of soy-based products with a solvent mixture comprised of methanol/water (80:20, v/v), at 10000*g* for 1 min (Brinkmann homogenizer, Polytron; Kinematica GmbH) while cooled in ice. The homogenate was filtered under reduced pressure through filter paper (Whatman #1), concentrated until methanol elimination under vacuum at 40 °C to ∼2 mL on a rotatory evaporator (Rotavapor RE 120, Büchi, Flawil, Sweden), and made up to 5 mL with distilled water. The extract was added to a 1 g of polyamide SC6 column (Macherey-Nagel Gmbh and Co, Düren, Germany) preconditioned with methanol (20 mL) and distilled water (60 mL). 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. 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 rotary evaporator to a volume of 0.2−0.4 mL, and then, the volume was adjusted to 2 mL with high-performance liquid chromatography (HPLC) grade methanol.[5] Aliquots of the samples were filtered through a 0.22 μm PTFE filter unit [poly(tetrafluoroethylene), Millipore Ltd., Bedford, MA] and analyzed by HPLC. The extractions were run in triplicate.

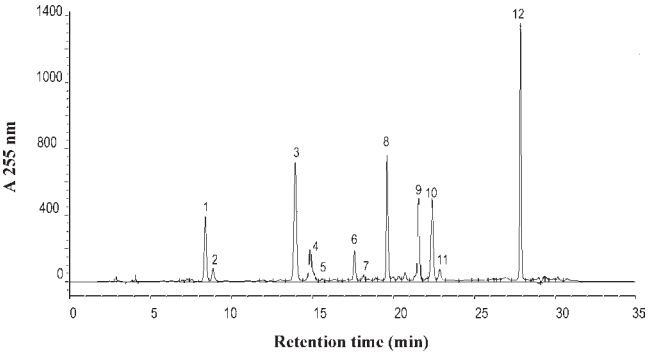

Figure 1. Typical HPLC chromatogram (255 nm) of soy-based products and retention times of the reference compounds: 1, daidzin; 2, glycitin; 3, genistin; 4, malonyldaidzin; 5, malonylglycitin; 6, acetyldaidzin; 7, acetylglycitin; 8, malonylgenistin; 9, acetylgenistin; 10, daidzein; 11, glycitein; and 12, genistein.

HPLC Quantitation of Isoflavones. Isoflavone separation and determination were performed according to Genovese and Lajolo[5] with a C18 NovaPak (30 cm × 4.6 mm id) column (Waters, Milford, MA) and a Hewlett-Packard 1100 system equipped with a diode array detector and the ChemStation software (version Rev. A.10.02[1757], Agilent Technologies, Palo Alto, CA). Identification was made based on the spectra and retention time in comparison to known standards, and quantitation was based on external calibration. The 12 isoflavone standards were from LC Laboratories (Woburn, MA). Calibration was performed by injecting the standards three times at five different concentrations ($R^2 \geq 0.999$). 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 weights between glycoside derivatives. The weight 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, as recommended by Song et al.[13] Figure 1 shows a typical chromatogram of soy-based products and the retention times for the reference compounds.

Antioxidant Capacity. *Folin−Ciocalteu Reducing Ability.* The Folin−Ciocalteu reducing ability was determined according to the method of Singleton et al.[14] The samples were extracted in a solvent mixture composed of methanol/water (70:30, v/v). The homogenate was filtered under reduced pressure through filter paper (Whatman #1). A 0.25 mL aliquot of adequately diluted extract was added to 2 mL of distilled water and 0.25 mL of the Folin−Ciocalteu reagent. After 3 min at room temperature, 0.25 mL of saturated sodium carbonate was added, and the tubes were kept at 37 °C for 30 min for color development. The absorbance readings were performed at 750 nm in a spectrophotometer Ultrospec 2000 UV−visible spectrophotometer (Amersham Biosciences, Cambridge, United Kingdom), and results were expressed as mg equivalents of catechin per 100 g of sample FW.

DPPH$^{\bullet}$ Scavenging Activity. The DPPH$^{\bullet}$ scavenging activity of soy food phenolics was assessed according to Brand-Williams et al.,[15] with some modifications.[16] Briefly, a 50 μL aliquot of the extract previously diluted and 250 μL of a methanol solution of DPPH$^{\bullet}$ (0.5 mM) were shaken, and after 20 min, the absorbance was measured at 517 nm using the Benchmark Plus microplate spectrophotometer (BioRad, CA). Results were expressed as μmols Trolox equivalents/100 g of sample FW.

Oxygen Radical Absorbance Capacity (ORAC) Assay. The ORAC assay was performed according to Dávalos et al.[17] for extraction of hydrophilic fraction. The original method was altered by Prior et al.[18] for extraction of lipophilic compounds. Briefly, the hydrosoluble extracts were diluted in 75 mM phosphate buffer (pH 7.4), and the liposoluble extracts were diluted in acetone/water (1:1, v/v) containing 7% randomly methylated β-cyclodextrin. An aliquot of extracts in appropriate dilution was mixed with a fluorescein solution (70 nM). After incubation (37 °C, 15 min), an AAPH solution (24 mM) was added to all of the tubes. The fluorescence was measured every 10 min following addition of AAPH for 80 min. The antioxidant activity was expressed as μmols Trolox equivalents/100 g of sample FW.

Statistical Analysis. Univariate Analysis. All of the analyses were performed in triplicate samples, and results were expressed as means ± standard deviations. First, the results were subjected to Levene's test to check for homogeneity of variances, while one-way ANOVA was applied for identification of contrasts among samples, and differences among means were analyzed by Fisher's LSD test ($p < 0.05$). To verify the univariate relationships between parameters, Pearson correlation coefficients (r) were calculated, and the significance level (p value) of such correlations was also provided.[11]

Chemometric Application. PCA, implemented in the Statistica 7.1 software (Stat-Soft Inc., Tulsa, OK), was the chemometric statistical technique used to analyze correlations among all results simultaneously. For this purpose, PCA was applied to the autoscaled data to separate the samples ($n = 15$) according to their values of ORAC, DPPH, total protein, Folin−Ciocalteu reducing ability, and total isoflavone content, for a total of 75 results. The data obtained for each parameter were adopted as columns, and the soy-based products were adopted as rows. Analyses were based on correlation, and variances were computed as $SS/(n - 1)$. Eigenvalues higher than 1.0 were adopted to explain the projection of the soy food samples on the factor-plane (1×2).

RESULTS AND DISCUSSION

Traditional foods have been modified to supply consumer expectations. Meat and milk are the most common ingredients excluded from formulation of these products. They are replaced by textured soy protein, soy isolate, and concentrate. These commercial soy-based products contain soybeans and/or soy protein ingredients as the major starting material and represent the "new generation" of soy foods. Their appearance and taste are very similar to conventional foods. Meat analogs (hamburger and sausage), dairy products (ice cream and yogurts), and frozen, ready-to-eat meals, such as lasagna and stroganoff, are the most representative and consumed among these foods.[7] In this way, after a survey made in the main supermarkets in Sao Paulo city, 15 products were selected among the most common marketed in Brazil and compared in relation to the soy-derived ingredients (Table 1).

Meat analog products, such as soy steak, soy hamburger, soy nuggets, soy kibe, and soy sausage, as well as frozen, ready-to-eat meals (soy lasagna, soy pasta, and soy stroganoff), are prepared with textured soy protein. This soy-derived ingredient is obtained through extrusion cooking of soy flour. During this process, quaternary structures of proteins open due to the moisture and high temperatures, proteins polymerize and reorient, and intermolecular bonds are set up, giving the final product a texture similar to meat. Meanwhile, enzymes, such as urease (which reduces the useful lifetime of the product), lipoxygenase (which produces off-flavors due to oxidation of the soybean oil), and the trypsin inhibitor (which reduces the digestibility of the protein), are destroyed. This process improves the biological value and modifies the functional properties of the product.[5]

Table 1. List of Soy-Derived Ingredients Used in the Formulation of Second Generation Soy-Based Products Commercialized in Brazil (Sampled from 2007 to 2009)[a]

soy-based products	soy protein ingredients	sample abbreviations for PCA
soy condensed milk	soybean extract	5
soy bread	soybeans (grain) and soy flour	12
soy cereal bar	soy protein isolate, soy flakes	1
soy chocolate	soybean extract	2
soy cookies (A brand)	soy protein isolate	3
soy cookies (B brand)	soy flour	4
soy steak	textured soy protein	6
soy hamburger	textured soy protein, soy protein isolate and concentrate	8
soy kibe	textured soy protein	13
soy lasagna	textured soy protein	9
soy nuggets (A brand)	textured soy protein and soy protein concentrate	10
soy nuggets (B brand)	textured soy protein	11
soy pasta	textured soy protein	14
soy sausage	textured soy protein	15
soy stroganoff	textured soy protein	7

[a] Available information in this table was obtained on the labels and corresponds to the data supplied by the manufacturers.

In soy condensed milk (extract) and soy chocolate, soy extract has been used as a cow's milk substitute. In the cereal bar and bread, soybean and soy protein ingredients have been incorporated to enrich the nutritional value of these products, because there is no partial or total substitution.

Soy-derived ingredients are also used as starting material for many conventional foods, such as meat products, breads, beverages, sauces, and soups, because of their functional properties, such as absorption, gel formation, and emulsification.[5]

The average protein and moisture contents (three different batches of each product) were determined (Table 2) and compared to the corresponding values on the product labels and to data from the Brazilian Food Composition Table.[19] In general, the protein content was in accordance with the information provided by suppliers. In comparison to conventional versions, some soy-based products (soy chocolate, soy kibe, and soy sausage) presented similar protein contents. However, other samples showed different amounts of protein in relation to regular products. Soy bread, soy cereal bar, and soy lasagna presented higher values (from 34 to 59% more). Soy condensed milk (extract), soy cookies (two brands), soy steak, soy hamburger, soy nuggets (two brands), soy pasta, and soy stroganoff showed lower amounts of protein (from 8 to 54% less).

The highest protein contents were detected in soy cereal bar, soy nuggets, soy kibe, and soy sausage (13.6−15.5 g/100 g FW). The consumption of 100 g of these industrialized soy-based products corresponds to approximately 56 g of cooked (boiled) soybeans, which is equivalent to 9.4 g of protein.[20] The FDA recommends a daily intake of 25 g of soy protein,[3] corresponding to six soy cereal bars, three soy sausages, eight soy nuggets, or eight soy kibes. The consumption of these products in combination makes it possible and easier to achieve the recommendation of soy protein intake.

Table 2. Moisture (%), Protein Content (%), Fat Content (%), and Calorie (kcal/100 g) of Second Generation Soy-Based Products Commercialized in Brazil (Sampled from 2007 to 2009)[a]

soy-based products	moisture	protein content	fat content[b]	calories[b]
soy condensed milk	23 ± 1 b	3.9 ± 0.2 a	8.0	300
soy bread	39 ± 1 c	12.4 ± 0.4 c,d	2.4	212
soy cereal bar	4.2 ± 0.3 a	15.5 ± 0.2 e	6.8	328
soy chocolate	1.7 ± 0.1 a	5.7 ± 0.3 a	32.8	548
soy cookies (A brand)	25 ± 1 b	4.0 ± 0.1 a	15.0	413
soy cookies (B brand)	28 ± 2 b	3.7 ± 0.2 a	14.0	410
soy steak	41 ± 1 c	11.1 ± 0.3 b,c	7.5	164
soy hamburger	50.8 ± 0.1 d	8.9 ± 0.1 b	3.75	80
soy kibe	60 ± 1 e	14 ± 1 c,d,e	3.75	209
soy lasagna	66 ± 1 f	8.8 ± 0.3 b	6.0	186
soy nuggets (A brand)	50 ± 3 d	11.4 ± 0.2 b,c	20.0	293
soy nuggets (B brand)	52 ± 2 d	13.6 ± 0.3 c,d,e	10.0	204
soy pasta	70 ± 4 f	8.5 ± 0.3 b	4.7	129
soy sausage	59.6 ± 0.2 e	15 ± 1 d,e	12.5	203
soy stroganoff	79 ± 1 g	5.0 ± 0.2 a	8.0	115
P value (Levene)	0.06	0.09	0.12	0.13
P value (ANOVA)	<0.001	<0.001	<0.001	<0.001

[a] Values are expressed as means ± SDs for triplicates. Means in the same column with common letters are not significantly different according to Fisher's LSD test ($p > 0.05$). [b] Fat and calorie contents according to label information supplied by the manufacturers.

The moisture content of soy-based products varied from 2 (soy chocolate) to 79% (soy stroganoff) and was compared to data of the same samples in their regular version.[19] Soy condensed milk (extract), soy bread, soy chocolate, soy cookies (two brands), soy nuggets (two brands), soy kibe, soy sausage, and soy stroganoff showed moisture contents similar to the regular products. Soy cereal bar, soy steak, soy hamburger, soy lasagna, and soy pasta had lower values than the conventional versions. The differences in moisture content were expected and possibly resulted from the alteration of various ingredients in the original formulation.

Among all of the second generation soy foods, soy chocolate, soy cookies (two brands), and soy nuggets (A brand) presented the highest amounts of fat and calories, indicating that total lipids had a significant contribution to the energy content of these products. Soy condensed milk and soy cereal bar were also rich in calories, probably due to the higher amounts of carbohydrates in their composition.

The isoflavone content and distribution (malonyl, acetyl, β-glycosides, and aglycones) of soy-based products are shown in Figure 2. Products that contained soy-derived ingredients showed a large variability in isoflavone contents, ranging from 18.1 to 2.4 mg/100 g (FW), and soy kibe and soy sausage presented the highest amount of isoflavones. A significant variation in the content of isoflavones was detected by Kuhnle et al.[21] in 115 foods including vegetarian substitutes. Phytoestrogens were detected in all analyzed foods; the average content was 20 μg/100 g (FW), and isoflavones represented a total of 6 μg/100 g. Soy cereal bar, soy cookies (A brand), and soy hamburger, which are made of soy protein isolate, presented aglycone percentages (25−38%) similar to soy protein isolate (31%).[2] Although there is a predominance of glycosylated isoflavones in soybeans, the endogenous β-glucosidase activity during the aqueous extraction process for isolate production leads to hydrolysis of β-glucosides into aglycones.[22]

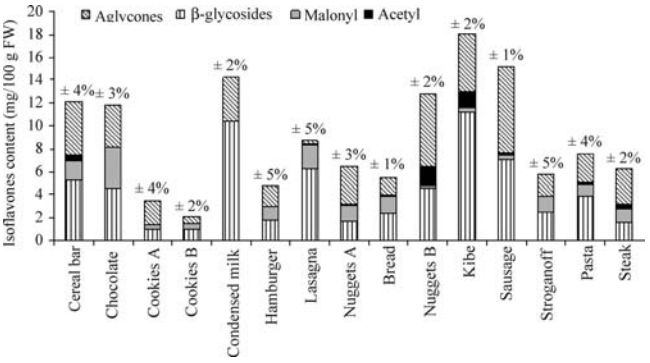

Figure 2. Isoflavone profile and content (mg/100 g FW) of second generation soy-based products. The standard deviation of total isoflavone content was expressed as a percentage, by means of values written above the column relative to the products.

Genistein derivatives were the highest in all products (59−66%), followed by daidzein derivatives (20−32%). Ratios among genistein, daidzein, and glycitein (3:2:1) were similar in all products. The same profile was found by Setchell et al.[23] in soy protein preparations and in several soy protein ingredients,[24] showing that the distribution of isoflavones was the same of that in soy cotyledons, indicating that only the profile of glycosylated isoflavones changed during the processing.[2,25]

In the soy nuggets (two brands), soy kibe and soy sausage, obtained by incorporation of textured soy protein, the percentage of acetylglycosides was in the range of 2−13%. These compounds are normally formed during the toasting of soybean flour or extrusion.[5] The aglycone contents (49−52%) observed in these products were similar to those reported in a previous study (44−59%).[1] Textured and isolated soy proteins used as ingredients in these products were analyzed concomitantly, showing isoflavone contents from 47 to 92 mg/100 g (FW), predominance of acetylglycosides in textured ingredients, and of aglycones in protein isolates (Figure 3).

The isoflavone forms differ in relation to absorption, bioavailability, and potential beneficial effects on health. For example, the extent of the antioxidant capacity of isoflavones is positively correlated to the number of hydroxyl groups in the isoflavone nucleus. Glycosidation of isoflavones depresses their antioxidant activity considerably.[28,29]

Previously, Barbosa et al.[30] reported that the chronic ingestion of dietary soy isoflavones has a positive effect on the antioxidant status in Wistar rats, enhancing the plasma antioxidant capacity and the antioxidant enzymes in some tissues. These effects are associated with the free form of isoflavones and not with protein-associated nor soy protein. On the other hand, soy consumption is not effective on biomarkers of oxidative stress in hypercholesterolemic humans. Although plasma total antioxidant activity was 10% higher with soy protein intake, regardless of dietary isoflavones, the effect on oxidative stress was masked by the other plasma antioxidants present in relatively high concentration.[31]

Although isoflavones are not destroyed by heating during processing of food, the high temperature alters isoflavone conjugation in these products. Heating treatment promotes the conversion (deesterification) of malonylglycosides to β-glycosides, more thermostable compounds.[5]

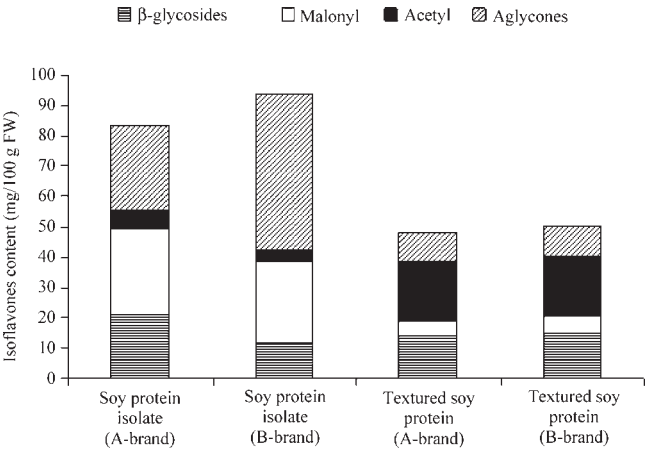

Figure 3. Total content of isoflavones (mg/100 g FW) of soy-derived ingredients used in processing of second generation soy-based products.

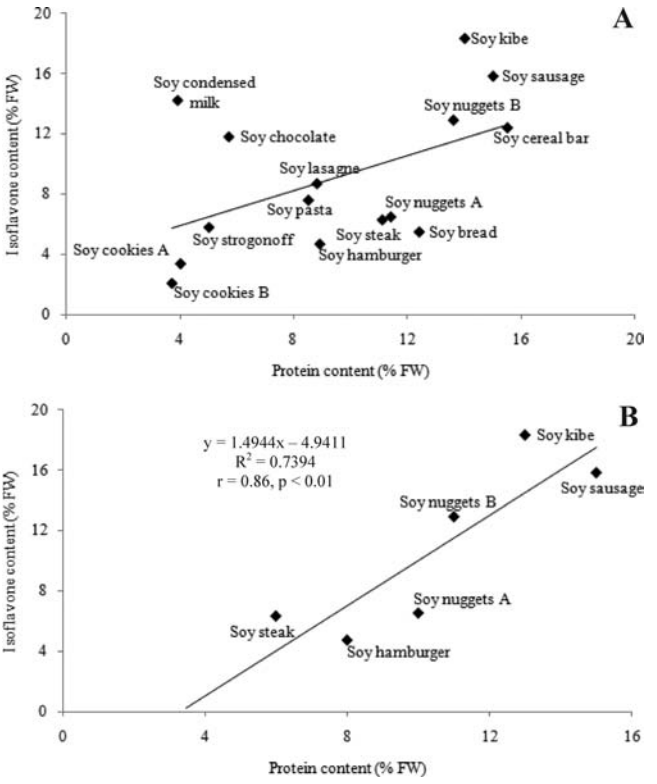

Figure 4. (A) Pearson's correlation coefficient (r) between protein and isoflavone contents of all second generation soy-based products. (B) Pearson's correlation coefficient (r) between protein and isoflavone contents of second generation soy-based products containing soy protein as the unique protein source.

The U.S. Department of Agriculture was responsible for publishing the first database containing information related to the isoflavone content of many foods. The available values in the database for soy sausage and soy nuggets are lower than those found here. Variations could be attributed to the composition of the products analyzed by the U.S. Department of Agriculture, which contain soy protein as a partial meat replacer. Thus, we expected a lower isoflavone content in these products in comparison to those products based only on vegetable protein (soy ingredients) as a source of protein. The soy hamburger analyzed by the U.S. Department of Agriculture does not have meat in its composition (total replacement by soy protein). The amounts of isoflavones were twice those in our sample, which also contains only soy proteins. The variety of the grain, the harvest season, and geographic location affect the isoflavone content and contribute significantly to the variability in the content of these compounds in foods containing soy or derivatives.[4,32]

The hamburger analyzed here contains protein isolate and textured protein concentrate as ingredients. Data from the U.S. Department of Agriculture table show that the protein concentrate obtained by alcohol extraction has about 90% less isoflavones than that obtained by aqueous extraction.[20] Thus, if the protein concentrate incorporated in the hamburger was obtained by alcohol extraction, its contribution in isoflavone content is very low, which explains the low amounts of these compounds in the products analyzed in this study.

Isoflavone contents of the second generation soy-based products are considerably lower than those in the other products derived from soybeans (isolated, concentrated, and textured soy protein). This profile was expected due to the use of many ingredients besides soy (dilution with nonsoybean ingredients) in the production of these samples. The addition of this grain or its derivatives may occur as a substitute for animal protein or to reduce the fat content of the products. However, the utilization of these ingredients should be limited to maintain similarity to conventional products.[7,26]

Pearson's correlation coefficient was used to investigate the association between isoflavones and protein amounts. The protein content had a moderate, but statistically significant, correlation with the isoflavones content ($r = 0.60$, $p < 0.01$) (Figure 4). The isoflavone content of these products is associated with their protein content, as much of the protein present is derived from soybeans or soy-based ingredients, which is in accordance with physicochemical interactions between the main storage soy proteins, glycinin and β-conglycinin, and isoflavones, through electrostatic binding or hydrophobic interaction.[22]

Similarly, Callou et al.[27] observed a high positive correlation ($r = 0.81$, $p < 0.05$) between protein and isoflavone content of soy-based beverages. The addition of other protein ingredients (egg, cheese, and milk) could contribute to the decrease in correlation coefficient, because they are not source of isoflavones.

Products containing soy-derived ingredients showed large variability in the antioxidant capacity determined by the DPPH and the Folin−Ciocalteu assays. The results ranged from 11 to 998 μmol of Trolox equivalent/100 g and from 32 to 575 mg of catechin equivalent/100 g, respectively (Table 3). Soy chocolate presented the highest antioxidant activity among the samples by either the DPPH or the Folin−Ciocalteu assay, 5−10 times higher than soy cereal bars.

To compare the antioxidant capacity of conventional and soy-based products, two products in their regular version, hamburger and sausage, were analyzed, and these foods had similar antioxidant activities to soy and/or derivative products assessed by Folin−Ciocalteu assay (71 ± 1 and 61 ± 1 mg catechin equivalent/100 g FW, respectively). However, the DPPH scavenging capacity of the conventional products was higher (144 ± 2 and 129 ± 1 μmol Trolox equivalent/100 g FW, respectively) than for all products containing soy and/or derivatives, except for chocolate. This fact can be attributed to the addition of sodium erythorbate, an antioxidant commonly used

Table 3. Antioxidant Capacity of Second Generation Soy-Based Products Assessed by Means of Folin−Ciocalteu Reducing Ability (mg of Catechin Equivalent/100 g FW) and DPPH Radical Scavenging Capacity (μmol of Trolox Equivalent/100 g FW)[a]

soy-based products	Folin−Ciocalteu	DPPH
soy condensed milk	71 ± 3 d	59 ± 5 e
soy bread	89 ± 3 c	38 ± 3 f
soy cereal bar	118 ± 5 b	81 ± 4 c
soy chocolate	575 ± 28 a	998 ± 10 a
soy cookies (A brand)	65 ± 2 e	30 ± 1 g
soy cookies (B brand)	51 ± 1 f	24 ± 1 h
soy steak	57 ± 2 f	31 ± 1 g
soy hamburger	43 ± 2 g	11 ± 1 i
soy kibe	72 ± 4 d	87 ± 1 b
soy lasagna	67 ± 2 e	31 ± 1 g
soy nuggets (A brand)	46 ± 2 f,g	18 ± 1 h
soy nuggets (B brand)	57 ± 2 f	11 ± 1 i
soy pasta	38 ± 1 g,h	21 ± 1 h
soy sausage	59 ± 1 f	75 ± 4 d
soy stroganoff	32 ± 2 h	20 ± 2 h
P value (Levene)	0.06	0.07
P value (ANOVA)	<0.001	<0.001

[a] Values are expressed as means ± SDs for triplicates. Means in the same column with common letters are not significantly different ($p > 0.05$).

in meat products. This compound is a synthetic isomer of vitamin C and is used at concentrations in the range 0.05−0.25%, which could interfere in the Folin−Ciocalteu and DPPH assays. The Folin−Ciocalteu assay was employed to determine the total phenolic content, and this method is not specific to these compounds. The Folin reagent can be reduced by the other components, such as ascorbic acid, tertiary amines, Cu(I), reducing sugars, or aromatic amino acids (tryptophan and tyrosine), forming a blue complex of molybdenum.[14] To investigate the contribution of hydrophilic and lipophilic fractions, the ORAC method was used to assess the total antioxidant capacity of these products (Figure 5).

Soy chocolate showed the highest values of total antioxidant capacity and hydrophilic and lipophilic antioxidant capacity assessed by the ORAC assay. Soy chocolate presented a total antioxidant activity six times higher than the majority of soy-based products analyzed here. Among those other soy foods, soy kibe also had a high antioxidant capacity, especially because of hydrophilic compound contribution.

The hydrophilic antioxidants accounted for more than 85% of the total antioxidant compounds of vegetable materials.[18] In these products, the hydrophilic fraction corresponded to 84−99% of the total antioxidant capacity.

Table 4 presents the correlation coefficients among the response variables, including the hydrophilic (ORAC-H) and lipophilic (ORAC-L) fractions of the ORAC assay. There was a high correlation among the values of antioxidant capacity determined by the three methods of Folin−Ciocalteu, DPPH, and ORAC, mainly for the hydrophilic fraction.

Numerous publications showed excellent linear correlation between the Folin−Ciocalteu reducing ability and the antioxidant activity assessed by DPPH assay. This fact is mainly due to the similarity between the mechanisms involved in both methods, which

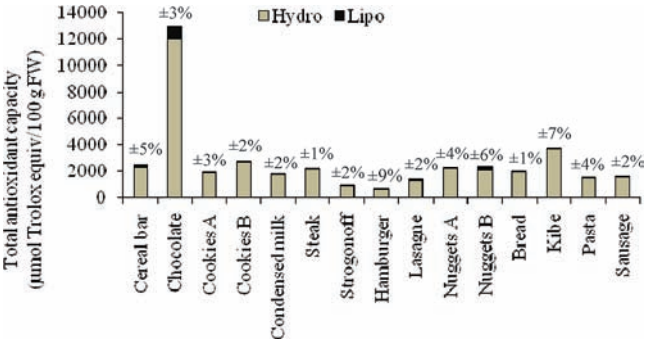

Figure 5. Total antioxidant capacity and both hydrophilic and lipophilic fractions of products containing soy and/or derivatives, obtained by means of ORAC, expressed as μmol Trolox equivalent/100 g sample (FW). The standard deviation of total isoflavone content was expressed as a percentage, by means of values written above the column relative to the products.

Table 4. Pearson's Correlation Coefficient (r) among the Three Different Methods for Determining Antioxidant Capacity and Isoflavone Content of Soy-Based Products

methods	correlation coefficient (r)	p value
Folin−Ciocalteu × DPPH	0.99	<0.01
Folin−Ciocalteu × ORAC	0.97	<0.01
Folin−Ciocalteu × ORAC-H	0.97	<0.01
Folin−Ciocalteu × ORAC-L	0.92	<0.01
DPPH × ORAC	0.97	<0.01
DPPH × ORAC-H	0.97	<0.01
DPPH × ORAC-L	0.91	<0.01
isoflavones × DPPH	0.24	<0.05
isoflavones × Folin−Ciocalteu	0.22	<0.05
isoflavones × ORAC	0.25	<0.05

is basically an electron transfer reaction.[27] The ORAC method is based on the transfer of hydrogen atoms from antioxidants to the radical AAPH.[17] This assay was originally developed to measure the antioxidant capacity of hydrophilic compounds, since it employs a hydrophilic radical generator and a hydrophilic detector. Modifications have been performed to improve the sensitivity of the method by using a new indicator and a specific component to enhance the solubility of lipophilic antioxidants, providing a more comprehensive estimate of the total antioxidant capacity of samples. However, the lipophilic fraction (ORAC-L) did not show good correlation with other methods for determining antioxidant capacity, because they are not able to measure the antioxidant capacities of liposoluble compounds.[26]

There was no significant correlation between these variables, which could be explained by the presence of other ingredients in these foods, which are also rich in phenolic compounds with antioxidant activity. Among them, we should mention the cocoa liquor and spices, as well as synthetic food additives, such as erythorbate, used as antioxidant agents.

Univariate approaches such as ANOVA are recognized worldwide to be very specific and robust to check for differences in a response variable. However, when more than 12 samples are evaluated by multiple variables, such as in this work, a multivariate approach is required to depict correlations among variables and also

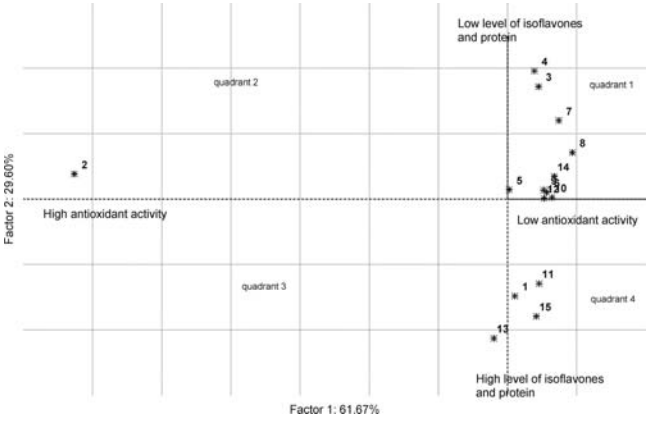

Figure 6. Scatter plot of PC 1 vs PC 2 of the main sources of variability between the soy-based foods. See Table 1 for the definition of sample abbreviations.

to try to group samples with similar characteristics. In this study, PCA was used to determine similarities among all soy-food products and show which variables contributed more to differentiation. The first principal component (PC) (eigenvalue 3.08) was associated with the Folin−Ciocalteu reducing ability and antioxidant activity toward DPPH and AAPH radicals, while the second PC (eigenvalue 1.48) was associated to the protein and isoflavone contents. The first two PCs explained up to 91.27% of all variability in the data set. Sample 2 (Figure 6) had the highest value of DPPH, ORAC, and phenolic compounds, while the samples present in the right side of the scatter plot presented a lower value of these parameters. Samples included in the first quadrant present very similar characteristics, low antioxidant activity and low contents of isoflavones and protein, while the samples included in the third and fourth quadrants present a high content of protein and isoflavones besides having low antioxidant properties. Moreover, it is possible to verify that the three antioxidant activity assays are associated (confirming the Pearson linear correlations) and that the soy chocolate is a very interesting product with a high antioxidant activity. The uniqueness of multivariate statistical techniques, such as PCA applied to food analysis, is that the researcher may observe correlations among all parameters and samples simultaneously, which enables the identification of important features of a food.

In conclusion, there is a wide variation among products containing soy and/or soy-based ingredients in relation to protein and isoflavone contents and antioxidant capacities. These industrialized foods do not present a significant content of isoflavones when compared with the grain, and their in vitro antioxidant capacity is not correlated with these compounds. However, they may represent alternative sources and provide soy protein, isoflavones, and vegetable fat for those who are not ready to eat traditional soy foods.

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