

## BIOACTIVE COMPOUNDS AND ANTIOXIDANT POTENTIAL OF SOY PRODUCTS\*

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■ **ABSTRACT:** The aim of this work was to evaluate the amounts of bioactive compounds in soybean and derived products and the antioxidant activity (AA) assessed by the methods of ABTS<sup>+</sup>, DPPH, FRAP and peroxidation of linoleic acid (PLA). The micronized soy protein (MSP), defatted soy flour (DSF) and textured soy protein (TSP) had a higher content of phenolic compounds and higher antioxidant activity (AA), than the other products. MSP and tofus had the highest content of flavonoids and phytic acid (PA), respectively. The AA correlated with total phenolics and flavonoids, but the PA can act synergistically chelating the pro oxidants ions iron and copper. The highest concentration of copper was in soy protein isolate, and of iron in an ingredient of soy fiber and soy germ. Many compounds present in soy products contribute for the AA, but the concentration and potential will depend on final preparation of the grain or ingredients before consumption.

■ **KEYWORDS:** Phenolic compounds; flavonoids; phytic acid; iron; copper.

### INTRODUCTION

The soybean [*Glycine max (L.) Merrill*] is an important commodity noticeable in the grain market due to its levels of oil, proteins, essential amino acids and secondary metabolites considered beneficial – phenolic compounds and flavonoids among others. The isoflavones are the principal compounds of the flavonoid group and are associated with numerous benefits to the human health like prevention of cancer, cardiovascular diseases, osteoporosis and menopause symptoms.<sup>1</sup>

The phenolic compounds are known as active antioxidant and as such they are able to scavenge free radicals (FR) – important due the deleterious effect of such reactive species, since they are able to oxidize bio molecules which may damage cells and cause tissue alterations. The antioxidants present in foods or supplements help the human body to reduce oxidative stress by different mechanisms of action, hindering the formation of free radicals, blocking

the decomposition of peroxides and hydroperoxides, or acting as metal chelator.<sup>18</sup>

Soybean grains have phytic acid (PA) generally associated with the protein bodies, whose concentration varies from 1 to 1.5%,<sup>17</sup> it is considered an efficient chelating agent able to interact with iron ion, blocking free bonds and hindering the catalysation of lipid peroxidation by the metal ion.<sup>28</sup> Besides PA, the flavonoids can chelate metal ions and there are some evidences that the chelates formed are more effective in scavenging free radicals than the isolated flavonoids.<sup>15</sup>

Consumption of soybean grain per capita in Brazil is not as significant as in Asian countries, despite all the healthy properties associated with its consumption. On the other hand, protein ingredients derived from soybeans have been used extensively in the industrialization of meat products, breads, beverages, soups, among others. The hydro soluble soy extract (soy milk) and tofu have been gaining market due to improvement in sensory characteristics. Thus, the objective of this study was to investigate how much of the phenolic compounds, flavonoids, phytic acid, copper and iron present in the soybean grain are retained in the soybean derivatives, and assess the antioxidant potential of the products using different methods.

### MATERIAL AND METHODS

#### Material and Reagents

The soybean variety studied was the BRS 267, supplied by EMBRAPA – Soja (Londrina - PR), and used as raw-material reference. Two different lots of defatted soybean flour (DSF), the other whole grain organic soybean flour (WOSF), soybean fiber flour (SFF), soybean fiber and germ flour (SFGF), three hydro soluble soy powders (soy milk, HSSP), two soybean protein isolate (SPI), four textured soy proteins (TSP), micronized soybean protein (MSP), and tofus were bought in a supermarket in Londrina, Paraná, Brazil for analysis, but they are representative of large industries with national distribution.

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The standards quercetin and gallic acid were purchased from Merck, (Darmstadt, Germany) and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-acid carboxylic), from Sigma Chemical Co. (Saint Louis, EUA). The reagents ABTS<sup>+</sup> (2,2-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid), DPPH<sup>•</sup> (2,2-diphenyl-1-picrylhydrazyl), TPTZ (2,4,6-tripyridyl-s-triazine) and potassium persulfate were obtained from Sigma-Aldrich Chemie (Steinheim, Germany) and the Folin-Ciocalteu reagent from Dinâmica (São Paulo, Brazil). The others were of analytical grade.

### Extraction of Antioxidant Compounds

One gram of the sample, previously grounded and sifted in a 40 mesh sieve, was extracted with 80% ethanol.<sup>14</sup> The pooled ethanolic extract obtained was used for the determination of total phenolic and flavonoid compounds and for the assessment of the antioxidant potential.

### Determination of the Total Phenolic Compounds

The quantification of the total phenolic compounds used the method of Folin-Ciocalteu, as described by Swain & Hillis,<sup>22</sup> using 2,5mL of Folin-Ciocalteu reagent (10%), 2,0mL of sodium carbonate (7,5%) and 0,5mL of extract. After 5 min at 50°C, the absorbance was read at 760nm against the blank (Folin-Ciocalteu reagent and sodium carbonate). The concentration of the total phenolic compounds was quantified using a standard curve prepared with gallic acid and expressed as mg gallic acid equivalent per 100g of sample (mg GAE/100g).

### Determination of Total Flavonoids

The level of total flavonoids was determined according to Boateng et al.<sup>6</sup> Thus, 0,5mL of extract was added of 3,5mL of deionized water, 150µL of NaNO<sub>2</sub> (50g/L), 150µL of AlCl<sub>3</sub> (100g/L) and 1mL of NaOH (1M). The solutions were shaken and the absorbance was read at 415nm. The blank solution had no sample. The level of total flavonoids was calculated based on the standard curve of quercetin (from 0.05 to 0.25mg/mL) and expressed as mg quercetin equivalent per 100g of sample (mg QE/100g).

### Determination of Phytic Acid

The extraction of phytic acid from grains and soy products was carried out according to Thompson & Erdman.<sup>24</sup> The amount of phytic phosphorous (PP) was determined in a spectrophotometer UV-VIS at 820nm (Cintra 20) according to Chen et al.<sup>9</sup> using the factor 3.55 related to 28.2% of PP present in PA molecule to convert phytic phosphorous into phytic acid. Phosphorus standard curve was a solution of K<sub>2</sub>HPO<sub>4</sub> with P concentrations varying from 0.9 to 9.0µg.

### Determination of Copper, Iron and Total Phosphorus

The analysis of Cu, Fe and total P was carried out by a nitric perchloric digestion (HNO<sub>3</sub>:HClO<sub>4</sub>/3:1) of the

samples and after proper dilution measured in a plasma emission spectrometry (ICP- ICAP 61E, Thermo Jarrel Ash Corporation).<sup>2</sup>

### Determination of the Antioxidant Activity

The scavenging activity of DPPH<sup>•</sup> radicals (2,2-diphenyl-1-picrylhydrazyl) was determined according to Brand-Williams et al.<sup>7</sup> In this analyse 1mL of acetate buffer (100mM, pH 5,5); 1mL of ethanol; 0,5mL of DPPH solution (250µM) and 50µL of extract were mixed. After 30 minutes the absorbance was read at 517nm. The control had no sample and the blank no DPPH solution. The calibration curve was set with a solution of Trolox, linear between 100 and 1000µM Trolox.

The antioxidant capability of the extracts with ABTS<sup>+</sup> free radical was carried out according to Thaipong et al.<sup>23</sup> using 30µL of extract and 3mL of ABTS solution. The absorbance was read at 734nm after 6 minutes, using ethanol as blank. A standard curve with different concentrations of Trolox was used for the calibration (from 100 to 2000µM).

The ferric reduction power (FRAP) of the extracts was assessed according to Benzie & Strain.<sup>5</sup> Then 2,7mL of FRAP reagent (2,5mL of TPTZ 10mM, 2,5mL of FeCl<sub>3</sub> 20mM and 25mL of acetate buffer 0,3mM pH 3,6) was mixed with 90µL of extract. After 30 minutes at 37°C the absorbance was read at 595nm against the blank (FRAP reagent). The standard curve was also prepared with Trolox, linear between 100 to 1000µM. All the results were expressed as Trolox antioxidant equivalent (µmol of Trolox/g of the sample).

The antioxidant activity assessed by the method of linoleic acid peroxidation (PLA) was carried out according to Lingnert et al.<sup>16</sup> using 2,0mL of linoleic acid emulsion and 20µL of extract. After 8h at 37°C the absorbance was measured at 234nm. The antioxidant activity was calculated using the formula  $AA = (ABS_{234} \text{ of control} - ABS_{234} \text{ of sample}) / (ABS_{234} \text{ of control})$ . The control had no sample and the blank no linoleic acid.

### Determination of Extracted Solids

The moisture content of the samples was determined by desiccation at 105°C until reaching a constant weight according to AOAC method.<sup>2</sup> The dry residuals of the alcoholic extracts were determined after evaporation in water-bath and drying in at 105°C until constant weight. The levels of quantified compounds and antioxidant activities were expressed taking into account the weight of dried samples.

### Statistical Analysis

The analyses were carried out in triplicate and the results were expressed as mean ± standard deviation. Analyses of variance (ANOVA) were conducted and differences among the samples means were analyzed by

Tukey test ( $p < 0.05$ ), the results were also analyzed by principal component analysis (PCA) using the software Statistica version 6.0.

## RESULTS AND DISCUSSION

### Total Phenolic Compounds and Flavonoids

The soybean grain (BRS 267 Embrapa) had concentration of phenolic compounds and total flavonoids equivalent to 187.8mg GAE/100g d.b. and 94.3mg QE/100g d.b. (Table 1). Barbosa et al.<sup>4</sup> found total phenolic compounds of 200mg catechin/100g d.b. in a Brazilian soybean and Boateng et al.<sup>6</sup> found approximately 90mg of catechin/100g d.b. for the American soybean variety studied. We considered BRS 267 sample as representative of the grains produced in Brazil.

The micronized protein, the defatted flour and textured proteins (Kinasoy and Mãe Terra) had higher concentrations of phenolic compounds (Table 1). MSP showed the highest level of flavonoids (126.6mg QE/100g d.b.) probably due to the processing that involved a double milling, in a hammer mill and in a roller mill, and heat treatment at 80°C until reaching a final humidity between 6 and 8%. The smaller particle size favours the extraction of bioactive compounds, resulting in a higher level when compared to other products. TSP had higher level of phenolic compounds and flavonoids than the SPI

(Table 1). Despite the drastic conditions employed in this processing, which associates high temperature, pressure and shearing, Singletary et al.<sup>21</sup> observed a non-significant reduction of 9% from the raw material (soybean protein concentrate) therefore pointing out that the isoflavones were not degraded during the extrusion. SPI has another purification step compared to protein concentrate the material usually used for extrusion. According to Wang & Murphy<sup>27</sup> the isoflavones are associated with soluble compounds, mainly proteins, but they also reported lower levels of isoflavones in SPI (90% of protein) probably due to losses caused during extraction, since the alkaline pH used for the extraction may change the protein molecular charge and conformation what could alter the associations with the isoflavones. Moreover the soybean variety used, the agricultural practices and the environment may also have contributed for these results.<sup>11,26</sup>

Powdered soy milk products, soybean fiber flour and soybean fiber and germ flour were different regarding the total phenolic compounds, which varied from 144.2 for SFF to 230.2mg of GAE/100g d.b. (HSSP Vitao), but the comparisons are among very different products, some used only as ingredients (SFF, SFGF) while the soy milk powder will be diluted before consumption. Powdered soy milk and freeze dried tofus are similar products, differing only in the coagulation step but there is reduction of the whey proteins in the tofus, and this resulted in lower concentrations of

Table 1 – Moisture (%), total phenolic (mg GAE/100g d.b.) and total flavonoids (mg QE/100g d.b.) in soybean and derived products.

Product/Brand	Moisture (%)	Total phenolic (mg GAE/100g)	Total flavonoids (mg QE/100g)
Soybean BRS 267	8.4 ± 0.2 <sup>c</sup>	187.8 ± 0.7 <sup>f</sup>	94.3 ± 1.7 <sup>c</sup>
DSF Vitao	6.8 ± 0.1 <sup>d,e,f</sup>	242.3 ± 5.7 <sup>a</sup>	104.1 ± 1.6 <sup>b</sup>
WOSF Jasmine	6.5 ± 0.3 <sup>d,e,f,g</sup>	186.1 ± 8.3 <sup>f</sup>	101.0 ± 1.7 <sup>b</sup>
HSSP Jasmine	6.2 ± 0.1 <sup>f,g</sup>	194.2 ± 4.2 <sup>f</sup>	66.3 ± 3.5 <sup>f,g</sup>
HSSP Kinasoy	5.8 ± 0.1 <sup>g,h</sup>	204.2 ± 5.7 <sup>e</sup>	55.5 ± 2.3 <sup>h</sup>
HSSP Vitao	5.8 ± 0.2 <sup>g,h</sup>	230.2 ± 5.4 <sup>b</sup>	71.4 ± 3.4 <sup>e,f</sup>
TSP Vitao	7.3 ± 0.2 <sup>d,e</sup>	222.9 ± 2.4 <sup>b,c</sup>	102.8 ± 2.9 <sup>b</sup>
TSP Kinasoy	7.3 ± 0.3 <sup>d</sup>	241.1 ± 4.0 <sup>a</sup>	99.8 ± 2.8 <sup>b,c</sup>
TSP Mãe Terra	6.3 ± 0.5 <sup>f,g</sup>	243.1 ± 2.2 <sup>a</sup>	100.1 ± 2.4 <sup>b,c</sup>
TSP Jasmine	6.4 ± 0.01 <sup>e,f,g</sup>	215.6 ± 3.1 <sup>c,d</sup>	78.9 ± 2.8 <sup>d</sup>
MSP Sadia	6.9 ± 0.1 <sup>d,e,f</sup>	243.5 ± 3.5 <sup>a</sup>	126.6 ± 1.7 <sup>a</sup>
SPI Solae	4.8 ± 0.1 <sup>i</sup>	138.4 ± 3.3 <sup>h</sup>	78.8 ± 2.0 <sup>d</sup>
SPI Nutrisoy	5.0 ± 0.1 <sup>h,i</sup>	136.4 ± 3.5 <sup>h</sup>	83.1 ± 2.5 <sup>d</sup>
SFF Vitao	7.0 ± 0.2 <sup>d,e,f</sup>	144.2 ± 1.2 <sup>h</sup>	72.3 ± 1.6 <sup>e</sup>
SFGF Visoy	6.8 ± 0.2 <sup>d,e,f</sup>	213.9 ± 1.1 <sup>d</sup>	104.2 ± 1.6 <sup>b</sup>
Tofu Nelson Kikuchi & CIA	83.8 ± 0.6 <sup>b</sup>	104.3 ± 6.3 <sup>i</sup>	27.9 ± 2.6 <sup>i</sup>
Tofu Agro Nippo – Soft	87.2 ± 0.9 <sup>a</sup>	171.3 ± 4.4 <sup>g</sup>	62.2 ± 3.1 <sup>g</sup>
Tofu Agro Nippo – Extra Soft	87.2 ± 0.3 <sup>a</sup>	163.5 ± 2.2 <sup>g</sup>	54.6 ± 2.5 <sup>h</sup>

Means values in the same column followed by the same letter are not significantly different (Tukey,  $p \leq 0.05$ ). DSF (defatted soybean flour); WOSF (whole grain organic soybean flour); HSSP (hydro soluble soy powders); TSP (textured soy proteins); MSP (micronized soybean protein); SPI (soybean protein isolate); SFF (soybean fiber flour); SFGF (soybean fiber and germ flour).

total phenolics but flavonoids were in the same range with the exception of tofu of Nelson Kikuchi & CIA.

The soybean fiber flour has lower level of phenolic compounds probably due to the low affinity of these compounds with insoluble carbohydrates.<sup>26</sup> The addition of soybean germ to the soybean fiber (soybean fiber and germ flour) provided higher level of such compounds since this portion of the grain – the hypocotyl – has high concentration of isoflavones, 5.5-6 times higher than in the cotyledon.<sup>25</sup>

Among the products analysed, the tofu (Nelson Kikuchi & CIA) had the lowest level of phenolic compounds and total flavonoids. The variation among the brands may be due to the processing, since the tofu mentioned is the only one from a local micro enterprise and could have suffered variation during its processing, such as a long period of maceration. Ciabotti et al.<sup>10</sup> reported losses of 38% of isoflavones during the processes of maceration, milling, thermal treatment, and coagulation of tofu.

Recent research have concluded that the consumption of isoflavones associated with proteins can reduce the risk of diseases.<sup>3</sup> Currently a claim is allowed stating that the ingestion of 25g of soybean protein associated to 30-50mg of isoflavone daily is able to reduce serum cholesterol level, when associated with a healthy diet.<sup>20</sup> In Brazil the same claim is recommended by ANVISA (Agência Nacional de Vigilância Sanitária).<sup>8</sup> Considering that most of flavonoids present in the soybean are isoflavones, it is possible to infer that the soybean grains and the whole grain organic soybean flour with approximately 40% of proteins and the defatted soybean flour containing 50% of proteins would provide 58.9, 59.0 and 48.5mg of total flavonoids, respectively, if the recommended protein intake (25g) was followed. The TSP and MSP had on the average 50% of protein and the daily intake of 25g of such nutrient can provide between 36.9 and 58.9mg of total flavonoids.

Considering the dilution of soy milk powder (30g of the product for 250mL water) and the protein content, it would be necessary approximately 500mL of soy milk to provide the recommended protein intake (25g), which would supply the recommended level of isoflavones (between 32.7 to 42.0mg of total flavonoids).

The SPI (90% protein) and the soybean fiber flour (40% protein) had lower concentration of flavonoids and it would be necessary to ingest 39-44.6g of the products, respectively, to achieve the minimum level of total flavonoids (30mg). In contrast to SPI and soybean fiber flour, the soybean germ flour (20% protein) would demand an intake of 125g, to achieve the recommended protein intake (25g), what would lead to a high level of flavonoids (121.4mg). The same occurs with tofu that presents a low level of proteins (between 7.5 and 10%) in comparison with the other products requiring therefore a high intake (from 250 to 333g), but still not enough to reach the recommended amount of flavonoids.

Thus, choosing just one product to reach the recommended levels of flavonoids must be carefully made, since the ingestion of several foods based on soybean

helps to achieve the recommended level of protein and flavonoids.

### Antioxidant Activity

The micronized protein had higher AA with the FRAP methodology, not different from TSP (Kinasoy) ( $p < 0.05$ ) in DPPH assay, or from TSP (Kinasoy and Mãe Terra) ( $p < 0.05$ ) with the ABTS<sup>+</sup> methodology (Table 2), probably due to the high level of phenolic compounds and flavonoids found in such soybean-based ingredients. Furthermore, the ethanolic extract of TSP had higher values of extracted dry residual, varying from 1.4 to 1.6% (Table 2), indicating a higher efficiency in the extraction of phenolic compounds which are responsible for AA.

The results obtained with DPPH methodology are in accordance with Barbosa et al.<sup>4</sup> In all assays soybean fiber had lower AA than the SFGF probably due to the higher level of isoflavones present in the soybean germ. Among the products of lower AA, the SFF, the SPI and tofus had lower content of phenolic compounds, showing that the loss of such substances during the processing led to the decrease of the AA.

Boateng et al.<sup>6</sup> when applying the FRAP methodology to several grains, including the soybean, demonstrated that the total phenolic compounds decreased after maceration; nevertheless they increased after roasting. According to Siddhuraju<sup>19</sup> the stability of the antioxidant compounds during the heating process could be due to the formation of products of Maillard reaction such as hydromethylfurfural (HMF) that has antioxidant activity. Such factors could explain the high antioxidant activity of TSP and MSP submitted to a thermal treatment.

The antioxidant activity assessed by the inhibition of the linoleic acid peroxidation has indicated that DSF, MSP and the soybean grains had high inhibition percentages – 85.6, 81.3 and 80.3%, respectively. Such AA was similar among the brands of TSP and HSSE, varying between 63.5 and 77.9%. The other products had lower inhibition capability (Table 2).

The phenolic compounds had a high correlation with the antioxidant activity determined by methods ABTS<sup>+</sup> ( $r = 0.93$ ;  $p < 0.05$ ), DPPH<sup>·</sup> ( $r = 0.89$ ;  $p < 0.05$ ), PAL ( $r = 0.82$ ;  $p < 0.05$ ) and FRAP ( $r = 0.74$ ;  $p < 0.05$ ). It is likely that the compounds measured by Folin – phenolic acids, polyphenols among other substances – are involved in the ABTS<sup>+</sup>, DPPH<sup>·</sup> and PLA free radicals scavenging and to a lesser extent in the ferric reducing power. The correlation of such methods with the level of total flavonoids was lower in all cases ABTS<sup>+</sup> ( $r = 0.69$ ;  $p < 0.05$ ), DPPH<sup>·</sup> ( $r = 0.62$ ;  $p < 0.05$ ), FRAP ( $r = 0.58$ ;  $p < 0.05$ ) and PLA ( $r = 0.55$ ;  $p < 0.05$ ). These data indicate that polyphenols among other phenolic compounds contribute to AA of soybean and its derivatives products, as well as substances not included in these groups such phytic acid, peptides and other not yet identified.

Regarding the methods of antioxidant assessment the assays of DPPH<sup>·</sup> and ABTS<sup>+</sup> were highly correlated

( $r=0.88$ ;  $p<0.05$ ), since both detect the free radical scavenging capability. Concerning the FRAP assay, the correlation with the methods DPPH<sup>·</sup> and ABTS<sup>·+</sup> were also significant  $r=0.82$ ;  $p<0.05$  and  $r=0.71$   $p<0.05$ . The FRAP assay consisted of a mechanism of action exclusively characterized by electron exchange, while the methods of DPPH<sup>·</sup> and ABTS<sup>·+</sup> may detect the electron exchange as well as hydrogen atoms transfer.<sup>14</sup> PLA method correlated with the methods ABTS<sup>·+</sup> ( $r=0.78$ ;  $p<0.05$ ), DPPH<sup>·</sup> ( $r=0.77$ ;  $p<0.05$ ) and FRAP ( $r=0.74$ ;  $p<0.05$ ).

Thus, the soybean-based products retain bioactive compounds present in the grain, which act as antioxidants as well as scavengers of free radical (ABTS<sup>·+</sup> and DPPH<sup>·</sup>), transferring electrons (FRAP) or hindering the propagation of the chain reaction associated with the peroxidation of linoleic acid.

### Phytic Acid and Minerals

All the products and the grain sample had PA concentrations that varied between 2.4 for the tofu of Agro Nippo- Soft and 1.1g/100g for the SFF, while the WOSF had a lower level of PA, reflecting the non-use of mineral fertilization. Tofu (Agro Nippo – Soft and Agro Nippo – Extra Soft) had higher levels of phytic acid (2.45 and 2.34g/100g d.b., respectively). Those high values in dry-basis are due to the extraction of phytic acid with the soluble proteins during the preparation of the soy milk and the precipitation of the PA along with the soy proteins in the coagulation step.<sup>17</sup>

The importance of PA concentration is related to its antioxidant function, especially in maintaining the iron in Fe (III) oxidation state and obstructing generation of hydroxyl radicals.<sup>12</sup> We determined iron and copper in the samples and soy grain since those ions may catalyze oxidation reactions, but may be chelated by PA thus having their pro oxidant activity suppressed.

The products analysed had different levels of iron and copper (Table 3). The soybean grains (BRS 267) had 1.50mg/100g d.b. and 10.97mg/100g d.b. of copper and iron, respectively. Iron is an essential micronutrient for most organisms, catalyzing numerous biochemical reactions. When free (Fe<sup>2+</sup>) is present it catalyzes the formation of hydroxyl radical (OH) from the super oxide anion (O<sub>2</sub><sup>-</sup>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), being necessary just one binding site available or occupied by an easily dissociable molecule such as water. The phytic acid hinders the formation of OH and decreases the lipid peroxidation speed due to its strong affinity for iron, forming a chelate and occupying all the binding sites.<sup>13</sup>

The antioxidant activities of flavonoids seem to be related not only with the free radical scavenging but also with the ability to chelate metals. According to Kostyuk et al.,<sup>15</sup> metals that catalyse the peroxidation reactions (Fe and Cu), can be chelated by flavonoids. There are evidences that the chelates formed are more effective in the free radical scavenging and suffer less oxidation than the free flavonoids, suggesting that the presence of the metal forms a more effective active centre in the scavenging of the superoxide anion.

Table 2 – Antioxidant activity estimated by the methods DPPH<sup>·</sup>, ABTS<sup>·+</sup> and FRAP (μmol de Trolox/g of sample d.b.), PLA (% of inhibition), and dry alcoholic extracted residuals - DAER (%) in soybean and derived products.

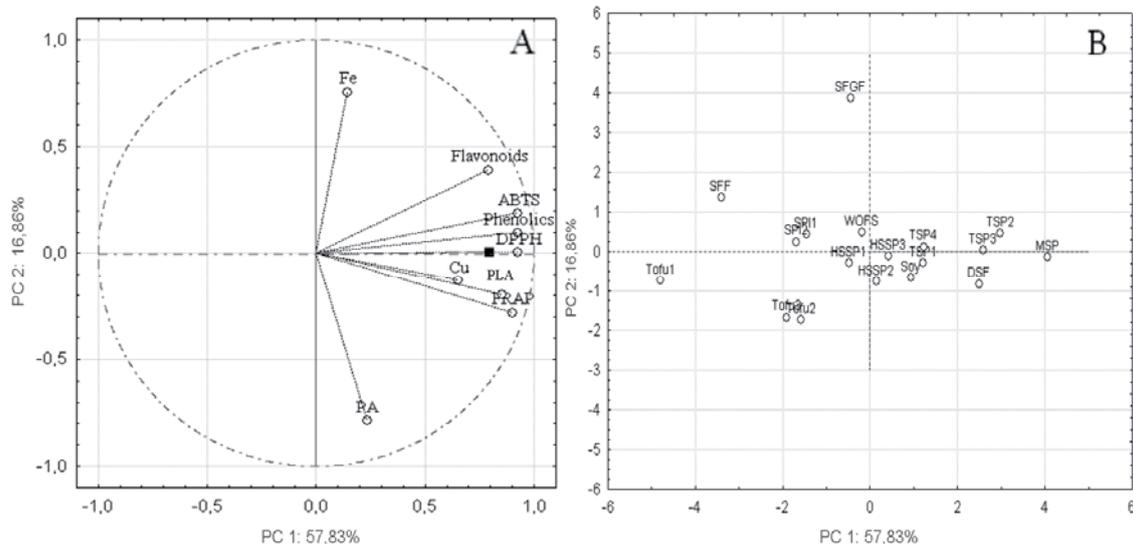
Product/Brand	DPPH <sup>·</sup> (μmol de Trolox/g)	ABTS <sup>·+</sup> (μmol de Trolox/g)	FRAP (μmol de Trolox/g)	PLA (%)	DAER (%)
Soybean BRS 267	4.0±0.1 <sup>e,f,g</sup>	7.1±0.2 <sup>f</sup>	8.4±0.1 <sup>e,f</sup>	80.3±1.4 <sup>a,b</sup>	1.1±0.0 <sup>e</sup>
DSF Vitao	4.2±0.2 <sup>d,e,f</sup>	9.2±0.3 <sup>b</sup>	9.5±0.1 <sup>c</sup>	85.6±0.8 <sup>a</sup>	1.0±0.0 <sup>e</sup>
WOSF Jasmine	2.9±0.1 <sup>i</sup>	5.4±0.4 <sup>g</sup>	6.7±0.2 <sup>h,i</sup>	69.9±0.8 <sup>d,e</sup>	1.1±0.0 <sup>f</sup>
HSSP Jasmine	3.5±0.1 <sup>g</sup>	7.7±0.1 <sup>e,f</sup>	6.5±0.3 <sup>h,i,j</sup>	65.3±3.5 <sup>e,f</sup>	1.3±0.0 <sup>e,d</sup>
HSSP Kinasoy	3.8±0.2 <sup>f,g</sup>	8.1±0.3 <sup>d,e</sup>	8.9±0.2 <sup>d,e</sup>	70.4±4.0 <sup>d,e</sup>	1.3±0.1 <sup>c</sup>
HSSP Vitao	4.4±0.2 <sup>d,e</sup>	8.9±0.3 <sup>b,c</sup>	6.3±0.3 <sup>i,j</sup>	73.6±3.6 <sup>c,d</sup>	1.3±0.1 <sup>c</sup>
TSP Vitao	4.7±0.3 <sup>c,d</sup>	8.7±0.2 <sup>b,c</sup>	7.3±0.3 <sup>g</sup>	63.5±2.3 <sup>f</sup>	1.2±0.0 <sup>d,e</sup>
TSP Kinasoy	6.3±0.3 <sup>a</sup>	10.5±0.2 <sup>a</sup>	10.9±0.1 <sup>b</sup>	69.1±1.0 <sup>d,e</sup>	1.5±0.0 <sup>b</sup>
TSP Mãe Terra	5.4±0.6 <sup>b</sup>	10.2±0.2 <sup>a</sup>	9.1±0.2 <sup>c,d</sup>	77.9±2.2 <sup>b,c</sup>	1.6±0.1 <sup>a</sup>
TSP Jasmine	5.1±0.1 <sup>b,c</sup>	8.4±0.4 <sup>c,d</sup>	8.2±0.1 <sup>f</sup>	65.2±3.8 <sup>e,f</sup>	1.5±0.0 <sup>a,b</sup>
MSP Sadia	6.6±0.2 <sup>a</sup>	10.0±0.2 <sup>a</sup>	12.4±0.3 <sup>a</sup>	81.3±3.1 <sup>a,b</sup>	1.4±0.0 <sup>b</sup>
SPI Solae	1.9±0.2 <sup>j</sup>	5.6±0.2 <sup>g</sup>	6.1±0.2 <sup>i,j</sup>	40.0±2.1 <sup>i</sup>	1.1±0.0 <sup>e</sup>
SPI Nutrisoy	1.7±0.1 <sup>j</sup>	4.1±0.3 <sup>h</sup>	6.0±0.5 <sup>j,k</sup>	45.5±1.5 <sup>h</sup>	1.1±0.0 <sup>e</sup>
SFF Vitao	1.8±0.3 <sup>j</sup>	4.1±0.1 <sup>h</sup>	3.1±0.1 <sup>m</sup>	45.5±1.4 <sup>h</sup>	0.7±0.0 <sup>g</sup>
SFGF Visoy	3.4±0.2 <sup>g</sup>	7.6±0.5 <sup>e,f</sup>	5.5±0.3 <sup>k</sup>	53.3±2.8 <sup>g</sup>	1.1±0.0 <sup>e</sup>
Tofu Nelson Kikuchi & CIA	1.7±0.1 <sup>j</sup>	0.8 ± 0.1 <sup>j</sup>	3.8±0.2 <sup>l</sup>	40.8±2.7 <sup>h,i</sup>	0.6±0.0 <sup>g</sup>
Tofu Agro Nippo – Soft	2.7±0.2 <sup>h</sup>	3.4 ± 0.2 <sup>i</sup>	7.4±0.5 <sup>g</sup>	52.4±2.8 <sup>g</sup>	1.0±0.0 <sup>f</sup>
Tofu Agro Nippo – Extra Soft	2.4±0.1 <sup>h,i</sup>	3.4 ± 0.2 <sup>i</sup>	7.0±0.4 <sup>g,h</sup>	53.9±2.8 <sup>g</sup>	1.1±0.0 <sup>e</sup>

Means values in the same column followed by the same letter are not significantly different (Tukey,  $p \leq 0.05$ ). DSF (defatted soybean flour); WOSF (whole grain organic soybean flour); HSSP (hydro soluble soy powders); TSP (textured soy proteins); MSP (micronized soybean protein); SPI (soybean protein isolate); SFF (soybean fiber flour); SFGF (soybean fiber and germ flour).

Table 3 – Content of PA (g/100g d.b.), total P, Cu and Fe (mg/100g d.b.) in soybean and derived products

Product/Brand	PA (g/100g)	P (mg/100g)	Cu (mg/100g)	Fe (mg/100g)
Soybean BRS 267	1.9 <sup>f</sup>	0.8 ± 0.0 <sup>d,e</sup>	1.5 ± 0.0 <sup>e,f</sup>	11.0 ± 0.2 <sup>g</sup>
DSF Vitao	2.1 <sup>c,d</sup>	0.8 ± 0.1 <sup>a,b,c</sup>	1.7 ± 0.1 <sup>b,c</sup>	10.3 ± 0.9 <sup>h,i</sup>
WOSF Jasmine	1.5 <sup>h</sup>	0.6 ± 0.0 <sup>h</sup>	1.5 ± 0.0 <sup>f</sup>	13.4 ± 0.8 <sup>f</sup>
HSSP Jasmine	1.5 <sup>h,i</sup>	0.6 ± 0.0 <sup>h</sup>	1.3 ± 0.1 <sup>g</sup>	7.6 ± 0.5 <sup>j</sup>
HSSP Kinasoy	1.5 <sup>h</sup>	0.6 ± 0.0 <sup>h</sup>	1.3 ± 0.1 <sup>g</sup>	7.1 ± 0.6 <sup>j</sup>
HSSP Vitao	1.4 <sup>i</sup>	0.6 ± 0.0 <sup>h</sup>	1.2 ± 0.0 <sup>h,i</sup>	6.8 ± 0.3 <sup>j</sup>
TSP Vitao	1.9 <sup>f</sup>	0.8 ± 0.0 <sup>f</sup>	1.6 ± 0.1 <sup>d,e</sup>	9.6 ± 0.9 <sup>i</sup>
TSP Kinasoy	2.0 <sup>e,f</sup>	0.8 ± 0.0 <sup>d,e,f</sup>	1.6 ± 0.0 <sup>d</sup>	20.2 ± 0.9 <sup>b</sup>
TSP Mãe Terra	2.0 <sup>f</sup>	0.8 ± 0.0 <sup>e,f</sup>	1.7 ± 0.0 <sup>d</sup>	15.2 ± 0.5 <sup>e</sup>
TSP Jasmine	1.9 <sup>f</sup>	0.8 ± 0.1 <sup>e,f</sup>	1.6 ± 0.1 <sup>d</sup>	17.8 ± 0.9 <sup>e</sup>
MSP Sadia	2.1 <sup>c</sup>	0.8 ± 0.1 <sup>c,d</sup>	1.8 ± 0.1 <sup>b</sup>	16.5 ± 0.5 <sup>d</sup>
SPI Solae	2.0 <sup>d,e,f</sup>	0.8 ± 0.1 <sup>a,b,c</sup>	1.9 ± 0.0 <sup>a</sup>	20.5 ± 0.7 <sup>b</sup>
SPI Nutrisoy	2.1 <sup>c,d,e</sup>	0.8 ± 0.0 <sup>a,b</sup>	1.8 ± 0.1 <sup>b</sup>	20.1 ± 0.5 <sup>b</sup>
SFF Vitao	1.1 <sup>j</sup>	0.5 ± 0.0 <sup>i,j</sup>	0.8 ± 0.0 <sup>j</sup>	13.9 ± 1.0 <sup>f</sup>
SFGF Visoy	0.6 <sup>k</sup>	0.4 ± 0.0 <sup>j</sup>	1.1 ± 0.0 <sup>i</sup>	26.8 ± 0.8 <sup>a</sup>
Tofu Nelson Kikuchi & CIA	1.7 <sup>g</sup>	0.7 ± 0.0 <sup>g</sup>	0.9 ± 0.0 <sup>j</sup>	9.6 ± 0.7 <sup>i</sup>
Tofu Agro Nippo – Soft	2.4 <sup>a</sup>	0.8 ± 0.1 <sup>b,c,d</sup>	1.3 ± 0.0 <sup>g,h</sup>	9.3 ± 0.8 <sup>i</sup>
Tofu Agro Nippo – Extra Soft	2.3 <sup>b</sup>	0.8 ± 0.0 <sup>a</sup>	1.2 ± 0.0 <sup>g,h</sup>	9.3 ± 0.9 <sup>i</sup>

Means values in the same column followed by the same letter are not significantly different (Tukey,  $p \leq 0.05$ ). DSF (defatted soybean flour); WOSF (whole grain organic soybean flour); HSSP (hydro soluble soy powders); TSP (textured soy proteins); MSP (micronized soybean protein); SPI (soybean protein isolate); SFF (soybean fiber flour); SFGF (soybean fiber and germ flour).



(A) Fe (Iron); Cu (copper); PA (phytic acid); PLA (Linoleic Acid Peroxidation). (B) Soy (Soybean BRS 267); DSF (defatted soybean flour Vitao); WOSF (whole grain organic soybean flour Jasmine), SFF (soybean fiber flour Vitao), SFGF (soybean fiber and germ flour Visoy), HSSP 1 (hydro soluble soy powders Jasmine), HSSP 2 (hydro soluble soy powders Kinasoy), HSSP 3 (hydro soluble soy powders Vitao), SPI 1 (soybean protein isolate Solae), SPI 2 (soybean protein isolate Nutrisoy), TSP 1 (textured soy proteins Vitao), TSP 2 (textured soy proteins Kinasoy), TSP 3 (textured soy proteins Mãe Terra), TSP 4 (textured soy proteins Jasmine), MSP (micronized soybean protein Sadia), Tofu 1 (Nelson Kikuchi & CIA), Tofu 2 (Agro Nippo – Soft), Tofu 3 (Agro Nippo – Extra Soft).

FIGURE 1 – Principal Component Analysis graphic representation of samples composition and antioxidant activity: projections of the analyses (A) and samples (B).

## Principal Component Analysis

In the Principal Component Analysis (PAC) the first two components explained 74.7% of the total variance. PC 1 was correlated to the concentration of phenolic compounds, flavonoids, dry extracted alcoholic residuals, and copper and by the determinations of AA (ABTS<sup>+</sup>, DPPH, PLA and FRAP) (Figure 1A). PC 2 was characterized by the level of iron and PA, and was not correlated to the antioxidant activity. Despite the different mechanisms of the assessing methodologies for antioxidant activity all methods characterized the samples similarly. The projections of the results of the analyses carried out and of the products studied, in the factorial scheme PC 1 x PC 2 is in Figures 1A and 1B.

The graphic of the samples placed products with high antioxidant activity positioned at the right side of the graph, which had also higher levels of phenolic compounds, flavonoids and copper. High levels of proteins characterize such soybean-based foods, confirming the association of the bioactive compounds to the protein content. The products TSP, HSSP and the soybean flours (organic and defatted) were placed close to the soybean grain (BRS 267), indicating that despite the probable difference of raw materials, the processing did not caused abrupt changes in the level of phenolic compounds and flavonoids and the antioxidant activity was maintained. The distance of MSP from such products is a consequence of its higher level of bioactive compounds and elevated AA, could be due to the concentration of such compounds, since lipids and carbohydrates of the grains are partially eliminated. Moreover, the easiness of extraction of the compounds from MSP may have contributed to these higher values.

However, despite the high protein content of SPI (90%), two brands were placed at the left side in the graph, showing that alkaline or alcoholic extraction removed the bioactive compounds, consequently, decreasing the antioxidant activity. Likewise, the tofus and products with soybean fiber were placed at the left side of the graph, the tofus in the lower quadrant due to their high level of PA. Finally the SFF and the SFGF were characterized for the higher concentration of iron.

## CONCLUSION

The soybean products maintain antioxidant compounds from the grains. In general, the products that have higher levels of phenolic compounds, flavonoids and antioxidant activity were the micronized protein, textured proteins, and the defatted flour, all products minimally processed. In the SPI the low level of antioxidant compounds is due to the losses caused by the extraction method but they have high levels of Fe and of PA. However, the dilution or reprocessing of such products at the moment of consumption will influence the real concentration and the antioxidant potential of the compounds present in the soybean products.

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BOLANHO, B. C.; BELÉIA, A. P. Compostos bioativos e potencial antioxidante em derivados de soja. **Alim. Nutr.**, Araraquara, v. 22, n. 4, p. 539-546, out./dez. 2011.

■RESUMO: O objetivo deste trabalho foi avaliar o teor compostos bioativos na soja e seus derivados e o potencial antioxidante (AA) avaliado pelos métodos ABTS<sup>+</sup>, DPPH, FRAP e peroxidação do ácido linoléico (PLA). Proteína de soja micronizada (MSP), farinha de soja desengordurada (DSF) e proteína de soja texturizada (TSP) apresentaram maior teor de compostos fenólicos e maior AA. MSP e tofus demonstraram maior teor de flavonóides e ácido fítico (AF), respectivamente. O potencial antioxidante correlacionou-se com o teor de fenólicos e flavonóides totais, porém o AF pode agir sinergicamente quelando pró-oxidantes ferro e cobre. A maior concentração de cobre foi encontrada no isolado protéico de soja, e de ferro no produto formulado com fibra e gérmen de soja. Muitos compostos presentes nos produtos à base de soja contribuem com a AA, no entanto a concentração e o potencial dependerão do preparo final do grão ou dos derivados no momento do consumo.

■PALAVRAS-CHAVE: Compostos fenólicos; flavonóides; ácido fítico; ferro; cobre.

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