Journal of Nutrition & Food Sciences

Huber et al., J Nutr Food Sci 2016, 6:5
DOI: 10.4172/2155-9600.1000551

Research Article Open Access

Phenolic Acid, Flavonoids and Antioxidant Activity of Common Brown Beans (*Phaseolus vulgaris* L.) Before and After Cooking

Karina Huber, Priscila Brigide*, Eloá Bolis Bretas and Solange Guidolin Canniatti-Brazaca

Food and Nutrition Department, Luiz de Queiroz College of Agriculture, University of São Paulo, Brazil

Corresponding author: Priscila Brigide, Agri-Food Industry, Food and Nutrition Department, Luiz de Queiroz College of Agriculture, University of São Paulo, Avenida Pádua Dias, 11 CP 9, CEP 13418-900 Piracicaba, SP, Brazil, Tel: 550211934294118; Fax: 550211934292552; E-mail: pbrigide@hotmail.com

Received date: July 23, 2016; **Accepted date:** September 06, 2016; **Published date:** September 12, 2016

Abstract

This study aimed to evaluate the impact of thermal processing and soaking on the profiles and contents of phenolic compounds in brown beans, as well as their antioxidant activity. We evaluated the antioxidant activity of extracts and the contents of phenolic acids and flavonoids by high-performance liquid chromatography (HPLC). With the exception of chlorogenic acid, the contents of all of the other phenolic acids were increased by cooking. Kaempferol was only detected in samples treated by soaking followed by cooking. Catechin and kaempferol-3-glucoside were found in all of the brown bean extracts. Cooking, with or without soaking, caused significant increases in the concentrations of quercetin and quercetin-3-glucoside only. The effect of the heat treatment increased the antioxidant activity and the concentrations of the phenolic compounds evaluated.

Keywords: 2,2-diphenyl-1-picrylhydrazyl (DPPH); 2,2'-azino-bis (ABTS); Polyphenols; Bioactive compounds; Thermal processing

Introduction

Similar to other legumes, beans possess large contents of bioactive compounds such as polyphenols [1]. These compounds are secondary metabolites in plants and are widely known for their antioxidant capacities. Polyphenols therefore play an important role in reducing the risk of cardiovascular disease, diabetes, some cancers, and Alzheimer and Parkinson's diseases [2]. The antioxidant activity of beans is due mainly to the reducing properties of polyphenols, which play an important role in the neutralisation or sequestration of free radicals and in the chelation of transition metals, acting against both the initiation and propagation of oxidative processes. The intermediates formed by the action of phenolic antioxidants are relatively stable due to the resonance of the aromatic rings in the structures of these substances [3].

Common beans possess antioxidant activity due to the presence of phenolic acids and flavonoids, mainly tannins [4].

It is known that the phenolic acids most commonly found in raw and cooked beans are gallic acid, vanillic, p-coumaric, ferulic, sinapic and chlorogenic acids and that they have great importance as precursors for the synthesis of other phenolic compounds in plants [3,5].

Flavonoids share a common structure consisting of two aromatic rings that are linked through three carbons, forming an oxygenated heterocycle. They are divided into six subclasses, depending on the type of heterocycle formed: Flavonols, flavones, isoflavones, flavanones, anthocyanidins and flavanols (catechin and proanthocyanidin). The main flavonoid representatives are quercetin and kaempferol in glycosylated forms. In raw and cooked beans, the main flavonoid representatives are catechin, kaempferol, quercetin, myricetin and procyanidins [3,5].

To improve the nutritional quality of beans, peeling, soaking, cooking and germination methods are used. Therefore, the present study aimed to evaluate the impact of cooking preceded by soaking and cooking without soaking on the profiles and contents of phenolic acids and flavonoids, as well as the antioxidant activity of brown beans.

Materials and Methods

Samples of the common bean (*Phaseolus vulgaris* L.) BRS9435-cometa cultivar (brown) were analysed raw and after thermal processing.

The beans were stored at refrigerator temperature (4°C) before the measurements and then they were ground and sieved. The beans intended for use as cooked samples were divided into two treatments: Treatment 1 involved soaking the beans for 10 h in distilled water, followed by an exchange of the water and then cooking in an autoclave at 121°C for 10 min; Treatment 2 involved cooking the beans in an autoclave at 121°C for 10 min. After cooking, the samples were lyophilised and stored at −26°C. Milling of the cooked beans was performed at the time of the analyses and under the same conditions as the raw samples (Treatment 3).

The polyphenol extraction was performed according to Cardador-Martínez et al. [6]. Five extractions were performed for each treatment.

The extracted material was separated into six fractions (A, B, C, D, E and F) using open column chromatography under vacuum according to the methodology proposed by Aparicio-Fernandez, Manzo-Bonilla, and Loarca-Piña [7]. Lyophilised extract in 0.5 g quantities was diluted in 1 mL of methanol and poured into an open column over silica gel (Sigma Aldrich, 13% $CaSO_4$). The reagents were added as shown in Figure 1 and the fractions were collected after each passage.

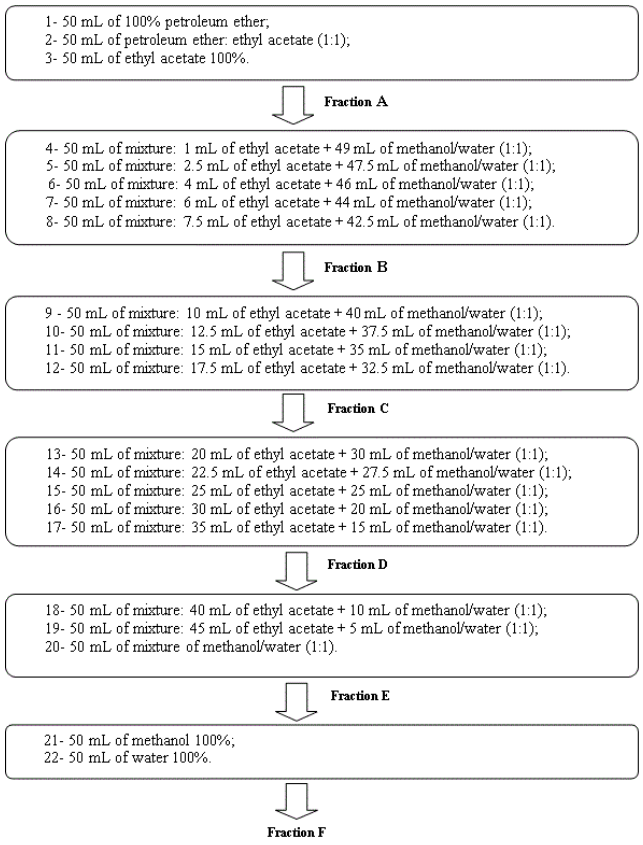

Figure 1: Polyphenol separation into six fractions (A-F) using open column chromatography under vacuum.

After the recovery of each fraction (except for fraction A which was discarded as a lipid-removal and column-cleaning fraction), the mixtures were placed in a flask to evaporate the solvents in a rotary evaporator. The fractions FB, FC, FD and FF were lyophilised for further analysis; however, Fraction FE did not generate sufficient phenolic compounds for analysis.

To measure antioxidant activity via 1,1-diphenyl-2-pycrylhydrazyl (DPPH) Sigma Chemical Co., we used the methodology proposed by Brand-Williams, Cuvier, and Berset [8]. The results were expressed in milligrams of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) Sigma Chemical Co. equivalent antioxidant capacity (TEAC) g^{-1} of the extract. We used the 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) Sigma Chemical Co. method proposed by Re et al. [9], Van Den Berg et al. [10] and Arts et al. [11], with modifications' to determine the antioxidant activity. The results were expressed in mg of Trolox-equivalent antioxidant capacity (TEAC) g^{-1} of extract.

To identify and quantify the phenolic acids, free phenolic acids were separated by high-performance liquid chromatography (HPLC) according to Xu and Chang [12], with modifications. A Shimadzu model 20 A, equipped with a UV detector (270 and 325 nm) was used. A Zorbax ODS Stablebond-C18 (Agilent Technology), 4.6 × 250 mm, 5 mm analytical column was used for the separation, at 40°C. The mobile phases used were A, 0.1% trifluoroacetic acid solution in water; B, 100% methanol and an isocratic gradient that include 20% phase A and 80% phase B with flow rates of 1.0 mL min^{-1}.

To identify the HPLC peaks, one stock solution (1 mg mL^{-1}) of each phenolic acid profile was individually prepared and diluted. These dilute solutions were injected (20 µL) separately, duplicating the conditions described above. The peak areas and their retention times were used to compare, identify and quantify these phenolic acids in the samples injected afterwards.

To prepare the stock solutions, 10 mg of each phenolic acid was dissolved in 10 mL of 80% methanol and then diluted in 80% methanol in the following solutions: 0.5, 1, 2.5, 5, 10 and 25 µg mL^{-1} of vanillic acid; 10, 25, 50 and 100 µg mL^{-1} of chlorogenic acid; 1, 2.5, 5, 10, 25, 50 and 100 µg mL^{-1} of sinapic acid and 1, 5, 10, 25 and 50 µg mL^{-1} of gallic acid. The content of each phenolic acid was expressed in micrograms per gram of extract (µg g^{-1}).

The chromatographic system and methodology used for the separation of flavonoids were the same as those used for the phenolic acids analysis. The oven temperature was 34°C. The mobile phases used were A, 0.1% acetic acid solution in water and B, 0.1% acetic acid in acetonitrile in the following concentration gradients and flow rates: 1.0 mL min^{-1} with 15% phase B and 85% phase A during the first 5 min; 1.5 mL min^{-1} with an increase in phase B to 29% and a reduction in phase A to 71% from 5 to 23 min; 1.0 mL min^{-1} with an increase in phase B to 35% and a reduction in phase A to 65% from 23 to 44 min; 1.0 mL min^{-1} with an increase in phase B to 50% and a reduction in phase A to 50% from 44 to 46 min and 1.0 mL min^{-1} with a reduction in phase B to 15% and an increase in phase A to 85% from 46 to 48 min.

To identify the HPLC peaks, a stock solution (1 mg mL^{-1}) of each phenolic acid profile was individually prepared and then diluted. These dilute solutions were injected (20 µL) separately and in duplicate under the conditions described above. The peak areas and their retention times were used to compare, identify and quantify these phenolic compounds in the subsequently injected extracts.

To prepare the stock solution, 10 mg of each flavonoid was dissolved in 10 mL of 80% methanol and then diluted in 80% methanol in the following concentrations: 5, 10, 25 and 50 µg mL^{-1} of catechin; 25, 50, 100 and 250 µg mL^{-1} of quercetin; 2.5, 5, 10, 25 and 50 µg mL^{-1} of kaempferol-3-glucoside; 10, 25, 50 and 100 µg mL^{-1} of kaempferol 3-O-rutinoside; 10, 25, 50 and 100 µg mL^{-1} of kaempferol and 10, 25, 50 and 100 µg mL^{-1} of quercetin-3-glucoside. The content of each flavonoid was expressed as micrograms per gram of extract (µg g^{-1}).

The statistical design was completely randomised. Analyses of the antioxidant activity were performed in duplicate, with two replicates for each extract and fraction and the identification and quantification analyses of the phenolic acids and flavonoids were performed in duplicate, with two replicates only for crude extracts of polyphenols. The results were analysed by the Tukey test at 5% probability.

Results

Some differences between the two methods can be observed. According to the DPPH method, both cooking processes resulted in higher antioxidant activity in the cooked beans compared to the extracts of the raw beans. For crude extracts of the beans, the results of DPPH ranged from 2.96 to 14.04 mg TEAC g^{-1} extract.

	DPPH			ABTS		
	Raw	**Cooked**	**Cooked and soaked**	**Raw**	**Cooked**	**Cooked and soaked**
Crude extract	8.73 ± 0.6[a]B[b]	12.65 ± 0.3A	13.74 ± 0.1A	22.30 ± 2.3A	29.51 ± 4.6A	20.28 ± 0.9B
Fraction FB	4.48 ± 0.5B	7.13 ± 0.1A	4.82 ± 0.2B	34.96 ± 21.2A	11.57 ± 0.1A	20.96 ± 0.8A
Fraction FC	6.05 ± 0.3A	6.25 ± 1.0A	7.26 ± 0.2A	46.70 ± 3.1A	29.31 ± 2.3B	19.72 ± 1.8B
Fraction FD	4.48 ± 3.3A	3.28 ± 1.3A	3.41 ± 0.1A	20.15 ± 0.5A	13.06 ± 0.2B	11.42 ± 1.0B
Fraction FF	0.53 ± 0.3A	0.87 ± 0.3A	1.37 ± 0.1A	13.48 ± 1.1A	15.57 ± 1.6A	10.92 ± 2.5A

[a]Means of two replicates ± standard deviation; [b]Means with different uppercase letters (s) in the horizontal direction (s) indicate that the fractions differ significantly ($p \leq 0.05$). FB, FC, FD, and FF: polyphenol fractions obtained by open column chromatography under vacuum.

Table 1: Antioxidant activity (mg TEAC g^{-1} of extract) of crude extracts and fractions of polyphenols from brown raw, cooked and cooked and soaked beans as measured by the DPPH and ABTS methods.

In the ABTS assays, the extracts ranged from 13.70 and 29.51 mg TEAC g^{-1} extract. Table 1 shows the antioxidant activity values according to the DPPH and ABTS methods.

The levels of phenolic acids present in the crude extract are shown in Table 2. It was found for phenolic acids that all extracts evaluated have the gallic acid ranged from 2203.55 to 3693.39 μg g^{-1} extracts of the beans, chlorogenic from 1272.04 to 4430,26 μg g^{-1} extracts of the beans, vanillic from 377.49 to 1404.45 μg g^{-1} extracts of the beans and sinapic from 88.52 to 382.37 μg g^{-1} extracts of the beans, and the first two were found at higher levels.

	Raw	**Cooked**	**Cooked and soaked**
Vanillic acid	498.17 ± 2.0[a]C[b]	779.83 ± 21.4A	563.97 ± 1.6B
Gallic acid	2872.37 ± 5.5C	3693.39 ± 44.2A	3510.18 ± 59.5B
Chlorogenic acid	3139.81 ± 6.3A	1812.56 ± 2.8B	1552.49 ± 5.3C
Sinapic acid	140.19 ± 8.3C	258.71 ± 3.9A	194.17 ± 1.2B

[a]Means of two replicates ± standard deviation; [b]Means with different uppercase letters (s) in the horizontal direction(s) indicate that the phenolic acid contents differ significantly ($p \leq 0.05$).

Table 2: Phenolic acids (μg g^{-1} extract) in crude extracts from raw, cooked and cooked and soaked brown beans.

Table 3 shows the results regarding the phenolic acid fractions of the crude extracts. All of fractions FC were indentified whith high level the phenolic acid. Table 4 shows the results of the analyses of the flavonoids in the crude bean polyphenol extracts. Almost all flavonoids were detected in almost all samples, except for kaempferol (0-311.20 μg g^{-1} of extract), wich was only detected in soaked and cooked samples. For extracts from white beans, flavonoids found in highest concentrations were catechin (547.33 μg g^{-1} of extract) and kaempferol-3-rutinoside 327.68 μg g^{-1} of extract; brown beans extracts showed mostly catechin 1886.61 μg g^{-1} of extract and kaempferol-3-glucoside 1690.77 μg g^{-1} of extract, and in extracts of black beans, mainly catechin 986.12 μg g^{-1} of extract, kaempferol-3-glucoside 307.60 μg g^{-1} of extract and quercetin-3-glucoside 343.65 μg g^{-1} of extract.

	Raw	**Cooked**	**Cooked and soaked**
Vanillic acid			
FB	783.74 ± 2.1[a]A[b]	-	1136.23 ± 8.7A
FC	768.06 ± 0.8A	628.71 ± 0.8A	688.81 ± 18.3A
FF	-	69.18 ± 2.7A	-
Gallic acid			
FB	3494.27 ± 3.8A	-	6475.18 ± 76.8A
FC	4530.60 ± 6.7A	3100.71 ± 1.7	4766.28 ± 42.6A
FF	-	Nd	-
Chlorogenic acid			
FB	1595.09 ± 0.3A	-	2050.96 ±1.2A
FC	2833.63 ± 3.4A	1325.83 ± 2.1	1994.51 ± 4.6A
FF	-	Nd	-
Sinapic acid			
FB	173.40 ± 2.1A	-	346.12 ± 18.1A
FC	195.73 ± 2.6A	148.87 ± 0.5A	156.61 ± 6.4A
FF	-	47.12 ± 0.4A	-

[a]Means of two replicates ± standard deviation; [b]Means with different uppercase letters in the horizontal direction and in the vertical direction for the same treatment differ significantly ($p \leq 0.05$) according to the means test. FB, FC and FF: fractions of polyphenols obtained by open column chromatography under vacuum that had higher antioxidant activities. Two fractions were used for each treatment. Nd: Not detected.

Table 3: Phenolic acid (μg g^{-1} of extract) fractions of polyphenols from raw, cooked and cooked-and-soaked brown beans.

	Raw	Cooked	Cooked and soaked
Kaempferol	0.00 ± 0.00[a]B[b]	0.00 ± 0.00B	127.41 ± 10.29A
Kaempferol-3-glucoside	1098.62 ± 37.57B	1690.77 ± 16.71A	1136.59 ± 24.50B
Kaempferol-3-rutinoside	13.62 ± 0.83C	69.78 ± 13.41B	176.81 ± 1.36A
Catechin	1105.77 ± 112.37B	1886.61 ± 73.03A	1428.83 ± 36.50B
Quercetin	9.58 ± 2.03A	10.67 ± 4.03A	12.14 ± 1.22A
Quercetin-3-glucoside	23.07 ± 5.54A	33.70 ± 9.58A	12.81 ± 5.44A
[a]Means of two replicates ± standard deviation; [b]Means with uppercase different letters(s) in the horizontal direction(s) differ significantly ($p \leq 0.05$).			

Table 4: Flavonoids (μg g^{-1} of extract) in crude extracts of polyphenols from raw, cooked and cooked-and-soaked brown beans.

The results for the flavonoid fractions in crude beans extracts are presented in Table 5. The highest level were for soaked and cooked beans.

Discussion

Some differences between the two methods can be observed. According to the DPPH method, both cooking processes resulted in higher antioxidant activity in the cooked beans compared to the extracts of the raw beans. Similar results were also reported in the study of Rocha-Guzmán et al. [4], who concluded that cooked beans removed of free radicals at a higher rate compared to raw beans. This increase in antioxidant potential after heat treatment with or without soaking may be due to the concentration of phenolic compounds in the cooking broth, which facilitates their extraction. In the work of Xu and Chang [12], the ability of the compounds scavenge free radicals as measured by DPPH was reduced by 28-36% in boiled beans under pressure compared to raw beans and reduced by 23-31% compared to beans that were soaked before cooking.

In the ABTS assays, the extracts and fractions from the raw beans did not have the lowest results. In the FC and FD fractions, cooking promoted a decrease in antioxidant activity, with or without soaking, compared to the extract of the same raw bean. Xu and Chang [12] attributed this decrease in antioxidant activity after thermal treatment to the possibility that chemical transformations, decomposition of phenolic compounds, formation of complexes between polyphenols and proteins and solubilisation of water-soluble antioxidants in the discarded soaking water occurred.

Although both results of the ABTS and the DPPH that the evaluated antioxidant has the ability to donate hydrogen, a comparative study of the FRAP, ORAC, DPPH and ABTS methodologies by Fernandez-Panchon et al. [13] indicated that the sensitivity of the ABTS radicals was lower compared to the DPPH radical following heat processing.

With the DPPH method, the antioxidant activities of the extracts were higher than those found for fractions. This is possibly due to synergy between all of the antioxidant compounds that were present in the crude extract, while no such synergy was possible in the isolated fractions. However, in the ABTS assays, the extracts did not did not contain higher fractions.

	Raw	Cooked	Cooked and soaked
Kaempferol			
FB	0.00	-	427.50[a] ± 0.41A[b]
FC	0.00	0.00	173.61 ± 5.35A
FF	-	0.00	-
Kaempferol-3-glucoside			
FB	1564.37 ± 0.35A	-	1813.33 ± 15.25A
FC	1888.64 ± 1.65A	1416.16 ± 4.56	1269.96 ± 22.53A
FF	-	Nd	-
Kaempferol-3-rutinoside			
FB	18.33 ± 0.02A	-	8.40 ± 1.29
FC	19.78 ± 0.06A	71.17 ± 2.97	Nd
FF	-	Nd	-
Catechin			
FB	1057.41 ± 1.43A	-	1097.99 ± 3.03A
FC	1594.51 ± 3.96A	1501.24 ± 168.31A	1466.67 ± 4.22A
FF	-	44.27 ± 0.37B	-
Quercetin			
FB	8.19 ± 0.04B	-	15.40 ± 0.64
FC	12.49 ± 6.72A	Nd	Nd
FF	-	10.76 ± 0.40	-
Quercetin-3-glucoside			
FB	9.46 ± 0.44A	-	16.48 ± 0.93A
FC	7.55 ± 0.20A	20.39 ± 0.06	19.28 ± 2.65A
FF	-	Nd	-
[a]Means of two replicates ±standard deviation; [b]Means with uppercase letters (s) that are different(s) in the vertical direction differ significantly ($p \leq 0.05$) according to the means test. FB, FC and FF: the fractions of these polyphenols that were obtained by open column chromatography under vacuum had higher antioxidant activities. Two fractions were used for each treatment. Nd: Not detected.			

Table 5: Flavonoid (μg g^{-1} of extract) fractions of polyphenols from raw, cooked, and cooked-and-soaked brown beans.

Using both methods, the fraction obtained with a more polar solvent (FF) had the lowest values of Trolox-equivalent antioxidant capacity. The polarity of the compounds in brown beans may affect their extraction from the feedstock [14]. Depending on the polarity of the solvent, more of one or another phenolic compound may be present in food and thus affect the antioxidant capacity of the extract.

Overall, the crude extracts showed similar antioxidant activities in relation to their respective fractions. The same finding was reported in the study by Beninger and Hosfield [14], who explained their results by noting that the flavonoids present in the crude extract were further concentrated in the extracts; this concentration may have been primarily responsible for the observed antioxidant activity.

In all of the samples analysed, gallic and chlorogenic acids were found to have the highest contents. These findings regarding beans have also been reported in the literature. The contents measured in this study are higher than those in other studies due to the units in which these data are expressed (per gram of extract in this study vs. per gram of food in others). Xu and Chang [5] reported contents of 89.64 and 32.93 μg of gallic acid g^{-1} in black bean that were raw and cooked under pressure for 10 min, respectively, and 226.1 and 89.9 μg of chlorogenic acid g^{-1} in black bean that were raw and cooked under pressure for 10 min, respectively. However, Luthria and Pastor-Corrales [15] evaluated 15 types of beans and detected only p-coumaric, sinapic and ferulic acids, the last showing the highest concentration. Ranilla et al. [16] detected chlorogenic acid in only three of twenty-eight cultivars analysed (between 2.8 and 5.6 mg 100 g^{-1}).

It was observed that cooking significantly increases the concentration of vanillic acid. By contrast, Diaz-Batalla et al. [1] found lower levels of vanillic acid in cooked beans than in raw beans for the 14 analysed cultivars. Aguilera et al. [17] found no vanillic acid in cooked beans, with or without soaking and found 10.71 μg g^{-1} in raw beans. This phenolic acid has the following desirable activities: antihelminthic activity, the prevention of sickle erythrocytes and the suppression of liver fibrosis in chronic liver diseases [5,18].

Similarly, higher concentrations of gallic acid were found in the extracts of cooked beans. Xu and Chang [5], however, reported contents of 83.17 μg g^{-1} in raw beans and 38.16 μg g^{-1} in cooked brown beans; both of these values are well below those reported here and indicate an effect of cooking on the content of this specific phenolic acid that is the opposite of that reported here. We note that recent studies report that gallic acid, in addition to its antioxidant properties, has the following biological effects: antineoplastic and bacteriostatic activity, repression of brain tumours, antitumor properties, induction of apoptosis in prostate carcinoma cells, antiangiogenic activity, inhibition of disaccharidases in the intestinal brush border of mammals and induction of apoptosis or necrosis of cancer cells [18]. Therefore, the consumption of beans is highly beneficial, as high contents of gallic were measured in the extracts that were analysed.

Large amounts of chlorogenic acid were measured in the extracts of raw beans. The action of this compound in the prevention of Alzheimer's disease via a reduction in apoptosis induced by amyloid-β cells. Additionally, chlorogenic acid displays anticholinesterase, anti-inflammatory and antioxidant activities. The phenolic compound can be easily oxidised via polyphenol oxidases leading to its interaction with NH_2 groups of proteins and amino acids, resulting in reducing the nutritional value of foods. The acid in question increases homocysteine levels in human plasma, which constitutes a risk factor for the onset of cardiovascular disease [18].

Regarding sinapic acid, the highest levels were found in extracts of cooked beans without soaking. In a study by Espinosa-Alonso et al. [19] sinapic acid as found at a concentration equal to 22.4 mg Kg^{-1} of different coloured beans. Campos-Vega et al. [2] reported that antioxidant activity is the main biological activity provided by sinapic acid.

We analysed the two fractions that had the highest antioxidant activity according to the DPPH and ABTS methods and these fractions varied according to the beans and treatment. Therefore, tests of the averages of duplicates that were injected into the chromatograph were performed.

In general, heat treatment increased the antioxidant activities and the concentrations of phenolic compounds evaluated. This finding is important because raw beans have antinutritional factors and should therefore be cooked before they are consumed. Soaking, on the other hand, had variable effects in each of the analyses.

Table 3 shows the results regarding the phenolic acid fractions of the crude extracts. We analysed the two fractions that had the highest antioxidant activity according to the DPPH and ABTS methods and these fractions varied according to the beans and treatment. Therefore, tests of the averages of duplicates that were injected into the chromatograph were performed.

In general, heat treatment increased the antioxidant activities and the concentrations of phenolic compounds evaluated. This finding is important because raw beans have antinutritional factors and should therefore be cooked before they are consumed. Soaking, on the other hand, had variable effects in each of the analyses.

It is observed that some phenolic acids were not present in either fraction evaluated. It must then be the case that the significant antioxidant activity of these fractions was due to other, unidentified materials present in the extracts evaluated.

The phenolic acids that were found in the highest concentrations were gallic and chlorogenic acid. Among the various phenolic compound fractions that were evaluated, the FB and FC fractions had higher scores compared to the FF, although many of the fractions did not differ significantly. This finding corroborates the results from the DPPH and ABTS methods, which indicated that the FF fraction had lower antioxidant activity and the results regarding the polarity of acids that were evaluated and solvents used in the fractionation. It is known that the phenolic acids quantified herein are typically polar.

The literature reports that chlorogenic, gallic and sinapic acid have high antioxidant activities and that the activity of vanillic acid is lower [20]. Thus, it is concluded that, due to the large fractions of chlorogenic and gallic acid measured in the extracts of the beans evaluated here, brown beans have high potential as antioxidants, as confirmed by the results of the DPPH and ABTS assays.

Table 4 shows the results of the analyses of the flavonoids in the crude bean polyphenol extracts. Most of the flavonoids examined were detected in almost all of the samples, except for the kaempferol, which was only detected in the soaked and cooked samples. However, in a study by Diaz-Batalla et al. [1], kaempferol was present at higher concentrations in raw beans (average of 52.3 μg g^{-1} of bean) compared to cooked beans (average of 27.2 μg g^{-1} of bean). Likewise, in a work by Amarowicz and Pegg [3], cooking promoted a reduction of this flavonoid by 5-71%. Romani et al. [21], who assessed the levels of various flavonoids in Italians beans, did not detect even trace amounts of quercetin-3-glucoside and this flavonoid as not detected in the extracts evaluated in this study either.

We mainly found catechin and kaempferol-3-glucoside flavonoids. Ranilla et al. [16] detected quercetin and kaempferol in brown beans and Lin et al. [22] detected kaempferol-3-glucoside. It is known that kaempferol is associated with a reduced risk of developing cancer and that kaempferol-3-glucoside possesses anticancer properties and

neuroprotective properties against diseases such as Parkinson's and Alzheimer's [2,18].

It was expected that higher concentrations of catechin would be detected because catechin is a type of procyanidin and is the main flavonoid present in beans [23]. However, Xu and Chang [5] did not detect this compound in raw or cooked brown beans.

Based on the data in Table 4, it is observed that, for all flavonoids, when there was a significant difference among the extracts from raw and cooked beans, the former always exhibited inferior results. The effect of soaking varies for each compound evaluated; however, the effect of this process on the increases in the concentrations of kaempferol and kaempferol-3-rutinoside was clear. These results are contrary to published reports claiming that cooking and soaking have a negative impact on flavonoid concentrations [1,5,17].

It is also notable that quercetin and quercetin-3-glucoside were not influenced by cooking, with or without soaking. Amarowicz and Peggy [3], however, found that the concentration of quercetin was reduced by 12-65% after the beans were cooked and Díaz-Batalla et al. [1] found mean values of 10.9 μg of quercetina g^{-1} in raw beans and 6.5 μg of quercetina g^{-1} in cooked beans. It is reported in the literature that quercetin has various effects in the prevention and treatment of some types of cancer, in the reversal of cognitive deficits and the inhibition of H_2O_2 and histamine generation [2,18].

It was expected that higher concentrations of catechin would be detected because catechin is a type of procyanidin and is the main flavonoid present in beans [23]. However, Xu and Chang [5] did not detect this compound in raw or cooked brown beans.

Based on the data in Table 4, it is observed that, for all flavonoids, when there was a significant difference among the extracts from raw and cooked beans, the former always exhibited inferior results. The effect of soaking varies for each compound evaluated; however, the effect of this process on the increases in the concentrations of kaempferol and kaempferol-3-rutinoside was clear. These results are contrary to published reports claiming that cooking and soaking have a negative impact on flavonoid concentrations [1,5,17].

It is also notable that quercetin and quercetin-3-glucoside were not influenced by cooking, with or without soaking. Amarowicz and Peggy [3], however, found that the concentration of quercetin was reduced by 12 to 65% after the beans were cooked and Díaz-Batalla et al. [1] found mean values of 10.9 μg of quercetina g^{-1} in raw beans and 6.5 μg of quercetina g^{-1} in cooked beans. It is reported in the literature that quercetin has various effects in the prevention and treatment of some types of cancer, in the reversal of cognitive deficits and the inhibition of H_2O_2 and histamine generation [2,18].

The results for the flavonoid fractions in crude beans extracts are presented in Table 5. It is noted that many of the fractions did not contain one or more of the phenolic compounds analysed; additionally, the behaviour of the kaempferol was similar to that observed for other polyphenols and this compound was present only in extracts of cooked and soaked beans.

Aparicio-Fernandez et al. [7] performed fractionations similar to those in this study, but they used methanol extracts of black beans. They detected kaempferol-3-glucoside, quercetin-3-glucoside and myricetin glycosylated only in the FB fraction, at ratios of 5.8, 4.1 and 6.7 respectively. These authors note that the presence of glycosylated myricetin in these beans was unexpected and has been rarely documented for these beans.

Catechin was the only flavonoid that was found in all of the analysed extract fractions; the fact that this compound belongs to the procyanidin group, which is present in virtually all beans, further explains this observation. A study by Aguilera et al. [17] reported concentrations of catechin in macerated raw and cooked beans of 142.58 μg g^{-1} and 76.25 μg g^{-1}, respectively; these results are very similar to those obtained in this study (Table 5). It is known that catechin assists in the reduction of cholesterol absorption in the intestine and in the inhibition of LDL cholesterol oxidation [2].

Virtually all of the flavonoids analysed occurred in lower concentrations in the FF fraction relative to the fraction analysed for the same treatment. This observation leads to the conclusion that most of these phenolic compounds were not extracted or were extracted in small amounts by the solvent used in each fraction.

Although Beninger and Hosflied [14] indicated that flavonoids exist mainly in the glycosylated form, the fractions evaluated in this study also contained the aglycone flavonoid.

The occurrence, in significant quantities, of almost every flavonoid analysed (especially after cooking, which reflects how beans are most commonly prepared for consumption) confirms the importance of including beans daily as part of a healthy diet that is rich in bioactive compounds because all of the analysed flavonoids demonstrate free radical scavenging activity and other biological effects, which were described above [2,18].

Brown beans contain all of the flavonoids and phenolic acids analysed in this study, making their consumption highly beneficial due to the rich variety of physiological activities that phenolic compounds exert.

Acknowledgment

The authors are thankful to the rice and beans department of the Brazilian agricultural company EMBRAPA for donating the beans and FAPESP for granting the scholarship and research funding process number 2009/14924-4.

References

1. Díaz-Batalla L, Widholm JM, Fahey-Junior GC, Castaño-Tostado E, Paredes-López O (2006) Chemical components with health implications in wild and cultivated mexican common bean seeds (*Phaseolus vulgaris* L.). J Agric Food Chem 54: 2045-2052.
2. Campos-Vega R, Loarca-Piña G, Oomah BD (2010) Minor components of pulses and their potential impact on human health. Food Res Intern 43: 461-482.
3. Amarowicz R, Pegg RB (2008) Legumes as a source of natural antioxidants. Eur J Lipid Sci Technol 110: 865-878.
4. Rocha-Guzmán NE, Annete H, González-Laredo RF, Ibarra-Pérez FJ, Zambrano-Galván, G, et al. (2007) Antioxidant and antimutagenic activity of phenolic compounds in three different colour groups of common bean cultivars (*Phaseolus vulgaris).* Food Chem 103: 521-527.
5. Xu BJ, Chang SKC (2009) Total phenolic, phenolic acid, anthocyanin, flavan-3-ol, and flavonol profiles and antioxidant properties of pinto and black beans (*Phaseolus vulgaris* L.) as affected by thermal processing. J Agric Food Chem 57: 4754-4764.
6. Cardador-Martínez A, Aalbores A, Bah M, Calderón-Salinas V, Castaño-Tostado E, et al. (2006) Relationship among antimutagenic, antioxidant and enzymatic activities of methanolic extract from common beans (*Phaseolus vulgaris* L.). Plant Foods Hum Nutr 61: 161-168.
7. Aparicio-Fernandez X, Manzo-Bonilla L, Loarca-Piña G (2005) Comparison of antimutagenic activity of phenolic compounds in newly

harvested and stored common beans *Phaseolus vulgaris* against aflatoxin B1. J Food Sci 70: 73-78.

8. Brand-Williams W, Cuvier ME, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. LWT- Food Sci Tech 28: 25-30.
9. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, et al. (1999) Antioxidant activity applying na improved ABST radical cation decolorization assay. Free Radic Biol Med 26: 1231-1237.
10. Van Den Berg R, Haenen GRMM, Van Den Berg H, Bast A (1999) Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures. Food Chem 66: 511-517.
11. Arts MJTJ, Dallinga JS, Voss HP, Haenen GRMM, Bast A (2004) A new approach to assess the total antioxidant capacity using the TEAC assay. Food Chem 88: 567-570.
12. Xu BJ, Chang SKC (2008) Total phenolic content and antioxidant properties of eclipse black beans (*Phaseolus vulgaris* L.) as affected by processing methods. J Food Sci 73: 19-27.
13. Fernandez-Panchon MS, Villano D, Troncoso AM, Garcia-Parrilla MC (2008) Antioxidant activity of phenolic compounds: from in vitro results to in vivo evidence. Crit Rev Food Sci Nutr 48: 649-671.
14. Beninger CW, Hosfield GL (2003) Antioxidant activity of extracts, condensed tannin fractions and pure flavonoids from *Phaseolus vulgaris* L. seed coat color genotypes. J Agric Food Chem 51: 7879-7883.
15. Luthria DL, Pastor-Corrales MA (2006) Phenolic acids content of fifteen dry edible bean (*Phaseolus vulgaris* L.) varieties. J Food Comp Anal 19: 205-211.
16. Ranilla LG, Genovese MI, Lajolo FM (2007) Polyphenols and antioxidant capacity of seed coat and cotyledon from Brazilian and Peruvian bean cultivars (*Phaseolus vulgaris* L.). J Agric Food Chem 55: 90-98.
17. Aguilera Y, Estrella I, Benitez V, Esteban RM, Martín-Cabrejas MA (2011) Bioactive phenolic compounds and functional properties of dehydrated bean flours. Food Res Intern 44: 774-780.
18. Rauter AP, Dias C, Martins A, Branco I, Neng NR, et al. (2012) Non-toxic *Salvia sclareoides* Brot. Extracts as a source of functional food ingredients: phenolic profile, antioxidant activity and príon binding properties. Food Chem 132: 1930-1935.
19. Espinosa-Alonso LG, Lygin A, Widholm JM, Valverde ME, Paredes-Lopez O (2006) Polyphenols in Wild and Weedy Mexican Common Beans (*Phaseolus vulgaris* L.). J Agric Food Chem 54: 4436-4444.
20. Aaby K, Hvattum E, Skrede G (2004) Analysis of flavonoids and other phenolic compounds using high-performance liquid chromatography with colorimetric array detection: relationship to antioxidant activity. J Agric Food Chem 52: 4595-4603.
21. Romani A, Vignolini P, Galardi C, Mulinacci N, Benedettelli S, et al. (2004) Germplasm characterization of zolfino landraces (*Phaseolus vulgaris* L.) by flavonoid content. J Agric Food Chem 52: 3838-3842.
22. Lin LZ, Harnly JM, Pastor-Corrales MS, Luthria DL (2008) The polyphenolic profiles of common bean (*Phaseolus vulgaris* L.). Food Chem 107: 399-410.
23. Kosinska A, Karamac M, Penkacik K, Urbalewicz A, Amarowicz R (2011) Interactions between tannins and proteins isolated from broad bean seeds (*Vicia faba* Major) yield soluble and non-soluble complexes. Eur Food Res Technol 233: 213-222.