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Effect of cooking in common bean cultivars on antioxidant activity and phenolic compounds

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ABSTRACT

The phenolic compounds found in beans have several biological effects that may help in the prevention of some human diseases. This the research aimed to determine the antioxidant activity in different bean cultivars and to identify and quantify flavonoids and phenolic acids in raw and cooked beans. The antioxidant activity was performed by the ABTS and DPPH method and the flavonoids and phenolic acids were identified by HPLC. The results were analyzed using an ANOVA and Tukey's test. For antioxidant methods, significant differences were observed between raw and cooked beans, and the cultivar Porto Real presented the highest contents for both methods and treatments. There were significant differences between bean cultivars and treatments regarding phenolic acids and flavonoids. These results suggest that cooking favored the extraction of polyphenols, important for human health. Common beans are an excellent source of phenolic compounds with antioxidant activity.

KEYWORDS: *Phaseolus vulgaris L.*; Phenolic acids; Flavonoids; Antioxidant.

INTRODUCTION

Legumes are important sources of macronutrients and micronutrients and have played an important role in the traditional diets of many regions throughout the world. In addition to their nutritional value, it has long been recognized that legumes are functional foods that both promote good health and have therapeutic properties (XU; CHANG, 2007).

Such as the phenolic compounds ingested through the diet have generated attention for their numerous biological effects, including the inactivation of free radicals, the complexation of metal ion pro-oxidants, the modulation of enzymatic activity, the inhibition of cell proliferation and interactions in the signal transduction pathways. Epidemiological studies have noted positive associations between the consumption of foods rich in phenolic compounds and the prevention of some human diseases. The predominant phenolic compounds in the seeds of legumes are flavonoids, phenolic acids and condensed tannins (procyanidins) (AMAROWICZ; PEGG, 2008).

Consumption of the phytochemicals in beans, particularly polyphenolic compounds, has been linked to reduced risk of diabetes, obesity, coronary heart disease, colon cancer and gastrointestinal disorders. Thus beans have earned the distinction as being good for health and this has lead to the increase in their production. Accurate identification and quantification of phenolic compounds in beans is critical in developing successful new value added bean based products for new market opportunities in the functional food and nutraceutical industry and currently there is great interest in examining the phytochemical composition of legumes (ROSS; BETA; ARNTFIELD, 2009).

The processing usually applied to beans not only improves its sensory aspects but also inactivates trypsin inhibitors and hemagglutinin and bioactive compounds, promoting changes in both physical characteristics and chemical composition. Beans are soaked before cooking to reduce cooking time and to make their texture softer (XU; CHANG, 2011).

This research had the aim to measure the antioxidant activity in different cultivars beans and identify and quantify flavonoids and phenolic acid by HPLC with the alterations that occur with the cooking.

MATERIALS AND METHODS

The common beans (*Phaseolus vulgaris* L.), BRS Supremo (black color), Carioca Porto Real, Mulatinho Pirata, Chumbinho Brasil and Carioca Perola (brown color), were donated by EMBRAPA (National Center for Research on Rice and Beans).

Beans raw and thermally processed were analyzed. For an analysis of the raw grains, the samples were ground in a knife mill and sieved through a 30 mesh to obtain flour. This flour was stored in sealed polyethylene bags and kept at refrigerator temperature (4 °C) until use.

The cooked beans were soaked for 10 h in distilled water and cooked in an autoclave at 121 °C for 10 min. After cooking, the beans were placed in an oven at 55-60 °C to dry and subsequently ground.

EXTRACTION OF POLYPHENOLS

Extraction of the polyphenols was performed according Aparicio-Fernandez, Manzo-Bonilla e Loarca-Piña (2005). A 10 g lyophilized grain sample was ground for each treatment and placed in a 500 mL conical flask with 100 mL of methanol. The mixture was agitated for 14 h at 70 rpm. The samples were then centrifuged (Centrifuge Model 204NR) for 10 min at 2500 rpm and the supernatant collected and placed in round bottom flasks. The flasks were attached to a rotary evaporator and brought to 35 °C under vacuum to evaporate the methanol. The extracts were then frozen (- 80 °C) and lyophilized.

RADICAL DPPH AND ABTS SCAVENGING ACTIVITY

The antioxidant activity of the different bean varieties was determined according to the method described by Brand-Willians, Cuvier and Berset (1995) using 1,1-diphenyl-2-picrylhydrazyl (DPPH). The results were expressed in mg TEAC g^{-1} sample. The antioxidant activity was also determined by 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) method described by Berg *et al*. (2006) and Re *et al*. (1999).

HPLC ANALYSIS OF COMPOSITION OF PHENOLIC ACID AND FLAVONOID

The profile and content of the free phenolic acids was determined using an HPLC according Xu and Chang (2009), with modifications. The chromatography system had a Shimadzu 20A equipped with a pumping system (model LC-20AT), an autosampler (model 20AHT-SIL), a column oven (model CTO-20A), a communicator (model CBM-20A) and a UV detector (270 and 325 nm) (model SPD-20A). An analytical column (Zorbax ODS-C18 Stablebond, Agilent Technology, 4.6 x 250 mm) was used for the separation. A 40 °C temperature was maintained for the column. The mobile phases used were A (0.1 % trifluoroacetic acid in aqueous solution) and B (100 % methanol) with an isocratic flow (20 % of phase A and phase B 80 %). The flow equaled 1 $mL.min^{-1}$.

To identify the peaks of the HPLC samples, individual stock solutions (1 $mg.mL^{-1}$) for each phenolic acid (Sigma-Aldrich) were prepared and diluted. Diluted solutions were injected (20 µL), separately and in duplicate under the conditions described above. The peak areas were recorded and the retention times used to compare, identify and quantify the phenolic acids in the samples.

To prepare the stock solution, 10 mg of each phenolic acid was dissolved in 10 mL of 80 % methanol. A series of dilutions with methanol was performed to reach final concentrations of: 0.5, 1, 2.5, 5, 10 and 25 $\mu g.mL^{-1}$ for vanilic acid; 10, 25, 50 and 100 $\mu g.mL^{-1}$ for chlorogenic acid; 1, 2.5, 5, 10, 25, 50 and 100 $\mu g.mL^{-1}$ for sinapic acid; and 1, 5, 10, 25 and 50 $\mu g\ mL^{-1}$ for gallic acid. The content of the phenolic acids was expressed in micrograms per gram of extract ($\mu g.g^{-1}$).

The flavonoids were analyzed using the same chromatographic system and methodology for the identification and quantification of the phenolic acids. An analytical column was used for the separation at 34 °C. The mobile phases, solvent A (0.1 % acetic acid solution in water) and solvent B (0.1 % acetic acid in acetonitrile), were used with the following flow rates and concentration gradients: 1.0 $mL.min^{-1}$, 15 % solvent B and 85 % solvent A during the first 5min; 1.5 $mL.min^{-1}$, solvent B

increasing to 29 % and solvent A increasing to 71 % between 5-23min; 1.0 mL.min^{-1}, solvent B increasing to 35 % and solvent A increasing to 65 % between 23 to 44 min; 1.0 mL.min^{-1}, solvent B increasing to 50 % and solvent A decreasing to 50 % between 44 to 46 min; 1.0 mL.min^{-1}, solvent B decreasing to 15 % and solvent A increasing to 85 % between 46 to 48 min.

To identify the peaks of HPLC samples, individual stock solutions (1 mg.mL^{-1}) of each flavonoid, were prepared and then diluted. Diluted solutions were then injected (20 µL), separately and in duplicate, under the conditions described above. The peak areas were recorded and their retention times used in the comparison, identification and quantification of the flavonoids in the extracts.

For the stock solutions, 10 mg of each flavonoid was dissolved in 10 mL methanol (80%). A series of dilutions with methanol were also performed: 5, 10, 25 and 50 µg.mL^{-1} for catechin; 25, 50, 100 and 250 µg.mL^{-1} for quercetin; 2,5, 5, 10, 25 and 50 µg.mL^{-1} for kaempferol 3-glucoside; 10, 25, 50 and 100 µg.mL^{-1} for kaempferol- 3-O-rutinoside; 10, 25, 50 and 100 µg.mL^{-1} for kaempferol; and 10, 25, 50 and 100 µg.mL^{-1} for quercetin 3-glucoside. The flavonoid content was expressed in micrograms per gram of extract (µg.g^{-1}).

The statistical design was completely randomized with three replicates per treatment. Used an analysis of variance with an F-test using the Statistical Analysis Software System (SAS), followed by a comparison using Tukey's test with $p < 0.05$.

RESULTS AND DISCUSSION

ANTIOXIDANT ACTIVITY DPPH AND ABTS – EXTRACT OF BEANS

For the antioxidant activity measured with DPPH assay, the highest levels were found for Perola and Porto Real, both for the raw grains as well as for the boiled material (Figure 1a). Cooking beans reduced the antioxidant capacity for all samples. The decrease in antioxidant activity with cooking may occur because the antioxidant compounds are partially modified during the cooking process.

For the crude extracts, the results ranged from 8.29 to 12.69 mg TEAC.g^{-1} for the extract from the raw beans and from 7.99 to 11.15 mg TEAC.g^{-1} extract for the cooked beans. The cultivars showed a significant difference between the two treatments, consistent with previous work that reported that the differences in the amounts of these compounds was related to farming techniques, the genetic characteristics of the plants (HAMINIUK *et al*., 2012) and the seed coat colors (BENEVIDES *et al*., 2017).

Similar to the results presented in Figure 1a, Huber *et al.* (2016) determined in crude extracts of the brown beans, results of DPPH between 2.96 to 14.04 mg TEAC. g^{-1} extract and in the ABTS assays, between 13.70 and 29.51 mg TEAC.g^{-1} extract.

Compared with the raw legumes, Preti, Rapa and Vinci (2017) found that the processes of steaming and boiling significantly decreased the total phenolic content and antioxidant activity as measured using DPPH and ABTS in green beans. It's possible that the thermal treatment promotes the decomposition of phenolics or influences in the formation of complex compounds between phenolics and proteins (HUBER *et al*., 2014), which might explain the results shown in Figure 1. However also it can increase their total content by increasing their availability for

extraction, inactivating the polyphenoloxidase or by transforming the fiber-bound phenols into free polyphenols.

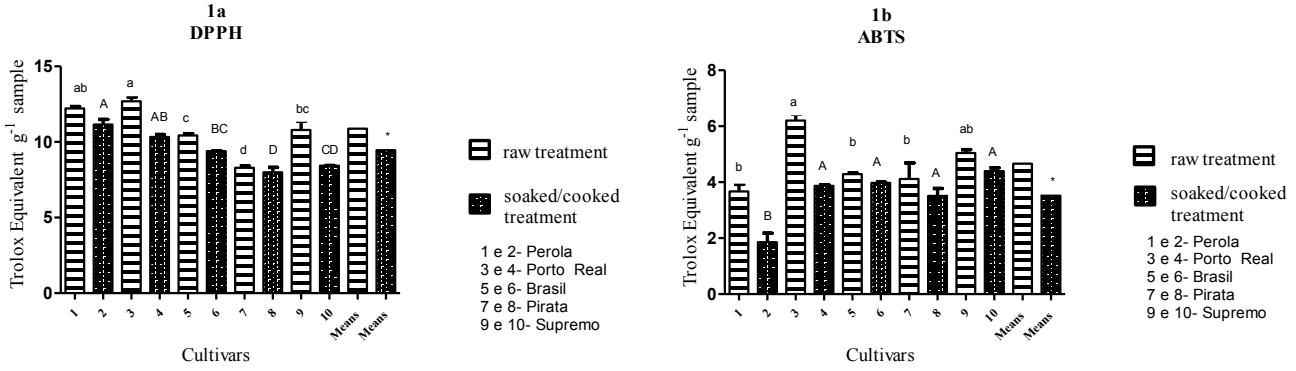

Figure 1 - Antioxidant activity of the polyphenol extracts from beans (1a – DPPH method and 1b – ABTS method, expressed as Trolox equivalent).

NOTE: Mean (n = 3); different lowercase letters in the column represent significantly different values for the raw beans; different capital letters in the column show a significant difference for the cooked beans ($P < 0.05$); different capital letters in the column are statistically significant ($P < 0.05$); average cultivars; ns shows no significant difference; * denotes a significant difference.

Huber *et al*. (2014) reported that the antioxidant activity values for extracts and fractions of raw bean were between 0.53 and 8.73, as expressed in TEAC using the DPPH method. These values are less than those found in work (Figure 1a). Instead, Jeng *et al*. (2010) evaluated the antioxidant activity by DPPH of the common bean cultivar Hwachia and the mutants induced by NaN_3 and found values ranging from 76.68 to 96.93 mg TEAC.g^{-1} on a dry basis.

Silva, Rocha e Canniatti-Brazaca (2009) found antioxidant activity values, expressed as TEAC.g^{-1} sample, for raw and cooked beans to be 22.57 and 12.18, respectively. These values are higher than those found in this work. Xu and Chang (2009) reported values of 0.78 and 0.97 mmol TEAC.g^{-1} for red and black beans, respectively, they found a high correlation between the phenolic compounds and the antioxidant activity. The results indicate that the process of cooking the beans decreases the DPPH values by 60.1 to 66.6%.

Cooking the extracts of the cultivars reduced the antioxidant activity. Consistent with these results, Xu and Chang (2009) observed that water used to soak the black beans had a dark color, indicating that some soluble components (components may include phenolic antioxidants) were leached into the cooking water. Huber *et al*. (2016) attributed this decrease in antioxidant activity to the possibility that chemical transformations, decomposition of phenolic compounds, formation of complexes between polyphenols and proteins and solubilization of water-soluble antioxidants in the discarded soaking water occurred.

Using the ABTS method (Figure 1b), the measured values of the Porto Real and Supremo cultivars differed from other cultivars with the raw treatment ranging from 6.20 to 3.65 mmol TEAC. g^{-1} and the cooked ranging from 1.85 to 3.97 mmol TEAC.g^{-1}. The cultivar Perola differed from the other beans, showing the lowest

level of antioxidant capacity. Cooking the extracts of the cultivars reduced the antioxidant activity.

The coefficient of correlation between the antioxidant activity and the phenolic content was 0.99 and 0.82 for the ABTS and the DPPH methods (Figure 1a and 1b), respectively, showing that the content of phenolic compounds is a good indicator of the *in vitro* antioxidant activity.

The Porto Real cultivar had the highest level for the raw bean, while the highest levels for the cooked beans were found in the Perola cultivar. Wolosiak *et al*. (2010) evaluated the antioxidant activity in fava beans using both ABTS and DPPH methods and found that the activity of the extracted substances was much higher for the ABTS than for the DPPH method. For raw beans, the value from ABTS was 264.2 mmol TEAC.g^{-1} and the value from DPPH was 98.4 mmol TEAC.g^{-1}. For steam cooked beans, the values were 237.4 for ABTS and 68 mmol TEAC.g^{-1} for DPPH.

According to Silva, Rocha and Canniatti-Brazaca (2009) the content of the polyphenols in beans may be related to some factors, in particular, the skin color of the beans, as darker colors are related to increased tannins and the cooking process causes changes in the antioxidant compounds, which influences in the reduction of these compounds during with cooking.

Jeng *et al*. (2010) evaluated the antioxidant activity by ABTS in common bean cultivar Hwachia and the mutants induced by NaN_3 and found values ranging from 238.5 to 385.3 mg TEAC. g^{-1}, on a dry basis.

Palombini *et al*. (2013) determined that the antioxidant activity by DPPH for five beans cultivars were values between 0.656 and 2.42 mmol TEAC.g^{-1}. This value is smaller than the activities found in this study (Figure 1a) for the two treatments. The differences between the present work and the results reported by authors may be due to differences in the variety of beans and the extraction method and solvent. However, studies using this particular analysis are scarce, making it difficult to compare data.

The antioxidants tests applied have the same principle based on the transfer of electrons by the antioxidant to the free radical, however, due to the particularities of the methods, as for example the ABTS method because it covers a longer wavelength range than the DPPH is able to detect the antioxidant activity of a greater number of phenolic compounds, the results of the tests can not be compared. According to Vasco, Ruales and Kamal-Eldin (2008), phenolic compounds react in different ways in the antioxidant assays, since the reactions depend on the hydrophilicity and the type of compound analyzed, the polarity of the different test systems and the radical source. Thus, due to this diversity of bases, types of compounds and antioxidant tests, no single method allows the exact extraction of the total antioxidant activity of an extract or food matrix. Two or more techniques are better used to infer the potential of extract or food.

Several studies (MELO *et al.*, 2011; VIEIRA *et al*., 2011) have observed a strong correlation between the total polyphenol content and the antioxidant activity. These results suggest that the total polyphenol content is a predictor of the *in vitro* antioxidant activity. The largest phenolic amounts are found in raw beans, which also has the highest antioxidant activity.

Siddhuraju (2006) reported differences in legumes after cooking. The antioxidant capacity after cooking influenced the Maillard reaction. The antioxidant capacity of the bean *Vigna aconitifolia* decreased after the cooking process. The raw beans have more antioxidant capacity, total phenols and tannins, showing the influence of the amount of tannins and polyphenols on the antioxidant capacity.

HPLC ANALYSIS OF COMPOSITION OF PHENOLIC ACID AND FLAVONOID- EXTRACT

In this work, chlorogenic acid was found to be present in greater amounts in the extracts of the raw beans, especially the black cultivars. For sinapic, gallic and vanillic acid the highest levels were found in extracts of the soaked/cooked grains.

Statistically significant differences were observed for the quantities of phenolic acids contained within the bean preparations depending on the type of treatment, due to thermal effects.

Thermal processing can cause physical and chemical complexations and changes in the reactions of the phenolic compounds, including the leaching of water soluble phenolic compounds, the phenolic release of complex shapes, the degradation of polyphenols, the breakdown and transformation of phenolic compounds, and the formation of products by the Maillard reaction, such as the formation of complex products from the phenolic compounds and proteins. It is believed that the antioxidant components of many foods can be significantly lost as a result of industrial sterilization, pasteurization and dehydration, as well as domestic cooking. However, the processing does not always result in the destruction of the antioxidant components. In some cases, the processing factors can induce the formation of compounds (SIDDHURAJU, 2006).

According to Aparicio-Fernandez, Manzo-Bonilla e Loarca-Piña (2005) the best separation of phytochemicals uses a 100% methanol extract from the bean coat with different fractionation methods. A direct chromatographic analysis using HPLC-MS was performed using the silica gel fractionation, enabling an improved identification of the compounds, especially the flavonols. These procedures were used in this work for the extraction and fractionation of these molecules.

As reported in the literature, phenolic acids were found in the samples, with gallic acid and chlorogenic acid representing the largest components. These results (Table 1) were higher than the values reported in the literature Xu and Chang (2008).

According to Espinosa Alonso *et al*. (2006), the phenolic composition of 62 wild and domestic Mexican beans were found to have wide variations in phenolic content relating to the genotypes not related to color. A higher amount of free phenolic acids were found in the shell than in the cotyledons of the beans, suggesting that the phenolic acids are mainly present in the seed hulls. The major phenolic acids found in the grains were analyzed to be ferulic, vanillic, p-hydroxybenzoic and sinapic acids, and at low amounts, the phenolic aldehyde vanillin, caffeic acid, syringic acid, and p-coumaric acid. However, Ombra *et al*. (2016) evaluated speckled and dark beans and detected only the, vanillic acid and caffeic acid in speckled bean and in dark bean, vanillic acid and syiringic acid, thermore, they identified in both beans the acids phenolics, caftaric acid and chicoric acid and chlorogenic acid in the dark bean.

Table 1 - Phenolic acid (μg.g^{-1}) extracts of the polyphenols from the raw beans and the cooked beans.

	Perola	Porto real	Brasil	Pirata	Supremo	Average
			Vanilic Acid			
Raw	261.63±4.2[a]c[b]	423.70±0.6b	565.62±0.3a	420.27±0.5b	618.44±3.5a	457.93B[c]
Cooked	672.94±1.3b	879.24±2.4a	880.64±0.1a	624.54±0.8c	333.72±0.8d	678.21A
			Gallic Acid			
Raw	1841.98±3.4d	2185.26±3.2c	2505.37±9.1b	1975.15±1.8cd	2823.70±1.4a	2266.29B
Cooked	3152.45±3.8c	3750.00a±3.2a	3462.39±4.0b	3063.37±2.9d	2431.64±3.1e	3191.97A
			Chlorogenic Acid			
Raw	1904.74±1.8c	2150.38±4.0bc	2376.74±2.0b	2342.40±2.0b	4548.6±7.6a	2664.69A
Cooked	1454.37±1.3e	1668.95±1.7d	1999.35±0.4c	2866.32±2.3a	2012.79±3.2b	2000.35B
			Sinapic Acid			
Raw	60.46±2.7e	118.99±1.6c	255.94±0.5a	165.27±0.0b	86.27±0.5d	137.39B
Cooked	248.57±0.3c	284.88±0.12b	406.23±2.2a	147.06±2.0e	211.69±1.9d	259.69A

NOTE: [a]Means of three replicates ± standard deviation; [b]Means with lowercase letter(s) differ significantly between cultivars, horizontally for the same treatment ($p \leq 0.05$); [c]Means with uppercase letter(s) differ significantly between raw and cooked vertically ($p \leq 0.05$).

Each phenolic acid was found in greater quantities with specific beans, indicating that the color of the seed is directly influenced by the composition of the phenolic acids. The genotype, farming practices, climatic conditions and maturity at harvest influence the profile of this phenolic compounds in the legume (VALDÉS *et al*., 2011).

For vanillic acid, there is a greater focus on the Supremo and Brasil cultivars. While cooking has been thought to significantly increase the concentration of this acid in most grains, Diaz-Batalla *et al*. (2006) found that 14 cultivars had lower levels of vanillic acid in the cooked beans compared with the raw beans. This study also showed that the highest values of vanillic acid were found in black beans. Aguilera *et al*. (2011) founded no vanillic acid in the cooked beans with or without maceration.

For gallic acid, the highest concentrations were found in the raw Supremo cultivar. Even higher concentrations of phenolic acid were found in the extracts of the cooked Porto Real beans. Xu and Chang (2007) reported that the gallic acid content was equal to 83.17 μg.g^{-1} for the raw brown beans and 38.16 μg.g^{-1} for the cooked brown beans. These results are lower than those measured in this work and inconsistent with these findings on the effects of cooking on the phenolic acid content.

In this work, chlorogenic acid was found to be present in greater amounts in the extracts of the raw beans, especially the black cultivars. Ranilla, Genovese and Lajolo (2009) detected chlorogenic acid in much lower quantities (between 2.8 and 5.6 mg.100g^{-1}) than those values found in this work. In addition, only three of the twenty-eight varieties analyzed contained chlorogenic acid. These differences may be explained by the different extraction techniques used in extracting the phenolic acids from the beans. Many studies in the literature examined the bean grain, while the present study assessed an extract of the bean. In the study by Espinosa-Alonso *et al*. (2006) the sinapic acid concentrations varied between 4.04 and 22.4 mg.kg^{-1} bean for the light and mixed colors, respectively.

Amarowicz *et al*. (2009) reported that the post-harvest processing and the storage conditions influence the flavonoid and phenolic acid content in foods. Consequently, the intake and bioavailability of the polyphenols can be modified. In addition, processed foods can also change the chemical form of the compound of interest to subsequently impact bioavailability.

The differences in grades between the various cultivars can be explained by the differences in the genotypes. The changes in the phenolic acid concentration in the seeds were determined by characterizing the relationship between the grain color and the expression of the genes in each cultivar (2009). Moreover, part of the grain sampled, grain handling and processing can influence also the levels (RAGAEE *et al*., 2014).

While nutritional values are determined from an analysis of the raw food, most beans are consumed in a cooked form. The nutritional values may also differ for the various genotypes. Nutrients in the beans can also be modified with various forms of preparation, such as a water soak, that can alter the food characteristics and the availability of the nutrients for absorption.

For the bean extract, the flavonoids kaempferol 3-3-glucoside and 3 rutinoside were found in higher concentrations in the Brasil cultivar for both treatments. For catechin, the Perola and Porto Real showed higher levels for the raw bean, while the Porto Real and the Brasil cultivars showed the highest levels for the cooked beans. The highest content of quercetin was observed in the Piratã, Porto Real and Supremo cultivars that were soaked and then cooked. Quercetin-3-glucoside showed a higher content in the Brasil and the Supremo cultivars with the cooked treatment showing an increased value. The flavonoids varied with the cultivars and the effect of cooking too (Table 2).

Díaz-Batalla *et al*. (2006), reported that kaempferol was present in higher concentrations in the raw beans (mean 52.3 $\mu g.g^{-1}$ bean) compared with the cooked beans (mean 27.2 $\mu g.g^{-1}$ bean). Amarowicz and Pegg (2008), also reported that cooking caused a reduction of this compound by 5-71%. These researches differ the results compared with the result found in this research.

In raw black beans, phenolic acids account for approximately 647.4 $\mu g.g^{-1}$ per bean and flavonoids account for approximately 677.4 $\mu g.g^{-1}$. After cooking the grains, these levels were reduced by 17.9 to 44.5% for the phenolic acids and by 12-65% for the flavonoids (VALDES *et al*., 2011). In the present study, the heat treatment increased the bioactive compounds by approximately 140.84 to 189.01 % for vanillic acid, gallic acid and sinapic acid, and decreased chlorogenic acid by 75.06 %. With the heat treatment, flavonoids increased from 176.52 to 325.25% for quercetin 3-glucoside, quercetin, catechin and kaempferol 3-glucoside. The heat treatment decreased kaempferol 3 - rutinoside by 17.9 %.

The heat treatment influenced the flavonoids, quercetin and catechin. While varying for each extract and compound evaluated, maceration increased the concentrations of this compounds, but not for kaempferol and kaempferol-3-rutinoside. These results agree to many literature studies claiming that the cooking and mashing process has a negative impact on the concentrations of flavonoids (DÍAZ-BATALLA *et al*., 2006; AGUILERA *et al*., 2011). Laparra, Glahn and Miller (2008) found that the kaempferol content of black beans ranged from 3.0 to 17.5 $mg.kg^{-1}$.

Table 2 - Flavonoids (µg.g^{-1}) extracts of polyphenols from raw beans and cooked beans.

Cultivar	Perola	Porto real	Brasil	Pirata	Supremo	Average
Kaempferol						
Raw	nd	nd	nd	nd	nd	nd
Cooked	279.3±1b	385.1±4b	1977.1± 4.2a	nd	nd	nd
Kaempferol-3-glicoside						
Raw	3.75±0d	458.73±4b	2505.58±2a	6.41±0d	93.39±0c	613.57A^{c}
Cooked	1075.86±4b	1126.20±4b	3591.01±4a	3.94±0c	119.86±5c	1183.37B
Kaempferol-3-rutinoside						
Raw	17.53±2c	1.37±0c	2191.0±2a	505.50±2b	6.32±2c	544.36ª
Cooked	133.89±1ab	111.02±1bc	142.02±2a	3.46± 0d	96.85±1c	97.45B
Catequin						
Raw	586.36±2ª	554.06±5a	211.42±5b	51.11±1c	78.34±0c	296.26B
Cooked	1034.80±4b	1446.57±2a	1163.55±2ab	568.63±3c	574.81±4c	957.67A
Quercetin						
Raw	5.56±0bc	1.58±0d	7.53±1b	16.85±3a	3.47±0cd	7.00B
Cooked	15.88±2bc	18.66±1ab	12.88±1c	5.01±1d	20.10±0a	14.50A
Quercetin-3-glicoside						
Raw	4.21±1b	3.65±0b	109.96±3a	3.18±0b	99.85±2a	44.17B
Cooked	21.40±1c	32.99±1c	146.23±11b	24.88±0c	164.33±1a	77.97ª

NOTE: [a]Means of two replicates ± standard deviation; [b]Means with lowercase letters (s) differ significantly between cultivars, horizontally on the same treatment ($p \leq 0.05$) by the test medium; [c]Means with uppercase letter(s) differ significantly between raw and cooked vertically ($p \leq 0.05$); nd - not detected.

Quercetin increase by cooking and maceration. Amarowicz and Pegg (2008) founded a reduction ranging from 12 to 65% for the concentrations of flavonoids after cooking. Díaz-Batalla *et al*. (2006) founded averages of 10.9 µg.g^{-1} of quercetin raw bean and 6.5 mg.g^{-1} of quercetin cooked beans. It appears that most of the pigment grains are directly related to the presence of flavonoids in a glycosylated form. Ranilla, Genovese and Lajolo (2009) detected quercetin and kaempferol in brown beans, kaempferol in white beans and quercetin and kaempferol in black beans. Lin and Tang (2007) founded myricetin-3-glucoside, quercetin-3-glucoside and kaempferol-3-glucoside in black beans as well as kaempferol-3-glucoside in brown beans.

Assessing the levels of some flavonoids in italians beans, Romani *et al*. (2004) identified only trace amounts of quercetin-3-glucoside. The distribution of these compounds in methanol extracts can be highly variable, depending on the skin color of the beans and the treatment type. This variation in results can be attributed to the influence that multiple factors, such as light, temperature, degree of plant nutrition and exposure to pathogens, exert on the changes in the phenolic content (DINELLI *et al*., 2006).

It appears that catechin is the only flavonoid found in all the analyzed extract fractions. This compound belongs to the procyanidin group, present in virtually all beans. A study by Aguilera *et al*. (2011) reported that the concentrations of catechin measured for raw and cooked white beans with maceration were 142.58 and 76.25 µg.g^{-1}, respectively.

Similar to the results found for the phenolic acids, the flavonoids may have had higher values than those cited in the literature because these compounds were obtained from extracts and not from the intact grain, as is most common in the literature.

CONCLUSIONS

The antioxidant activities by the DPPH method had high levels for both treatments for the Perola and Porto Real cultivars. An analysis using the ABTS method demonstrated that the raw beans from the Supremo and Porto Real cultivars showed high antioxidant activity. All cultivars except the Perola bean were measured to have statistically similar values for the soaked/cooked treatment. The antioxidant activity of the beans decreased with cooking. The ABTS and DPPH methods showed good repeatability and were used to determine the antioxidant activity of the samples. Among the beans tested, the highest antioxidant activity was obtained from the Porto Real cultivar for both methods.

For the phenolic acids, all cultivars showed significant amounts of gallic acid. The Brasil cultivar contained a larger amount of vanillic and sinapic acids for the raw bean than the soaked/cooked beans. The Supremo cultivar had a high content of gallic acid and chlorogenic acid for the raw beans. The soaked/cooked Porto Real cultivar contained high levels of gallic acid. The soaked/cooked Pirata cultivar had high levels of chlorogenic acid. There was a significant difference between the treatments (raw and soaked/cooked) in the measurements for gallic acid. The soaked/cooked treatments had high concentrations of vanillic acid, gallic acid, sinapic acid and chlorogenic acid, but showed low levels for the soaked/cooked treatment.

Of the flavonoids tested, only kaempferol was present in the three cultivars that were soaked/cooked. The remaining flavonoids were also analyzed. The Brasil cultivar that was soaked/cooked had a higher content of kaempferol. Both the raw and the soaked/cooked Brasil cultivar had three kaempferol glycosides and kaempferol 3 rutinoside. The catechin content was high in the Porto Real for both treatments. The quercetin levels were high in the raw Pirata grain. Quercetin was also found in the Supremo cultivars and the Porto Real grains that were soaked/cooked. Quercetin 3 glycoside was found in the Brazil and Supremo cultivar with both the raw and the soaked/cooked beans. The heat treatment increased the concentrations of catechin and quercetin, increasing the activity of these compounds. In general, the heat treatment changed both the antioxidant activities as well as the amounts of flavonoids and phenolic acids.

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Efeito da cocção em cultivares de feijão na atividade antioxidante e compostos fenólicos

RESUMO

Os compostos fenólicos encontrados nos feijões apresentam diversos efeitos biológicos que podem auxiliar na prevenção de algumas doenças humanas. Assim a pesquisa objetivou determinar a atividade antioxidante em diferentes cultivares de feijão e identificar e quantificar flavonoides e ácidos fenólicos em feijões cru e cozido. A atividade antioxidante foi realizada pelo método ABTS e DPPH e os flavonoides e ácidos fenólicos foram identificados por HPLC. Os resultados foram analisados por meio da ANOVA e teste de Tukey. Para os métodos antioxidantes foram observadas diferenças significativas entre feijão cru e cozido, e a cultivar Porto Real apresentou os maiores teores para ambos métodos e tratamentos. Houve diferença significativa entre as cultivares de feijão e tratamentos em relação a ácidos fenólicos e flavonoides. Esses resultados sugerem que a cocção favoreceu a extração de polifenóis, importantes para a saúde humana. O feijão comum é fonte excelente de compostos fenólicos, com atividade antioxidante.

PALAVRAS-CHAVE: *Phaseolus vulgaris L.*; Ácidos fenólicos; Flavonoides; Antioxidante.

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