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From the market to the plate: Fate of bioactive compounds during the production of *feijoada* meal and the impact on antioxidant capacity

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ABSTRACT

Consumption of plant foods are known to reduce the risk of developing non-communicable diseases. However, many foods are not consumed in their natural state being processed and cooked before ingestion. The objective of this work was to analyze bioactive compounds (phenolics, flavonoids, tannins, proanthocyanins, and anthocyanins), as for individual phenolic acids present in the main ingredients of *feijoada* whole meal from their uncooked state until the ensemble of the whole meal and evaluate the impact of cooking on antioxidant activity. Analysis of *Feijoada* whole meal, composed by black beans, rice, kale, cassava flour, and orange showed that black beans are the main provider of phytochemicals and antioxidant activity, with the highest values for phenolics, tannins, and anthocyanins. For flavonoids, kale was the main source. Phenolic acids were mainly observed in black beans and were identified as mainly protocatechuic, chlorogenic, caffeic and ferulic acids. The antioxidant activity, as for the bioactive compounds analyzed, was progressively reduced during processing. For the other ingredients, on the other hand, no significant difference was observed after cooking. As expected, the hydrolyzable fractions showed significantly higher amounts of phenolics and antioxidant activity than soluble fractions. Altogether, *feijoada* whole meal provides a variety of beneficial health compounds due to its rich composition of distinct food groups such as cereals, legumes, vegetables, and fruits suggesting that the evaluation of meal as traditionally consumed can be important to identify phytochemicals in the dietary pattern of a population.

1. Introduction

Consumption of plant foods has long been related to reduce risk of developing non-communicable diseases. High in fiber, low in saturated fats, plant foods also contain a variety of bioactive compounds which exert high antioxidant activity. Although initial research focused mainly on fruits and vegetables, recently grains and legumes have also been intensively investigated (Jonnalagadda et al., 2011; Okarter & Liu, 2010).

Legumes are important sources of proteins in the diet and constitute a staple food in many developing countries. In addition, it has high fiber content, is low in dietary fat, rich in minerals such as iron, and also contains numerous phytochemicals with useful biological activities. The consumption of legumes has been correlated with reduced risk of mortality from cardiovascular disease and coronary heart disease in different countries in the world (Menotti et al., 1999; Nöthlings et al., 2008). Flight and Clifton (2006) concluded that the consumption of legumes at least four times a week showed a protective effect against coronary diseases and cardiovascular diseases when compared to one time a week intake. This effect was associated with natural antioxidant presents in legumes which resulted in potent antioxidant activity shown in different varieties of legumes (Akond et al., 2011; Campos-Vega, Loarca-Piña, & Oomah, 2010).

Among the major antioxidant contributors to the beneficial effects of legumes in cardiovascular health are phenolic compounds. Phenolic compounds are characterized as having a hydroxyl (—OH) radical linked to an aromatic group and can be divided in different subclasses according to their chemical compounds. Among the main phenolics present in legumes are phenolic acids and flavonoids, especially anthocyanins and proanthocyanidins (condensed tannins). These flavonoids, other than having high antioxidant activity, are also related to the bean color and tend to have a higher concentration in the seed coat. Dark colored legumes, such as black beans, has shown to have higher antioxidant activities than pale colored legumes, such as green peas, due in part for its higher amount of condensed tannins (Wang, Melnyk, Tsao, & Marcone, 2011).

Black beans are the most commonly consumed legume in Brazil and it constitutes the basic diet when combined to rice. In addition, the most traditional meal in Brazil, *feijoada*, is constituted of black beans combined with rice, kale, cassava flour and orange, all consumed together as one meal.

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Although literature shows beneficial effects of the consumption of legumes, such as black beans, legumes are necessarily submitted to procedures such as soaking and cooking prior to consumption which can result in losses and/or modifications of the antioxidants compounds presents. In this way, the present study evaluates the fate of bioactive compounds and antioxidant activity of black beans and other main ingredients which composes *feijoada* meal from its uncooked state until its ready to be consumed.

2. Materials and methods

2.1. Chemicals and reagents

Folin–Ciocalteu reagent, tannic acid, HPLC grade methanol, acetic acid, and trifluoroacetic acid (TFA) were purchased from Merck (Berlin, Germany). Catechin, gallic acid, protocatechuic acid, chlorogenic acid, caffeic acid, ferulic cid, and cinnamic acid were purchased from Sigma-Aldrich, Inc. (St. Louis, MO). All other reagents were analytical grade.

2.2. Sample preparation

Feijoada whole meal is a traditional Brazilian meal consisted of black beans (*Phaseolus vulgaris* L.), rice (*Oryza sativa* L.), Brazilian kale (*Brassica oleracea* L. acephala D.C.), cassava (*Manihot esculenta*) flour, and orange (*Citrus sinensis* L. Osbeck), as the main food ingredients. Additionally, jerked beef was included during the cooking of black beans (amount approximately 30% of cooked *feijoada* whole weight, ready-to-eat). All food ingredients were purchased from the main markets of Rio de Janeiro, Brazil, and were chosen based on the common varieties consumed by the population. All ingredients were acquired in July 2009, during its harvest period, and taken to the Dietetic Laboratory of the Nutrition Institute of the Federal University of Rio de Janeiro for the preparation of meals. All ingredients, except orange, were cooked individually based on laboratory standardized technique using standard technical cards, which controlled the amount and type of ingredients used, preparation method, time and temperature of cooking. For black beans, soaking for 10 hours with 1:2 weight and water volume ratio was made prior to cooking. After cooked, each food ingredient was weighed and mixed as the food portion (Fig. 1). The final *feijoada* meal was constituted by 63.6% cooked black beans, 18.9% cooked rice, 6.5% cooked kale, 6.1% orange, and 4.9% roasted cassava flour, by total weight. The *feijoada* meal was thoroughly mixed in a blender, lyophilized and stored at −40 °C until analyses. Each individual food component of the meal, uncooked and cooked, was also mixed in a blender, lyophilized and stored at −40 °C until analyses. Black beans soaking water was directly used for analysis. Three different cooking batches were prepared for each ingredient, resulting in three final meals. For each meal triplicates were done for each analytical parameter.

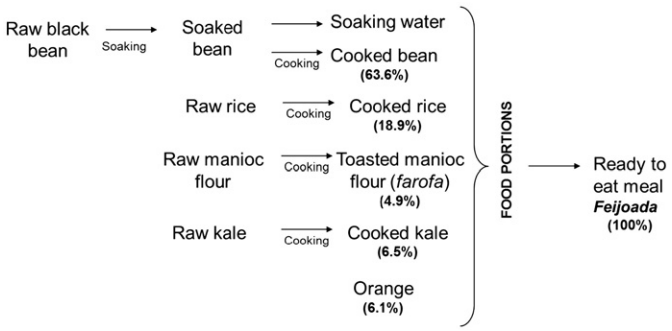

Fig. 1. Fluxogram of feijoada cooking and assembling. Values in parenthesis represent the contribution of each ingredient to *feijoada* whole meal.

2.3. Polyphenol extraction and quantification

The polyphenol extraction and quantification were conducted according to previous work from our laboratory (Faller & Fialho, 2010), with some modifications. Briefly, for 100 mg of each sample, 500 mL of extracting solution was added to a screw-capped cryotube vials. For the extraction of soluble polyphenols, a 50% methanol (methanol: water, 50:50, v/v) was used; for hydrolyzable polyphenols, a 1.2 M HCl 50% methanol (methanol: water, 50:50, v/v) was added. The samples were placed in a water bath at 90 °C for 180 min with constant shaking. After 3 h the samples were removed from the water bath and cooled at room temperature (approximately 2 min). The volume was filled up to 1 mL with 95% methanol and the Eppendorfs were centrifuged at 12,000 ×*g* for 5 min. The supernatants, considered to be soluble extracts (SE) or hydrolyzable extracts (HE) were used for bioactive compounds and antioxidant capacity analysis.

Polyphenol quantification methodology was modified to incorporate tannins analysis. Phenolic quantification in both SE and HE were made using Folin–Ciocalteu's reagent and tannic acid as standard. In test tubes, 50 μL of SE or HE were added to 450 μL distilled water. To the mixture, 0.25 mL of Folin–Ciocaulteu's aqueous solution (1:1, v/v) and 1.25 mL 20% sodium carbonate solution. The tubes were vortexed and let to stand at room temperature for 40 min after which samples were read in spectrophotometer at 725 nm. Results were expressed as mg of tannic acid equivalent (TAE)/mg dry weight (DW).

2.4. Total tannins quantification

Tannins were quantified according to Makkar, Bluemmel, Borowy, and Becker (1993). In test tubes 100 mg of polyvinylpolypyrrolidone (PVPP) were weight to which 1 mL from SE and HE and 1 mL of distilled water were added. The tubes were vortexed and let to stand at 4 °C for 15 min. After this period the tubes were agitating in vortex and centrifuged at 3000 ×*g* for 10 min. The supernatants were used for the quantification of phenolics. Since PVPP precipitates tannins the amount of tannins present in samples is a result of the difference between phenolics analysis before and after the addition of PVPP. In test tubes 200 μL of the supernatant were added to 300 μL distilled water, 0.25 mL Folin–Ciocaulteu's aqueous solution (1:1, v/v) and 1.25 mL 20% sodium carbonate solution. The same procedure for phenolics quantification was applied as describes in Section 2.2. For tannins quantification a larger amount of supernatant to adjust to the standard curve since during tannins precipitation with PVPP the sample is diluted. Tannin fraction was a result of the difference on phenolics analysis before and after the precipitation with PVPP, and both extracts, with or without tannins were used for antioxidant capacity analysis.

2.5. Condensed tannins quantification

Condensed tannins analysis was made using the buthanol-HCL method according to Wolfe, Terrill, and Muir (2008). Solutions of buthanol and chloridric acid (HCl) (95:5, v/v) and ferric ammonium sulfate (2% ferric ammonium sulfate in 2 N HCl) were made previously. For this protocol is necessary that the samples absorbance do not exceed 0.6. In this way, SE were diluted 1:5 (100 μL SE + 400 μL 50% methanol) and HE was diluted 1:10 (50 μL HE + 450 μL 50% methanol), both resulting in a total of 500 μL which were added to test tubes. To the 0.5 mL of diluted samples 3 mL of buthanol-HCl and 1 mL of ferric ammonium sulfate solutions were added. The tubes were vortexed and set in a heating block set at 97–100 °C for 1 hour. The tubes were than cooled in an ice bath and absorbance recorded at 550 nm. The reading was subtracted from blank constituted of the absorbance of the unheated mixture. Condensed tannins

were expressed as leucocyanidin equivalent (LE) calculated based on the equation below:

$$\text{Condensed tannins} : (\text{A550} \times 78.26 \times \text{dilution factor}) / \text{sample concentration}$$

Assuming that the effective El%, 1 cm, 550 nm of leucocyanidin is 460. Dilution factor was considered the total volume, 0.5 mL divided by the sample volume used, as for SE 0.5/0.1 = 5 and for HE 0.5/0.05 = 10.

2.6. Total flavonoid quantification

Flavonoids quantification was estimated according to methodology described by Kim, Chun, Kim, Moon, and Lee (2003). In test tubes, 4 mL distilled water was added to 1 mL diluted sample (1:1, v/v). To this mixture 0.3 mL of 5% sodium nitrate and after 5 min, 0.3 mL of 10% aluminium chloride. than 2 mL of 1 M sodium hydroxide were added and the volume completed to 10 mL with distilled water. The samples were read using spectrophotometer at 510 nm using catechin as standard and values were expressed as mg of catechin equivalents (CE)/mg.

2.7. Anthocyanin quantification

Monomeric anthocyanins were analyzed using the differential pH assay according to Sadinova, Stintzing, Kammerer, and Carle (2009). In test tubes 0.2 mL of samples, diluted as necessary, were added in duplicates. To distinct test tubes 1.8 mL of 0.025 M potassium chloride (pH 1.0) or 1.8 mL of 0.4 M sodium acetate (pH 4.5) were added. Spectrophotometric readings of samples at both pHs were made at 520 nm and 700 nm. Monomeric anthocyanins were quantified according to the equation below:

$$\text{Total monomeric anthocyanins(A)} = (\text{A520–A700 nm})\text{pH 1.0} - (\text{A520–A700 nm})\text{pH 4.5}$$

Concentrations of anthocyanins in samples were expressed as cyanidin-3-glucoside as below:

$$\text{Monomeric anthocyanins(mg/100 g)} = A \times MW \times DF \times 100 \text{ divided by}(\varepsilon \times 1)$$

Where A = absorbance, MW = molecular weight (449.2), DF = dilution factor and ε = molar extinction coefficient (26.900).

2.8. Phenolic acids extraction and analysis

Free phenolic acids were quantified by HPLC according to Queiroz, Lopes, Fialho, and Valente-Mesquita (2011), Queiroz, Silva, Lopes, Fialho, and Valente-Mesquita (2011). Briefly, 500 mg of lyophilized samples were extracted with 5 mL of methanol, water, acetic acid and BHT (85:15:0.5:0.2, v/v/v/w) for 4 hours at room temperature. Samples were then brought to dryness in a rotary evaporator at 40 °C under vacuum. The residue was re-dissolved in 1 mL 25% methanol and filtered with a 0.2 μm PTFE syringe before free phenolics analysis. The analyses were done using a Shimadzu Chromatography workstation and C18 Waters Symmetry column (250 mm × 4.6 mm, 5 μm). The flow rate was 1.0 mL/min. Mobile phase A was water: 0.02% TFA and phase B methanol: 0.02% TFA. The gradient conditions were as follows: 0–5 min, 25% B; 5–10 min, 25–30% B; 11–16 min 30–45% B; 17–18 min, 45% B; 19–40 min, 45-85% B; 41–50 min, 80–25% B. The detection weight length was set to 270 nm. Authentic standards of acids included gallic, protocatechuic, chlorogenic, caffeic, ferulic, and cinnamic acids at concentrations 0.25, 0.5, 1, 2.5, 5, 10, 25, 50 and 100 μg/mL. Phenolic acid contents were expressed as micrograms per gram (μg/g) on a dry weight basis.

2.9. Antioxidant capacity analysis

The antioxidant capacity was evaluated by three methods: ferric reducing antioxidant power (FRAP), DPPH and β-carotene bleaching method were made according to the methodology previously described (Queiroz, Lopes, Fialho, & Valente-Mesquita, 2011) with slight modifications. The FRAP assay measures the antioxidant potential of compounds to reduce the Fe^{+3} in acidic pH. FRAP reagent was freshly prepared from 300 mM acetate buffer (pH 3.6), 20 mM ferric chloride and 10 mM TPTZ made up in 40 mM HCl. All three solutions were mixed in the ratio 10:1:1. For each sample, 20 μL were pipeted into 96 well plates to which 280 μL of FRAP reagent was added. The absorbance was read at 630 nm after 30 min in the absence of light. A calibration curve of AA (0.1–1 mM) was used, and results were expressed as vitamin C equivalent antioxidant capacity (VEAC). For the DPPH assay, 100 μL of either SE or HE and 3.9 mL of 100 μM DPPH solution were mixed. The absorbance was set at 517 nm and it was recorded at 5, 10, 20, 40 and after 60 min. The antioxidant capacity was represented as percentage of radical inhibition remaining after each time according to the equation: % DPPH = (A0 − At) × 100/A0, where A0 represents the absorbance of DPPH solution alone measured at zero time, and At is the absorbance for each sample after each time of reaction. The β-carotene bleaching method is based on β-carotene oxidation induced by the products from linoleic acid oxidative degradation. Solutions were prepared by mixing 1 mL of β-carotene/linoleic acid system solution and 0.1 mL of SE or HE. The mixture was kept in a water bath at 50 °C. Spectrophotometric readings were made at 470 nm after the mixing and then at 30 min intervals until 120 min. The antioxidant capacity was represented as percentage of oxidation inhibition, in relation to control oxidation (no antioxidants), according to the equation: % oxidation inhibition = (Oxidation control − Oxidation sample) × 100/oxidation control, where 'oxidation control' is the degradation rate of control sample (no antioxidants), and 'oxidation sample' is the degradation rate for each sample.

2.10. Statistical analysis

The statistical analysis of the results was done using Graph Pad Prism 5.0 for Windows to apply one-way ANOVA followed by the Tukey test. Pearson's correlation and linear regression was done between bioactive compounds and antioxidant activity. Trends were considered significant when the means of compared sets differed at $P<0.05$.

3. Results and discussion

Modifications in bioactive compounds quantities were measured during cooking and production of *feijoada* whole meal. The main bioactive compounds groups analyzed in black beans during its cooking process, from raw black beans to the *feijoada* meal, are shown in Table 1. Raw black beans showed significantly higher amounts of polyphenols, tannins, flavonoids, proanthocyanidins, and anthocyanins when compared to each processing step, in both soluble and hydrolyzable extracts. Soaking seems to have a greater impact on bioactive compounds in soluble extract, as for after cooking, no significant difference was found between soaked and cooked black bean samples.

For hydrolyzable extracts the same was observed, with the exception of tannins which were significantly reduced after being cooked when compared to the soaked sample. However, for polyphenols and tannins, soaked and cooked beans losses were higher than 70% when compared to raw beans in the soluble extract, were as for the

Table 1
Bioactive compounds in black beans during cooking process.

	Raw	Soaked	Cooked	*Feijoada*	Soaking water
Soluble extracts					
Polyphenols (mg TAE/g)	17.78 ± 0.58a	4.83 ± 0.91b	4.98 ± 0.17b	3.83 ± 0.52bc	3.44 ± .027c
Tannins (mg TAE/g)	11.50 ± 0.75a	2.59 ± 1.14b	1.12 ± 0.87b	1.08 ± 0.46b	1.47 ± 0.93b
Flavonoids (mg CE/g)	2.32 ± 0.20a	0.93 ± 0.09b	1.19 ± 0.08b	0.83 ± 0.03b	0.82 ± 0.05b
Proanthocyanidins (mg LE/g)	13.21 ± 0.58a	1.23 ± 1.19b	1.35 ± 0.14b	nd	0.96 ± 0.30b
Anthocyanins (μg/g)	129.60 ± 10.89a	17.30 ± 4.38b	6.20 ± 2.26b	11.10 ± 0.99b	1.30 ± 0.00b
Hydrolyzable extracts					
Polyphenols (mg TAE/g)	24.82 ± 3.55a	16.55 ± 0.75b	14.09 ± 1.58b	14.85 ± 0.71b	4.76 ± 0.29c
Tannins (mg TAE/g)	15.70 ± 2.45a	11.57 ± 1.53b	5.15 ± 0.33 cd	7.55 ± 1.57c	3.41 ± 0.95d
Flavonoids (mg CE/g)	2.72 ± 0.13a	1.70 ± 0.06b	1.69 ± 0.06b	1.67 ± 0.08b	0.86 ± 0.03c
Proanthocyanidins (mg LE/g)	10.56 ± 1.66a	4.50 ± 1.27b	1.72 ± 0.54b	nd	nd
Anthocyanins (μg/g)	243.00 ± 22.34a	60.50 ± 5.66b	38.30 ± 3.39b	30.40 ± 1.41b	26.30 ± 3.96 b

Values with no letters in common in a row are significantly different ($p<0.05$). Nd — values not detected. Values expressed as grams in a dry weight basis.

hydrolyzable extract those losses were between 30% and 40%. The same can be observed for flavonoids, where losses were slightly lower in hydrolyzable extracts, approximately 37.5%, compared to 48.7% of the soluble extract.

Anthocyanins were markedly reduced in the first step of processing, during black beans soaking and continued during cooking. In the soluble extract, anthocyanins content after soaking reduced 86.6% and after cooking the losses reached 95.2% of the anthocyanin content of the raw black beans. Similar to the other phytochemical groups, higher anthocyanin content was obtained in hydrolyzable extracts. This reduction is expected since anthocyanins are highly water soluble compounds. However, despite the dark purple color observed in the soaking water, it does not seem to contain much of these phenolic compounds. Anthocyanins are known for being reactive compounds, readily reacting with other constituents or simply degrading by action of oxygen, light, enzymes, pH modifications and high temperature processing (McGhie & Walton, 2007). In this way, anthocyanins were shown to be the most sensitive compound studied, with losses occurring during soaking step and further during cooking.

Reduction in phenolic contents in legumes has been cited in literature (Xu & Chang, 2008a). Xu and Chang (2008b) also showed losses over 70% of the original dry bean polyphenol content when boiled at atmospheric pressure, as occurred in this study. Also, the authors showed that some of the phenolic compounds are released in cooking water, as observed by Ranilla, Genovese, and Lajolo (2009) when they submitted Brazillian and Peruvian beans to cooking. In *feijoada* meal black beans are consumed together with its cooking water which suggests being an important characteristic for increasing bioactive compounds intake in the diet.

Bioactive compounds in *feijoada* whole meal ingredients, complementary to black beans are shown in Table 2. Anthocyanins were not observed in any of the ingredients as expected, since this phytochemical is known as a pigment characteristic of certain foods. For all bioactive compounds analyzed orange was the ingredient with the higher values, followed by kale, for both raw and cooked samples.

Fruits and vegetables are known as high in phytochemical contents especially when compared to grains and tubers, such as white rice and cassava, which constitute the *feijoada* meal. However, it is important to point out that for grains, such as rice, and starch rich foods such as cassava; on contrary to fruits and vegetables, most polyphenols are found in the bound form or attached to a component of food matrix, this results in higher values for hydrolyzable extracts than soluble as seen in this study (Adom & Liu, 2002; Saura-Calixto, Serrano, & Goñi, 2007; Serrano, Goñi, & Saura-Calixto, 2007).

Free phenolics were analyzed and the chromatogram for the standards and samples can be visualized in Fig. 2A and B. The reduction in the raw black beans free phenolic acids is also observed in Table 3 where each individual phenolic acid is quantified. Gallic and/cinnamic acids were not observed in any of the samples tested. For raw black beans small amounts of protocatechuic, chlorogenic, caffeic and ferulic acids were identified, similar to a previous report (Xu & Chang, 2009). Interestingly, after processing only caffeic and ferulic acids were observed in both cooked bean and *feijoada* whole meal which is consistent with the work of Xu and Chang (2009) where after boiling amounts of protocatechuic acid was no longer detectable and chlorogenic acid reduced by half in black beans, which can suggest a higher sensitivity to thermal processing by these two phenolic acids.

On contrary to what can be assumed, of thermal procedure reducing phytochemical contents, in cassava and kale samples, ferulic acid values increased after cooking. Ferulic acid is commonly found linked to cell wall as well as other food matrix components from which heat from cooking procedure could result in breakage of its bounds. The release of free ferulic acid has been reported to be related to intrinsic characteristics of the food matrix partially explaining why the increase was only observed for kale and cassava (Adam et al., 2002).

Table 2
Bioactive compounds in raw and cooked *Feijoada* Ingredients.

	Raw rice	Cooked rice	Raw cassava flour	Toasted cassava flour	Raw kale	Cooked kale	Orange
Soluble extracts							
Polyphenols (mg TAE/g)	2.99 ± 0.55	2.28 ± 0.5	2.64 ± 0.55	2.68 ± 0.47	19.77 ± 1.71	22.12 ± 1.96	29.52 ± 4.56
Tannins (mg TAE/g)	nd	nd	nd	nd	11.31 ± 3.38	8.33 ± 1.09	22.25 ± 4.35
Flavonoids (mg CE/g)	3.69 ± 0.37	3.96 ± 0.05	4.89 ± 0.05	6.29 ± 0.23*	15.28 ± 0.28	13.23 ± 0.11*	0,31 ± 0,14
Anthocyanins (μg/g)	nd	nd	nd	nd	nd	nd	nd
Hydrolyzable extracts							
Polyphenols (mg TAE/g)	6.38 ± 0.4	1.83 ± 0.15	2.54 ± 0.31	4.12 ± 0.90	29.55 ± 4.65	22.36 ± 1.42	40.19 ± 0.87
Tannins (mg TAE/g)	nd	nd	nd	nd	16.46 ± 1.42	14.75 ± 1.65	29.25 ± 1.54
Flavonoids (mg CE/g)	4.71 ± 0.09	5.85 ± 0.11*	6.94 ± 0.09	8.36 ± 0.19*	13.71 ± 0.58	15.06 ± 0.09	9.53 ± 0.81
Anthocyanins (μg/g)	nd	nd	nd	nd	nd	nd	nd

Values marked with * are significantly different ($p<0.05$) after cooking. Nd — values not detected. Values expressed as grams in a dry weight basis.

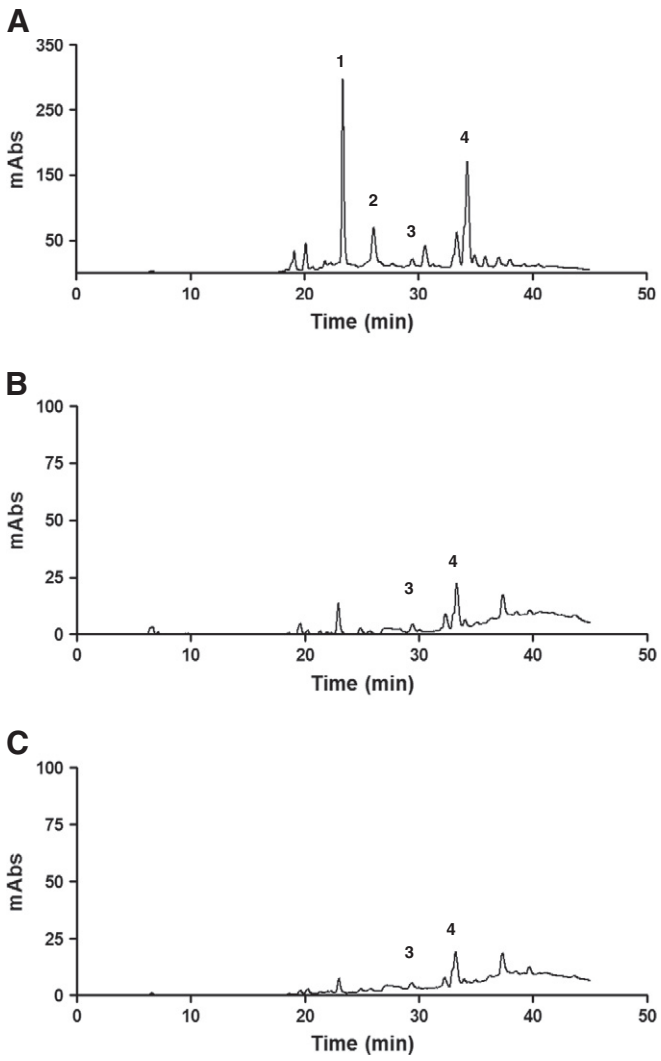

Fig. 2. Phenolic acids in black beans during cooking. (A) raw black beans; (B) cooked black beans; and (C) *feijoada* meal chromatograms. Showing protocatechuic acid (1), chlorogenic acid (2), caffeic acid (3), and ferulic acid (4).

Table 3
Phenolic acids in *Feijoada* ingredients during cooking process and final meal[a].

	Protocatechuic acid	Chlorogenic acid	Caffeic acid	Ferulic acid
Raw bean	2.69	11.04	0.96	6.52
Cooked bean	nd	nd	0.18	2.07
Feijoada	nd	nd	0.18	2.25
Raw rice	nd	nd	nd	5.92
Cooked rice	nd	nd	nd	4.30
Raw cassava flour	nd	nd	0.96	0.15
Toasted cassava flour	nd	nd	nd	5.04
Raw kale	nd	nd	31.57	0.15
Cooked kale	nd	nd	2.64	117.10
Orange	nd	Nd	4.79	3.73

[a] Values expressed as μg/g dry weight; nd — values not detected

Ferulic acid was the only phenolic acid analyzed which was detected in all ingredients of the *feijoada* whole meal and with the exception of black beans, the amount detected was higher in the cooked ingredients compared to the raw ingredients. This can be explained by the fact that ferulic acid is usually present in foods linked by ester linkages to polysaccharides, or ester bonds to lignin in cell wall (Tilay, Bule, Kishenkumar, & Annapure, 2008). These linkages could both be released during cooking and other processes resulting in a higher detection as observed for legumes in general (Amarowicz & Pegg, 2008).

Antioxidant activity of black beans during processing are seen in Table 4, measured by FRAP assay and by beta-carotene/linoleic acid assay. The highest antioxidant activity for the DPPH assay was observed for the raw black beans (Fig. 3A), followed by the cooked, soaked beans, and *feijoada* (Fig. 3B, C and D, respectively). During the entire processing of black beans, the samples before the removal of tannins resulted in higher antioxidant activity in both soluble and hydrolyzable extracts than after the removal when evaluated by the DPPH assay (Fig. 3A–D).

In both methods, FRAP and DPPH, hydrolyzable extracts showed higher antioxidant activity when compared to the soluble extracts, which is in agreement with the higher polyphenols and flavonoids values in those fractions. Higher bioactive compounds and antioxidant activity in hydrolyzable samples are expected especially in the black beans samples. As mentioned before, cereals and legumes are known to have a large proportion of their phenolics found in a bound form, linked to cell wall and other structural components (Adom & Liu, 2002). The extraction of the hydrolyzable fraction uses acidic conditions probably resulting in a more efficient extraction of the bound phenolics.

For black beans, all bioactive compounds analyzed showed high correlation value with antioxidant activity measured by FRAP for the soluble extract, ranging from $R^2 = 0.97$ for tannins to $R^2 = 0.99$ for polyphenols and flavonoids. For hydrolyzable extract, however, the bioactive compounds showed more different results, with the highest value being for proanthocyanins ($R^2 = 0.96$), followed by polyphenols, ($R^2 = 0.91$), flavonois ($R^2 = 0.89$), and tannins ($R^2 = 0.82$).

Reduction in tannins and proanthocyanins, during soaking and cooking (Table 1), can be positive in a nutritional point of view since they are known anti-nutrients, interacting with both minerals and proteins, resulting in a lower absorption of both. However, tannins and proanthocyanins have also been associated with beneficial aspects such as inhibition of cancer cell lines proliferation (Bawadi, Bansode, Trappey, Truax, & Losse, 2005). Regarding the antioxidant activity measured by the beta-carotene oxidation method, there was no clear relation with the processing of black beans probably due to the extraction methodology applied which did not focus on obtaining the lipophilic compounds presents in the sample.

Table 4 also shows the results for the antioxidant activity, by FRAP and beta-carotene/linoleic acid assays for the other ingredients that together with black beans compose *feijoada* whole meal. As observed for the black beans, for all ingredients hydrolyzable extracts showed significantly higher antioxidant activities than soluble extracts when measured by FRAP. Correlation values between bioactive compounds from the other ingredients of feijoada whole meal and FRAP antioxidant activity showed a different behavior than black beans. Flavonoids were the bioactive compounds with the highest correlation, with $R^2 = 0.64$ and $R^2 = 0.93$ for soluble and hydrolyzable extracts, respectively. Polyphenols in this case, showed a lower correlation value, $R^2 = 0.50$ and $R^2 = 0.43$, for soluble and hydrolyzable extract respectively.

No pattern was observed, however, for the beta-carotene/linoleic acid assay where the samples, both soluble and hydrolyzable extracts, and before and after cooking were not significantly different. For black beans, as well as for the other ingredients, no positive correlation was established between the bioactive compounds analyzed and beta-carotene/linoleic acid assay. This was probably due to the extraction methodology using aqueous methanol solution that does not prioritize the extraction of lipophilic compounds.

Previous work from our group showed that cooking does not necessarily affect negatively the antioxidant activity of foods. When five different vegetables were evaluated by DPPH two actually showed

Table 4
Antioxidant capacity of *Feijoada* meal ingredients during cooking process.

	FRAP (μM VEAC/mg)				% Betacarotene Oxidation Inhibition	
	Soluble extracts		Hydrolyzable extracts		Soluble extracts	Hydrolyzable extracts
Black beans	w/ taninns	w/o taninns	w/ taninns	w/o taninns		
Raw	73.19 ± 2.43a	20.54 ± 3.35[a]	190.99 ± 7.91a	19.02 ± 2.20a	38.47 ± 11.18ab	72.28 ± 1.40a
Soaked	16.94 ± 1.23b	3.62 ± 2.40b	96.00 ± 2.96b	9.54 ± 3.86b	9.85 ± 8.17a	64.13 ± 2.38a
Cooked	20.26 ± 1.87b	8.87 ± 3.76b	89.21 ± 1.93bc	16.05 ± 1.95a	56.13 ± 9.51b	18.41 ± 3.77b
Feijoada	13.30 ± 0.63c	7.74 ± 2.80b	83.23 ± 4.14c	15.90 ± 2.18a	48.25 ± 6.29b	63.75 ± 0.66a
Soaking water	13.12 ± 0.76c	2.35 ± 0.90b	40.27 ± 1.67d	3.26 ± 1.40c	60.13 ± 1.64b	82.96 ± 1.32c
Ingredients	Soluble extracts		Hydrolyzable extracts		Soluble extracts	Hydrolyzable extracts
Raw rice	11.82 ± 1.20		18.68 ± 0.59 *		50.20 ± 2.64	53.71 ± 1.43
Cooked rice	16.60 ± 6.61		30.36 ± 7.91 *		52.12 ± 1.47	55.00 ± 0.39
Raw cassava flour	10.71 ± 2.53		30.64 ± 2,53 *		51.56 ± 0.92	41.54 ± 15.80
Toasted Cassava flour	12.76 ± 1.03		28.76 ± 2.90 *		52.66 ± 0.11	44.89 ± 13.07
Raw kale	30.31 ± 3.73		107.52 ± 14.06 *		55.41 ± 1.84	53.71 ± 6.56
Cooked kale	33.03 ± 6.38		108.39 ± 10.12 *		52.89 ± 1.17	43.55 ± 18.01
Orange	18.80 ± 1.47		52.48 ± 0.38 *		62.96 ± 5.10	56.77 ± 6.23

Values with no letters in common in a column are significantly different ($p<0.05$) between black bean cooking stages. Values marked with * are significantly different ($p<0.05$) between soluble and hydrolyzable fractions. VEAC: vitamin C equivalent antioxidant capacity.

an increase in the antioxidant activity when compared to the uncooked match, suggesting that thermal processing could be positive in some cases depending on the food matrix and other intrinsic characteristics (Faller & Fialho, 2009a, 2009b).

Analyzing *feijoada* whole meal regarding the contribution of each ingredient to the intake of bioactive compounds and antioxidant activity the importance of black beans can be observed (Fig. 4). Black beans are the main providers of phenolics, tannins, and antioxidant activity measured by FRAP (Fig. 4A, C, and D, respectively), not only for its high content of phytochemicals but as a result of its large amount in *feijoada* whole meal, approximately 63% in weight. Interestingly, however, for flavonoids (Fig. 4B), despite kale representing only 6.5% in weight of the whole meal it is responsible for providing 34% of the total amount of flavonoids from *feijoada* whole meal. In this way, it is important not only to evaluate the amount of phenolics present in a certain food but also the importance and participation of this specific food in a whole diet context (Faller & Fialho, 2009a, 2009b).

4. Conclusions

Feijoada whole meal is a typical Brazilian meal composed mainly by plant foods, including in a single meal cereal, legume, vegetable and fruits, which makes it a potential source of bioactive compounds

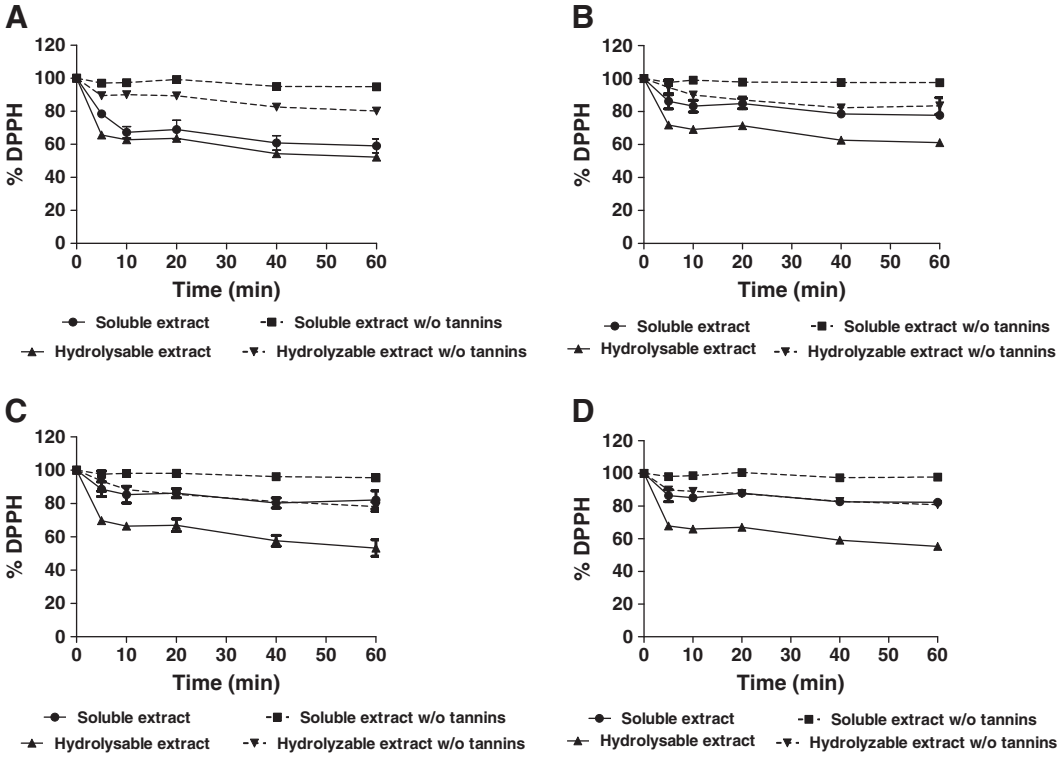

Fig. 3. Antioxidant capacity measured by DPPH of black beans during cooking procedure before and after removal of tannins. (A) Raw black beans; (B) soaked black beans; (C) cooked black beans and (D) *Feijoada* whole meal.

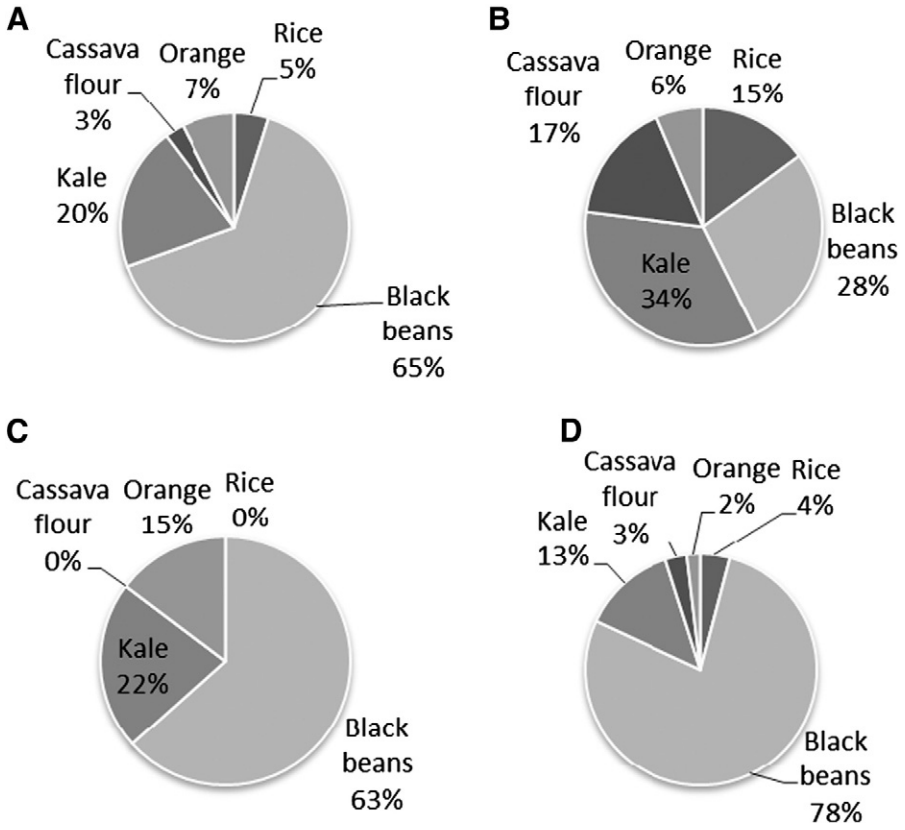

Fig. 4. Contributions of each ingredient to bioactive compound content and antioxidant activity of cooked *feijoada* whole meal. (A) total polyphenols; (B) total flavonoids; (C) tannins; (D) antioxidant capacity (FRAP).

in the diet of the population. It is shown to provide a variety of phytochemicals being the black beans as the main contributor, having kale only as the main provider of flavonoids in the whole meal. Despite the fact that processing of black beans, during soaking and cooking, results in a partial loss of bioactive compounds and antioxidant activity, the same was not as evident for the other ingredients of the meal. The different behaviors of each ingredient resulted in a final product, *feijoada* whole meal, with important levels of phenolics and other compounds with antioxidant activity. Due to this variety of plant foods in *feijoada* whole meal composition, a suggestion of a single serving of approximately 300 g would represent an intake of 1.1 g of polyphenols, as TAE, and around 250 mg of flavonoids, as CE, values similar to the daily estimation intake of polyphenols in some populations (Ovaskaien et al., 2008; Scalbert & Williamson, 2000). The analysis of bioactive compounds in individual food ingredients is important, however, when evaluating a whole meal it results in a better perspective to the context in which the meal is included, identifying the main contributors in a specific dietary pattern and importance of traditional foods to bioactive compounds intake in some populations.

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