Phenolic compounds and antioxidant activity of two bean cultivars 
(*Phaseolus vulgaris* L.) submitted to cooking

Compostos fenólicos e atividade antioxidante de duas cultivares de feijões 
(*Phaseolus vulgaris* L.) submetidos à cocção

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1 Introduction

Besides its excellent nutritional composition, the common bean contains bioactive compounds that provide antioxidant properties (HELBIG et al., 2003; PERRON; BRUMAGHIM, 2009). These substances are known as phenolic compounds and are mostly found in the tegument of colored beans (XU et al., 2007).

Phenolic compounds originate in the secondary metabolism of plants as responses to ecological and physiological pressures.
They are linked to the pigmentation of vegetables and are capable of attributing anti-pathogenic action, while also promoting growth and development (KHODDAMI et al., 2013). These compounds are composed of at least one aromatic ring, together with simple or polymer hydroxyls, which provide them with their antioxidant capabilities (SADEGHI et al., 2015). Several thousand phenols have been described in the literature and the main groups consist of simple phenols, benzoic and cinnamic acids, coumarins, tannins, lignins, lignans and flavonoids (KHODDAMI et al., 2013).

Despite the benefits promoted by phenolic compounds (e.g. reduction in lipid oxidation, prevention of atherosclerosis, arterial hypertension and cancer), it is important to evaluate their stability after processing, because factors such as temperature and time can affect their concentration (ZARGHAM; ZARGHAM, 2008; HUANG et al., 2013; MILEO; MICCADEI, 2016). In relation to the effect of processing on the phenolic profile of beans, Telles et al. (2017) affirmed that raw beans (Phaseolus vulgaris) can present significant values of chlorogenic, gallic and protoocatechuic acid. On the other hand, Huber et al. (2014) identified higher concentrations of kaempferol, catechin, vanillic, gallic and sinapic acid in beans after cooking.

Besides the processing, some anti-nutritional factors present in legumes, such as condensed tannins and phytic acid, can also interfere in the nutritional value and antioxidant capacity of the phenolic compounds, through the formation of insoluble complexes with minerals, proteins and amides (HELBIG et al., 2003; PERRON; BRUMAGHIM, 2009). In order to increase the nutritional value of beans, biofortification has been used to produce beans with higher amounts of minerals, mainly iron and zinc (e.g. Pontal bean), in comparison with conventional cultivars (VAZ-TOSTES et al., 2016).

Bean is a staple food in Brazil, and there is a need for studies that evaluate the behaviour of the antioxidant activity of different cultivars of beans in relation to the processing conditions commonly applied (cooking). Therefore, the aim of the present study was to determine the amount of phenolic compounds and tannins in a biofortified cultivar and in a commercial cultivar of the common bean (Phaseolus vulgaris L.), using both raw and cooked samples, as well as to determine the antioxidant activity of their extracts and phenolic fractions.

## 2 Material and methods

### 2.1 Bean cultivars and processing

Two bean cultivars (Phaseolus vulgaris L.) were used in this experiment: the Pontal bean (carioca beans biofortified with iron and zinc), which was kindly donated by EMBRAPA- National Center of Research on Rice and Beans; and the commercial common bean (carioca beans acquired in a local market in Piracicaba, São Paulo, Brazil).

Depending on the preparation technique, the beans (Phaseolus vulgaris L.) were divided into the following treatment protocols: Processing 1: raw Pontal bean; Processing 2: raw commercial bean; Processing 3: cooked Pontal bean; Processing 4: cooked commercial bean.

The raw beans were ground in a knife mill (Marconi, Piracicaba, Brazil) and sifted through a 30-mesh sieve in order to obtain a flour, which was stored in polyethylene bags, properly closed and maintained under refrigeration (4 °C).

The cooked beans were first soaked in deionized water, in a 1:3 proportion (w/v) for 12 hours. Subsequently, the soaking water was discarded and a new aliquot of water was added in the proportion of 1:2 (w/v). The beans were cooked in an autoclave at 121 °C for 10 minutes and then frozen (-20 °C) and freeze-dried (E-C Modulyo Apparatus Inc, New York, USA) for 48 h. The dried beans were ground, sifted through a 30-mesh sieve and stored in polyethylene bags at 4 °C.

### 2.2 Total phenolic compounds

The total phenolic compounds content was determined according to Deshpande and Cheryan (1987) using the Folin Ciocalteu reagent, anhydrous sodium carbonate and catechin. The same methodology was used by Cardador-Martinez et al. (2002) in earlier studies of beans. The polyphenol fractions were separated by vacuum liquid chromatography in silica gel, as described by Aparicio-Fernandez et al. (2005).

### 2.3 Extraction

The extracts were obtained according to Cardador-Martinez et al. (2002). Thus, 10 g of ground, freeze-dried beans from each processing were placed in a conical flask (500 mL) with 100 mL of methanol and shaken for 24 hours at 70 rpm (25 °C). Subsequently, the samples were centrifuged (Novatecnica, NT825, Piracicaba, Brazil) for 10 minutes at 2500 rpm. The supernatant was collected in round-bottom flasks and heated in a rotary evaporator at 35 °C under vacuum to evaporate the methanol. The extracts obtained were frozen (-20 °C) and freeze-dried.

### 2.4 Separation

Six fractions were obtained from the freeze-dried extracts as described by Aparicio-Fernandez et al. (2005): 0.5 g of the freeze-dried extract was diluted in methanol and transferred to a vacuum liquid column with silica gel, which was submitted to different reagents (methanol,
Phenolic compounds and antioxidant activity of two bean cultivars (*Phaseolus vulgaris* L.) submitted to cooking

Silva, M. O. et al.

Petroleum, ether and ethyl acetate) in different proportions, to obtain the fractions.

After extraction, the fractions were placed in round-bottom flasks and the reagents evaporated in a rotary evaporator at 35 °C under vacuum. The extracts obtained were frozen, freeze-dried and used to determine the antioxidant capacity by ABTS and DPPH methods.

### 2.5 Tannins

The tannins were determined as described by Price et al. (1980), based on extraction with methanol and a colorimetric reaction with 1% vanillin solution in methanol and 8% HCl in methanol (1:1 v/v) at 30 °C for 20 minutes. The tannin content was determined at 500 nm using a spectrophotometer (Shimadzu, UV-vis, model UV-1800; Tokyo, Japan). The results were expressed as mg Eq g⁻¹ of sample.

### 2.6 Antioxidant activity

#### 2.6.1 DPPH

The antioxidant capacity of the extracts and fractions from the different beans was determined according to Brand-Williams et al. (1995), using 1,1-diphenyl-2-picrylhydrazyl (DPPH). The method is based on the reduction of the stable radical DPPH• through the action of the antioxidants present in the food matrix.

The antioxidant activity of the extracts was determined in 0.002 mg of the raw beans and in 0.006 mg of the cooked beans. For the fractions, the antioxidant activity was measured in 0.002 mg of sample. The samples were diluted in 2 mL of ethanol and 500 µL of this solution distributed in tubes. Subsequently, 3 mL of ethanol and 300 µL of DPPH were added to the tubes. After 45 minutes of storage in the dark, the antioxidant activity was determined at 517 nm in a spectrophotometer (Shimadzu, UV-vis, model UV-1800; Tokyo, Japan). The results were expressed as Trolox equivalent antioxidant capacity (TEAC) g⁻¹ of sample.

#### 2.6.2 ABTS

Ethanol was used in the preparation of the Trolox solutions, the polyphenol extracts and their fractions. Aliquots of 20 µL of each solution, composed of 0.01 g (extract) and 0.014 g (fraction) were diluted in 2 mL of ethanol and added to 2 mL of the ABTS solution. The absorbance was determined at 734 nm and was recorded every minute for a total of six minutes. The results were compared with the initial absorbance, which was determined using 20 µL of ethanol and 2 mL of the ABTS solution. The reduction in absorbance after six minutes was calculated. The results were expressed as Trolox equivalent antioxidant capacity (TEAC) g⁻¹ of sample (ARTS et al., 2004).

### 2.7 Data analysis

The analyses were carried out in triplicate. A completely randomized statistical design was used, with the results expressed as the mean ± standard deviation. The analysis of variance (F test) and the comparison of means (Tukey’s test, p < 0.05) were applied using the Software Statistical Analysis System- SAS (version 9.2).

### 3 Results and discussion

#### 3.1 Phenolic compounds and tannins

Regarding to the phenolic compounds content (Table 1), statistical differences were observed between the cultivars in both processing forms (raw and cooked beans), and the highest value was reported for the raw Pontal bean (3.44 mg g⁻¹). After cooking, the Pontal bean still presented higher phenolic compounds values than the commercial bean. It was noted that cooking reduced phenolic compounds by 64 and 69%, respectively in the Pontal and commercial cultivars. Silva et al. (2009) studied the Pontal bean and found 4.51 mg g⁻¹ of phenolic compounds in raw beans and 0.49 mg g⁻¹ in cooked beans. On the other hand, Delfino and Canniatti-Brazaca (2008) studied the common bean and reported 0.24 mg g⁻¹ of phenolic compounds in raw beans and 0.04 mg g⁻¹ of phenolic compounds in cooked commercial beans, lower values than those of the present study. According to Granito et al. (2007), processing such as cooking may promote degradation amongst the aromatic rings of the phenolic compounds, leading to polymerization reactions or structural breaks, which are reflected in the lower phenolic content of the cooked beans. Ranilla et al. (2009) affirmed that other factors such as the soaking and draining stage can reduce the total phenolic content and antioxidant capacity of the beans. In treatments without soaking and where the cooking water was not discarded,

<table>
<thead>
<tr>
<th>Phenolic compounds (mg g⁻¹)</th>
<th>Tannins (mg Eq g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Raw</strong></td>
<td><strong>Cooked</strong></td>
</tr>
<tr>
<td>Pontal</td>
<td>3.44 ± 0.57b(A)</td>
</tr>
<tr>
<td>Commercial</td>
<td>1.88 ± 0.16a(A)</td>
</tr>
</tbody>
</table>

*Data represent mean ± standard deviation (n = 3). Different lowercase letters in the same column indicate a statistical difference (p < 0.05) between the cultivars. Different uppercase letters in the same row for the same analysis indicate a statistical difference (p < 0.05) between the sample processing.*

Table 1. Phenolic compound and tannin contents of raw and cooked beans (*Phaseolus vulgaris* L.) (dry weight basis).

Braz. J. Food Technol., Campinas, v. 21, e2016072, 2018
specific phenolic compounds such as flavonols and phenolic acids could be detected.

For tannin content, higher values were also reported for the raw Pontal bean (2.15 mg Eq g\(^{-1}\)). Cooking significantly reduced the tannin content in beans (99% for Pontal and 96% for commercial). Tannins are the most studied phenolic compounds in beans and their reduction was desirable since they are primarily considered to be an antinutritional factor. Depending on their contents, tannins can affect the nutritional value of this legume (FERNANDES et al., 2010). Petry et al. (2010) affirmed that high levels of polyphenols (e.g. tannins) inhibit iron absorption which can contribute to iron deficiency in countries such as Brazil, where beans are a staple food. Toledo et al. (2013) observed that polyphenols can interact with protein and interfere with digestibility of beans, decreasing the hydrolysis of phaseolin.

Soaking and cooking are processing that can influence the amount of tannins. According to Ramirez-Gárdenas et al. (2008), cooking can promote a significant decrease in tannins of bean, and when cooking is prepared without using the soaking water, this reduction is even more expressive than when the beans are cooked with the soaking water or are not soaked. Mariotto-Cezar et al. (2013) studied the domestic processing associated with the common bean and they also reported that soaking, followed by cooking, is the most recommended treatment for the reduction of tannins in legumes. After reviewing different studies, Fernandes et al. (2010) concluded that soaking and discarding of the soaking water was the most effective procedure to reduce the tannins.

The reduction caused by soaking and cooking is not the result of the chemical destruction of tannins, but is due to modifications in the solubility and reactivity characteristics of other molecules. Proteins, oligosaccharides and lipids are responsible for the formation of insoluble complexes, which inhibit the action of digestive enzymes in the body, thereby reducing the bioavailability of nutrients, and making it difficult to determine the tannins using analytical methods (HELBIG et al., 2003). Furthermore, during the soaking and cooking processing, tannins may migrate from the tegument and cotyledons of the beans into the soaking water or the cooking water (MKANDA et al., 2007), which are often discarded before the cooking and consumption of the beans.

Despite their effect on the nutritional value of food products, tannins are phenolic compounds that are also engaged in antioxidant activity and can contribute to human health, including the maintenance of the intestinal microbiota (CARDONA et al., 2013) and the prevention of degenerative diseases, such as cardiovascular disease (including hypertension), cancer, diabetes, Alzheimer disease, cataracts, and age-related functional decline (ZARGHAM; ZARGHAM, 2008; HUANG et al., 2013).

Nevertheless, soaking and discarding of the soaking water is still the most recommended procedure since soaking does not completely eliminate the tannins from the beans; therefore, the antioxidant activity attributed to this compound is partially preserved (FERNANDES et al., 2010). However, it is noted that there are few studies that evaluate the interactions of tannins with other nutrients in the human organism. Researches should be carried out to investigate both the positive and negative effects of ingesting these compounds on human health, and their influence on the bioavailability of other nutrients.

### 3.2 Antioxidant activity

#### 3.2.1 Crude extract

No statistical difference was observed between the raw beans for antioxidant activity using the DPPH method (Table 2). However, after cooking, a reduction in the antioxidant activity of both cultivars was observed, and the commercial bean exhibited lower antioxidant capacity. Strong positive correlations were observed between DPPH and phenolic compounds \((r = 0.8703)\) and tannins \((r = 0.9995)\) for cooked Pontal beans (Table 3). Xu and Chang (2008) correlated the reduction in the antioxidant activity of cooked beans to the dissolution of soluble antioxidants in the soaking water, when it is not used during cooking, as well as the effect of temperature. Silva et al. (2009) affirmed that the decrease in the scavenging of free radicals (DPPH•) after cooking occurs due to partial alterations in the structure of the phenolic compounds.

On the other hand, Huber et al. (2014) observed an increase in the potential antioxidant activity of the common bean \((Phaseolus vulgaris L.)\) after cooking, for treatments

<table>
<thead>
<tr>
<th></th>
<th>DPPH (µM TEAC g(^{-1}))</th>
<th>ABTS (mM TEAC g(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw</td>
<td>Cooked</td>
</tr>
<tr>
<td><strong>Pontal</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw</td>
<td>33.62 ± 0.64(^{ab})</td>
<td>26.83 ± 0.14(^{ab})</td>
</tr>
<tr>
<td>Cooked</td>
<td>33.38 ± 0.42(^{ab})</td>
<td>29.25 ± 0.18(^{ab})</td>
</tr>
<tr>
<td><strong>Commercial</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^{\text{a}}\)Data represent mean ± standard deviation \((n = 3)\). \(^{\text{b}}\)Different lowercase letters in the same column indicate statistical difference \((p < 0.05)\) between the cultivars. \(^{\text{c}}\)Different uppercase letters in the same row for the same analysis indicate statistical difference \((p < 0.05)\) between the sample processing.
with and without soaking. The authors stated that the concentration of phenolic compounds in the cooking water may facilitate the extraction of the phenolic compounds, when compared with raw beans.

The DPPH values found by Silva et al. (2009) for both raw and cooked beans were 22.57 and 12.18 µM TEAC g⁻¹, respectively. Xu et al. (2007) characterized the phenolic compounds and antioxidant activities of U.S. produced cool season legumes and found DPPH values in the common bean ranging from 1.48 in navy beans to 18.95 µM TEAC g⁻¹ for black turtle beans. The values reported by both studies were lower than those of the present research (Table 2).

Concerning to the determination of the antioxidant activity using the ABTS method (Table 4), the highest antioxidant activity was found in the commercial bean (18.04 mM TEAC g⁻¹). In general, the ABTS method showed stronger correlations with phenolic compounds and tannins than the DPPH method. According to Floegel et al. (2011), the difference in antioxidant activity between the DPPH and ABTS methods is due to the type of phenolic compounds determined. The spectrum of DPPH exhibits a maximal absorbance peak at 515 nm, which is only capable of measuring the antioxidant capacity of lipophilic compounds. Meanwhile, the ABTS spectrum exhibits maximal absorbance at a range of wavelengths (414, 654, 754 and 815 nm), and is capable of detecting the antioxidant activity of a greater quantity of phenolic compounds (both lipophilic and hydrophilic), which reflect in the different levels of antioxidant activity determined by these two methods for each cultivar.

Amongst the raw beans, the commercial bean presented higher antioxidant activity than the Pontal bean for the ABTS method. However, the values for antioxidant activity determined by ATBS decreased drastically after cooking and no significant differences were observed between the cultivars. Silva et al. (2009) also reported that thermal processing could influence the ABTS results for some cultivars of Phaseolus vulgaris L.

Few studies have provided data related to the antioxidant activity of beans based on the use of the ABTS method. When it is used, they only provide results in the form of the percentage of inhibition, which hinders comparison of the results found in the present study.

### Table 3. Correlation between DPPH, ABTS, tannins and phenolic compounds in biofortified and common beans.

<table>
<thead>
<tr>
<th></th>
<th>Phenolics</th>
<th>Tannins</th>
<th>Phenolics</th>
<th>Tannins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw</td>
<td>Cooked</td>
<td>Raw</td>
<td>Cooked</td>
</tr>
<tr>
<td><strong>DPPH</strong></td>
<td>0.8526</td>
<td>0.8107</td>
<td>0.8703</td>
<td>0.9995</td>
</tr>
<tr>
<td><strong>ABTS</strong></td>
<td>0.9996</td>
<td>0.9948</td>
<td>0.6148</td>
<td>0.9339</td>
</tr>
</tbody>
</table>

3.2.2 Fractions

According to Aparicio-Fernandez et al. (2005) the separation of polyphenols into their fractions using vacuum liquid chromatography in silica gel enables a simplified and faster separation of the phenolic compounds. It also facilitates the analysis of the antioxidant capacity of each fraction according to the predominant type of phenolic compound.

Due to the low yield of fractions F1 and F5, it was not possible to determine their antioxidant activity. The analyses of fractions 2, 3, 4 and 6 of the raw and cooked Pontal and commercial beans showed that the phenolic compounds present in fraction 3 of the Pontal beans exhibited the greatest capacity to scavenge the DPPH radical (Table 4). Aparicio-Fernandez et al. (2005) stated that fraction 3 is the one that most closely represents the types of phenolic compounds found in beans, and it is composed of proanthocyanidins (64% catechins and epicatechins), anthocyanins (13%) and flavonoids (17%). Regarding to the raw commercial bean, the greatest antioxidant activity was found in fraction 2, which is mainly composed of proanthocyanidins (85%).

The thermal processing reduced the antioxidant activity of most of the fractions, with the exception of fraction 6 of Pontal bean and fraction 3 of commercial bean. Xu and Chang (2008) attributed this reduction in activity of certain fractions to possible chemical modifications, damaged phenolic compounds and the formation of complexes between polyphenols and proteins.

Huber et al. (2014) analysed white common beans (Phaseolus vulgaris L.) and recorded the greatest antioxidant activity for fraction 6, when using raw beans and the DPPH method. For cooked and soaked beans, no differences were found between the fractions, except for fraction 3, which presented the lowest antioxidant activity amongst the samples. As in the present study, Huber et al. (2014) reported a large variation between the results obtained for antioxidant activity, depending on the composition of the fraction analysed.

In relation to ABTS method, fraction 2 exhibited the greatest antioxidant activity in both raw and cooked beans (Table 5). Cooking had no effect on fractions 2 and 4 of...
Phenolic compounds and antioxidant activity of two bean cultivars (*Phaseolus vulgaris* L.) submitted to cooking

Silva, M. O. et al.

In general, for both methods of antioxidant activity, cooking affected the capacity of compounds to scavenge the DPPH and the ABTS radicals in most fractions. According to Helbig et al. (2003), cooking promotes the formation of insoluble complexes, which are formed by bonding between phenolic compounds and proteins and removed in the water during cooking, and are either free or polymerized. These different forms of behaviour could explain the oscillations in the antioxidant activity of beans in relation to the methods used (Tables 4 and 5).

**4 Conclusions**

The thermal processing has a significant effect on phenolic compounds, tannins and the antioxidant activity, both for commercial and biofortified beans.

It was noted that the reduction in the antioxidant activity was associated with the reduction in tannins and total phenolic compounds in beans. The raw biofortified bean (Pontal) was the cultivar that exhibited higher levels of phenolic compounds and tannins in its composition.

For cooked beans, no significant difference was observed between the cultivars by ABTS. However, higher antioxidant activity was found for commercial beans by DPPH.

Regarding to the fractions, in general, there was a decrease in the antioxidant activity by DPPH, except for fraction 6 of the Pontal beans and fraction 3 of the commercial beans. No significant differences were observed in fraction 4 after cooking for both cultivars.

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Phenolic compounds and antioxidant activity of two bean cultivars (*Phaseolus vulgaris* L.) submitted to cooking
Silva, M. O. et al.


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Silva, M. O. et al.


