



Revista Ciência Agronômica

ISSN: 0045-6888

ccarev@ufc.br

Universidade Federal do Ceará
Brasil

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Revista Ciência Agronômica, vol. 48, núm. 5, diciembre, 2017, pp. 824-831
Universidade Federal do Ceará
Ceará, Brasil

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Effect of cooking on the bioactive compounds and antioxidant activity in grains cowpea cultivars¹

Efeito da cocção no conteúdo de compostos bioativos e atividade antioxidante nos grãos de cultivares de feijão-caupi

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ABSTRACT - The present study evaluated the effect of cooking on the levels of bioactives compounds and antioxidant activity in grains cowpea cultivars. The analysis were performed on the raw samples and after cooking in pressure cooker. Regarding the bioactive compounds present, the grain cultivar BRS Aracê exhibited the highest levels of total phenolic compounds (mg/100 g) both before and after cooking, 205.10 ± 2.89 and 150.62 ± 2.64 , respectively. Spermine and spermidine were identified in the cultivars (mg/kg) BRS Milênio in the amount of 120.5 in crude and 50.4 in cooked grain, in the BRS Tumucumaque in the amount of 116.2 in crude and 47.9 in cooked grain, exhibited significant losses of these compounds after cooking. It was not detected the presence of anthocyanins and flavonoids in the grain cultivars. For the antioxidant activity were observed different behaviors for each grain cultivar in the two methods evaluated. The grain cultivar BRS Aracê presented the highest antioxidant activity before cooking according to both methods tested ($\mu\text{mol TEAC}/100 \text{ g}$), 614.7 ± 5.43 (DPPH) and 660.1 ± 7.98 (ABTS). The grain cultivar BRS Xiquexique 419.8 ± 6.80 exhibited the highest antioxidant activity and the grain cultivar BRS Milênio 552.1 ± 4.78 after cooking. A strong correlation between the antioxidant activity and phenolic content and total flavonoid was found. It is concluded that the grains cultivars maintained important nutritional and functional characteristics following processing, recommending that the cowpea consumption with broth cooking for retaining compounds with antioxidant properties.

Key words: *Vigna unguiculata*. Functional food. Antioxidants. Processing.

RESUMO - O presente estudo visou avaliar a influência do cozimento no conteúdo de compostos bioativos e atividade antioxidante nos grãos de cultivares de feijão-caupi. As análises foram realizadas em cultivares cruas e após o cozimento em panela de pressão doméstica. Para os compostos bioativos, o grão da cultivar BRS Aracê apresentou os maiores conteúdos de compostos fenólicos totais antes ($205,10 \text{ mg}/100 \text{ g} \pm 2,89$) e após ($150,62 \text{ mg}/100 \text{ g} \pm 2,64$) o cozimento ($p < 0,05$). Foram identificadas as poliaminas espermina e espermidina nas cultivares (mg/kg), destacando-se a BRS Milênio no grão cru com 120,5 e no grão cozido de 50,4, e BRS Tumucumaque no grão cru de 116,2 e no grão cozido de 47,9, com perdas significativas ($p < 0,05$) após o cozimento. Não foi detectada a presença de antocianinas e flavonóis nos grãos das cultivares. Para a atividade antioxidante, observaram-se comportamentos diferenciados para cada grão das cultivares nos dois métodos avaliados. Antes do cozimento, o grão da cultivar BRS Aracê apresentou maior atividade antioxidante pelos dois métodos avaliados ($\mu\text{mol TEAC}/100 \text{ g}$) $614,7 \pm 5,43$ (DPPH) e $660,1 \pm 7,98$ (ABTS). O grão da cultivar BRS Xiquexique exibiu elevada atividade antioxidante de $419,8 \pm 6,80$ e o grão da cultivar BRS Milênio de $552,1 \pm 4,78$, após o cozimento. Foi constatada forte correlação entre a atividade antioxidante e o teor de fenólicos e flavonóides totais. Concluiu-se que após o processamento, os grãos das cultivares mantiveram características nutritivas e funcionais relevantes, recomendando-se o consumo do feijão-caupi com o caldo de cocção para retenção de compostos com propriedades antioxidantes.

Palavras-chave: *Vigna unguiculata*. Alimento funcional. Antioxidantes. Processamento.

DOI: 10.5935/1806-6690.20170097

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Recebido para publicação 14/08/2016; aprovado em 16/11/2016

¹Parte da Dissertação de Mestrado da primeira autora apresentada ao Programa de Pós-Graduação em Alimentos e Nutrição da Universidade Federal do Piauí, Campus Ministro Petrônio Portela, em Teresina-PI

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INTRODUCTION

Cowpea (*Vigna unguiculata* [L.] Walp.) is one of the most important legumes produced in tropical and subtropical regions worldwide, especially in the developing countries of Africa, Latin America and Asia. This species provides the main source of proteins, calories, dietary fiber, minerals and vitamins for a large segment of the world's population (PHILLIPS *et al.*, 2003).

This legume is also known as crowder-pea, southern pea or black-eyed pea. Because of its hardness, it is well known for its adaptability to water, heat and salt stress and is widely grown by small- and medium-scale producers in the Brazilian Northeast and Northern regions, where it represents is a key source of income and employment (FREIRE FILHO *et al.*, 2005).

Among legumes, the common bean is characterized as a food with good nutritional value and high levels of bioactive compounds with significant antioxidant activity, including flavonoids, anthocyanins, proanthocyanidins and isoflavones, and some phenolic acids (SILVA *et al.*, 2009).

Cooking of this legume leads to loss of cellular structure integrity, with migration of components occurring through leaching, resulting in a reduction of its phytochemical constituents. Furthermore, heat treatment can promote thermal degradation, and nutrient loss may occur via the action of enzymatic or non-enzymatic factors, including light and oxygen (VOLDEN *et al.*, 2009). The effects vary depending on the cultivar and treatment, as studies have shown that cooking significantly reduces the levels of phenolic compounds and antioxidant activity (assessed by *in vitro* assays) (XU; CHANG, 2011).

In light of the above considerations, together with the importance of cowpea in Brazilian eating habits (especially in the Northeast), its nutritional and functional characteristics (particularly regarding bioactive compounds), the scarcity of data on the levels of these compounds in grain cowpea grown in Brazil and the effect of cooking on new grains cultivars, the present study aimed to evaluate the effect of cooking on the levels of bioactive compounds in grains cowpea cultivars.

MATERIALS AND METHODS

Samples

Grains cowpea cultivars from two different lots were provided by the Department of Genetic Resources and Breeding of the Brazilian Agricultural Research Corporation, Mid-North (Embrapa Meio-Norte), Teresina

- Piauí (PI), Brazil, and maintained in the Laboratory of Bromatology and Food Biochemistry, Department of Nutrition/Center for Health Sciences/Federal University of Piauí (Universidade Federal do Piauí - UFPI) at 8 °C in polyethylene bags until analysis.

Four grains cowpea cultivars were analyzed before and after cooking: BRS Aracê, BRS Tumucumaque, BRS Milênio and BRS Xiquexique. The grains raw samples were analyzed within a one-week interval after they were received, and the cooking step was conducted after completing the raw bean analyses. The raw grain cowpea was ground in a rotor mill cyclone type (Tecnal, Model TE-651/2, Piracicaba-SP, Brazil) until a homogenous powder was obtained (0.5 mesh). The cowpeas were cooked at a bean:water ratio of 1:3 (w/v) in a 2 L domestic pressure cooker, for 13 minutes, over medium heat, after constant steam output through the pressure valve. The cooking broths resulting from boiling the four cultivars were stored in plastic containers (50 ml) at a temperature of 8 °C for subsequent analyses.

Analysis of bioactive compounds

The extracts of grain cowpea samples were initially prepared according to the method described by Rufino *et al.* (2010), using the solvents 50% methanol (50:50, v/v) e 70% acetone (70:30, v/v) and Milli-Q water.

The levels of phenolic compounds in the extracts were determined spectrophotometrically using the Folin-Ciocalteu reagent and absorbance readings of the samples were conducted at 765 nm in a spectrophotometer (BEL, Model 1102, Monza, Milan, Italy). The results are expressed as grams of gallic acid equivalents (GAE) per 100 g of sample. The concentration of total phenolic compounds was assessed through interpolation of the absorbance using a previously constructed gallic acid standard curve (SINGLETON; ROSSI, 1965).

The method described by Blasa *et al.* (2006) was used to assess total flavonoids and the absorbance was then measured at 425 nm in a spectrophotometer (BEL, Model 1102, Monza, Milan, Italy). Different concentrations of quercetin (0-100 mg/L) were used to construct a standard curve, and the results are expressed as milligrams of quercetin equivalents (mg QE)/100 g sample.

Analysis of the levels of total anthocyanins (TA) was performed following the pH-difference method (GIUSTI; WROLSTAD, 2001). The absorbance was measured in a spectrophotometer (BEL, Model 1102, Monza, Milan, Italy) at the peak wavelength of each sample and at 700 nm, in buffer solutions at pH 1.0 and pH 4.5, and using distilled water as a blank. The results are

expressed as cyanidin-3-glucoside (cy-glu-3) equivalents per 100 grams of dry sample.

The levels of total flavanols were assessed colorimetrically using the vanillin method (PRICE *et al.*, 1978), and absorbance readings were performed in a spectrophotometer (BEL, Model 1102, Monza, Milan, Italy) at 500 nm. Catechin was used as a standard, and the results are expressed as milligrams of catechin equivalents/100 g of sample.

Ten bioactive amines were assessed, including spermidine, spermine, putrescine, cadaverine, serotonin, histamine, tyramine, tryptamine, phenylethylamine and agmatine. The method employed to separate, detect and quantify the amines was high-performance liquid chromatography (HPLC), with an ion-pair reversed-phase column, according to Adão and Glória (2005).

Antioxidant activity

Antioxidant activity was assessed using the DPPH free radical scavenging method, developed by Brand-Williams *et al.* (1995) the absorbance was then measured at 515 nm in a spectrophotometer (BEL, Model 1102, Monza, Milan, Italy). A standard curve was constructed using Trolox at different concentrations (0-100 mg/L) as a reference. The results are expressed as μmol Trolox equivalent antioxidant capacity (TEAC) per 100 g of sample.

The assay with the ABTS• radical was conducted according to Re *et al.* (1999). The absorbance was measured in a spectrophotometer (BEL, Model 1102, Monza, Milan, Italy) at 734 nm. The results are expressed in μmol TEAC per 100 g sample.

Statistical analysis

A database was created using the Statistical Package for the Social Sciences (SPSS), version 17.0. Analysis of variance (ANOVA) was performed, and

means were compared using Student's *t*-test (two means) to assess the significance of the differences between the two means and Tukey's test (three or more means) to assess the existence of significant differences between the means of three or more grain cultivars. Pearson's correlation coefficient was applied. The significance level adopted was $p < 0.05$ for all tests. All analyzes were performed in triplicate (ANDRADE, 2010).

RESULTS AND DISCUSSION

Bioactive compounds

Regarding bioactive compounds, Table 1 presents the levels of total phenolic compounds assessed in the grain, before and after cooking, and in the respective cooking broths.

The concentrations of phenolic compounds in raw beans were higher than in cooked beans in all of the cultivars evaluated. Thus, cooking significantly reduced ($p < 0.05$) the levels of this type of bioactive compound (Table 1).

For the raw beans, a significant difference between the four studied cultivars was observed, with the BRS Aracê cultivar exhibiting the highest levels of phenolic compounds (205.10 mg/100 g), followed by the BRS Xiquexique cultivar (199.05 mg/100 g). The BRS Milênio cultivar presented the lowest concentration of these compounds (132.83 mg/100 g; Table 1).

Marathe *et al.* (2011) analyzed grain legumes including the common bean, cowpea, chickpea, soybean and pea, among others, and classified them into three different groups according to the levels of phenolic compounds observed. Based on this previous study, the raw grain cowpea cultivars assessed in the present study may be classified as showing moderate levels of phenolic compounds (> 100 and < 200 mg GAE/100 g), except

Table 1 - Total phenolic compounds in raw and cowpea cooked grains and in the cooking broths

Cultivars	Processing (mg GAE*/100 g)		Cooking broth (mg GAE*/100 g)
	Raw	Cooked	
	Mean \pm SD	Mean \pm SD	Mean \pm SD
BRS Milênio	132.83 \pm 3.12 aA	96.97 \pm 0.13 bA	51.32 \pm 0.67 cA
BRS Aracê	205.10 \pm 2.89 aB	150.62 \pm 2.64 bB	35.95 \pm 0.34 cB
BRS Tumucumaque	177.07 \pm 0.78 aC	126.58 \pm 1.98 bC	30.96 \pm 0.51 cC
BRS Xiquexique	199.05 \pm 1.98 aD	144.38 \pm 1.78 bD	42.40 \pm 0.12 cD

*Gallic acid equivalents (GAE). Mean of three replicates \pm standard deviation (SD); Lowercase letters compare the type of processing applied and uppercase letters compare between cultivars within each processing. Means followed by different lowercase letters (in columns) and different uppercase letters (in lines) are statistically different ($p < 0.05$)

for the cultivar BRS Aracê, which exhibited a high level of these compounds (> 200 mg GAE/100 g). The BRS Milênio grain cultivar presented the lowest level of phenolic compounds (< 100 mg GAE/100 g) among the cooked cultivars.

All of the cooked grain cultivars differed significantly ($p < 0.05$) regarding the levels of phenolic compounds, with the highest concentration being observed in the cultivar BRS Aracê, 150.62 mg/100 g, followed by the cultivar BRS Xiquexique, with 144.38 mg/100 g. The presence of phenolic compounds was detected in all of the cooking broths, with the highest levels being observed in the cooking broth of the cultivar BRS Milênio (51.32 mg/100 g; Table 1).

As shown in Table 1, the present study recorded higher levels of phenolic compounds than were found by Adebooye and Singh (2007), who evaluated the effect of cooking on the levels of phenolic compounds in two varieties of cowpea and recorded values ranging from 40 to 50 mg GAE/100 g in cooked grains. Giami (2005) analyzed four lines of cowpea and recorded levels ranging from 99 to 196 mg/100 g in the raw lines and from 52 to 78 mg/100 g in the cooked lines.

Results similar to those recorded in the present study, i.e., significant decreases in the levels of phenolic compounds, were observed in studies conducted by Adebooye and Singh (2007), Giami (2005) and Kalpanadevi and Mohan (2013), who evaluated the effect of cooking on the levels of such compounds in different cowpea cultivars.

Several factors may affect the levels of phenolic compounds in legumes, including genetic and environmental factors and factors inherent to the conditions applied to extract these compounds from the food matrix, including the type of solvent used. Such factors may explain the differences observed in the levels of these compounds in the present work compared to other studies.

Although the levels of phenolic compounds decreased after cooking, they remained significant, considering the sum of the levels recorded in the cooked grains cultivars and their respective cooking broths. This finding shows that even after cooking, the analyzed grains cultivars remain key health-promoting factors, maintaining their function. The levels of flavonoids in the raw and cooked grains cultivars and in the cooking broths are shown in Table 2.

Cooking caused a significant decrease in the levels of total flavonoids ($p < 0.05$). Raw grains of BRS Xiquexique (67.96 mg/100 g) and BRS Milênio (65.02 mg/100 g) presented the highest levels of flavonoids among the raw grains of cultivars. The cultivar BRS Milênio (52.34 mg/100 g) exhibited the highest levels of flavonoids among the cooked grains cultivars, whereas the cultivar BRS Tumucumaque exhibited the lowest levels of these compounds both before and after cooking, at 45.80 and 36.11 mg/100 g, respectively. A significant transfer of flavonoids ($p < 0.05$) to the cooking broth was observed for the cultivar BRS Milênio (24.27 mg/100 g; Table 2).

Despite the decrease in the levels of flavonoids after cooking, the cowpea grains maintained significant levels of these compounds. The evaluated cowpea cultivars exhibited higher levels of flavonoids than were observed by Barreto *et al.* (2009) in Brazilian tropical fruits, including loquat (24.3 ± 0.2 mg QE/100 g), jackfruit (18.3 ± 2.9 mg QE/100 g), nectarine (23.7 ± 1.2 mg EQ/100 g) and starfruit (42.6 ± 2.3 mg QE/100 g).

Wang *et al.* (2008) reported high levels of total flavonoids in cowpea grains samples in 2004 (441.9 μ g/g) and 2005 (252.9 μ g/g), when analyzing 40 accessions of selected legumes, including cowpea. Cowpea grains contains high levels of the flavonoids myricetin and quercetin and low levels of genistein, kaempferol and daidzein.

Cooking may promote the destruction of bioactive compounds, including flavonoids, or they may be eliminated into the cooking broth. Such effects may

Table 2 - Levels of total flavonoids in raw and cowpea cooked grains and in the cooking broths

Cultivars	Processing (mg QE*/100 g)		Cooking broth (mg QE*/100 g)
	Raw	Cooked	
	Mean \pm SD	Mean \pm SD	Mean \pm SD
BRS Milênio	65.02 \pm 0.23 aA	52.34 \pm 0.06 bA	24.27 \pm 0.01 cA
BRS Aracê	58.35 \pm 0.11 aB	42.56 \pm 0.19 bB	17.20 \pm 0.08 cB
BRS Tumucumaque	45.80 \pm 0.31 aC	36.11 \pm 0.25 bC	14.90 \pm 0.03 cC
BRS Xiquexique	67.96 \pm 0.54 aDA	41.01 \pm 0.44 bDB	20.17 \pm 0.01 cD

*Quercetin equivalents (QE). Mean of three replicates \pm standard deviation (SD); Lowercase letters compare the type of processing applied and uppercase letters compare between cultivars within each processing. Means followed by different lowercase letters (in columns) and different uppercase letters (in lines) are statistically different ($p < 0.05$)

explain the decrease in the levels of flavonoids observed in this study after cooking.

Table 3 shows the level of polyamines in grains cowpea cultivars before and after cooking and in the cooking broths. The polyamines spermine and spermidine were quantified in the analyzed cowpea grains. Thermal processing significantly changed ($p < 0.05$) the levels of polyamines in cowpea, which decreased after cooking.

The levels of polyamines were significantly different ($p < 0.05$) between all of the raw grains of the cultivars analyzed (Tabela 3), with the highest levels of spermidine being observed in the cultivar BRS Milênio (106.98 mg/kg), followed by BRS Tumucumaque (79.37 mg/kg) and BRS Aracê (74.68 mg/kg). The highest levels of spermine were observed in the raw grains of BRS Xiquexique (43.43 mg/kg), followed by BRS Tumucumaque (36.80 mg/kg) and BRS Aracê (25.26 mg/kg).

The highest levels of spermidine were observed in the cultivar BRS Milênio (37.67 mg/kg), whereas this cultivar exhibited 12.68 mg/kg spermine, which was the lowest level assessed among the cooked cultivar grains. Low amounts of polyamines were recorded in the cooking broths, with no spermine being detected in the cooking broths of cultivars BRS Aracê, BRS Tumucumaque and BRS Xiquexique (Table 3).

The cultivars BRS Milênio (120.5 mg/kg) and BRS Tumucumaque (116.2 mg/kg) displayed the highest levels of total polyamines (spermine + spermidine) in the raw grains. Significant losses of these substances were observed after cooking, ranging from 53.3-60.2%. Significant differences were observed between the raw ($p = 0.003$) and cooked ($p = 0.028$) grains of cultivars regarding the levels of total polyamines.

The presence of spermidine and spermine was expected in the grains cowpea cultivars because polyamines are naturally present in plant foods. The higher levels of spermidine compared with spermine were also expected, corroborating the results of Kalac and Krausová (2005). In a literature review, these authors found spermidine levels ranging from 7.7 to 8.8 mg/kg in cooked green beans, from 33.2 to 62.1 mg/kg in soybean and from 2.9 to 88.4 mg/kg in green peas. These values are indicative of the importance of the levels of these compounds in the raw cowpea cultivars examined in the present study, which are comparatively higher.

Results similar to the present study were observed by Lima *et al.* (2006) when they evaluated 10 foods typically consumed by Brazilians, including common beans (*Phaseolus vulgaris* L.). The authors reported a predominance of spermine and spermidine in the beans, with a decrease of these substances occurring after the legume was cooked. The levels of spermidine ranged from 1.30 to 0.85 $\mu\text{g/g}$ and the levels of spermine from 2.62 to 2.28 $\mu\text{g/g}$ in raw and cooked beans, respectively.

Considering the data recorded in the present study, additional studies are needed to identify and quantify the levels of polyamines in beans and their performance, taking into account changes in storage and processing conditions, as these data are important for diet planning in the nutritional management of healthy patients or patients with specific pathologies.

Total anthocyanins and flavanols were undetectable in the grains cowpea samples both before and after cooking in the present study.

Similar results were reported in a study by Ranilla *et al.* (2009), in which no condensed tannins were detected

Table 3 - Levels of polyamines in grains of cowpea cultivars before and after cooking and in the cooking broth

Amines	Cultivar	Processing (mg/Kg)		Cooking broth (mg/Kg)
		Raw	Cooked	
		Mean \pm SD	Mean \pm SD	Mean \pm SD
Spermidine	BRS Milênio	106.98 \pm 9.75 aA	37.67 \pm 2.19 bA	2.44 \pm 0.10 cA
	BRS Aracê	74.68 \pm 2.41 aB	28.54 \pm 2.54 bB	1.57 \pm 0.02 cB
	BRS Tumucumaque	79.37 \pm 4.87 aC	30.32 \pm 13.40 bC	0.86 \pm 0.00 cC
	BRS Xiquexique	69.15 \pm 3.99 aD	29.47 \pm 2.32 bDBC	1.09 \pm 0.01 cD
Spermine	BRS Milênio	13.53 \pm 3.91 aA	12.68 \pm 1.11 bA	1.94 \pm 0.01 c
	BRS Aracê	25.26 \pm 3.44 aB	17.84 \pm 1.53 bBCD	nd*
	BRS Tumucumaque	36.80 \pm 2.45 aC	17.59 \pm 2.33 bCBD	nd*
	BRS Xiquexique	43.43 \pm 4.88 aD	15.32 \pm 2.56 bDBC	nd*

Lowercase letters compare the type of processing applied and uppercase letters compare between cultivars within each processing. Means followed by different lowercase letters (in columns) and different uppercase letters (in lines) are statistically different ($p < 0.05$). * Not detected

in cooked black and brown beans. It was suggested that this result arose from the formation of insoluble complexes between proteins and tannins and between carbohydrates and tannins in whole grains, leading to failure of extraction of the compounds with the solvent and thus non-detection of these compounds in the method using the vanillin reagent.

In a study addressing the profile of condensed tannins (or proanthocyanidins) in six different cowpea genotypes, Ojwang *et al.* (2013) also did not detect these compounds when using the HCl-vanillin test in white and green cowpea genotypes. These authors suggested that the accumulation of these compounds is genetically controlled.

Antioxidant activity

The antioxidant activity of grains cowpea cultivars analyzed using the DPPH and ABTS free radical scavenging methods, before and after cooking, is shown in Table 4. A significant decrease was observed in the antioxidant activity of the studied cultivars after cooking.

Xu and Chang (2012) observed antioxidant activity, ranging from 107 $\mu\text{mol TEAC}/100\text{ g}$ in yellow soybean to 1,940 $\mu\text{mol TEAC}/100\text{ g}$ in black bean, when using the DPPH method to analyze health-promoting effects related to the antioxidant activity of 13 legumes consumed in the United States, including peas, lentils, soybeans, garbanzo beans, cowpeas and common beans. The levels determined in the present study using the DPPH method were higher than the values observed by those authors in yellow pea samples (358 $\mu\text{mol TEAC}/100\text{ g}$), garbanzo beans (294 $\mu\text{mol TEAC}/100\text{ g}$), green peas (277 $\mu\text{mol TEAC}/100\text{ g}$) and yellow soybeans

(107 $\mu\text{mol TEAC}/100\text{ g}$) and lower than those recorded in cowpeas (707 $\mu\text{mol TEAC}/100\text{ g}$).

Xu and Chang (2009), who analyzed the effect of thermal processing on the antioxidant properties in grains of common beans, observed decreases in DPPH values ranging from 46-67%. Furthermore, these authors concluded that cooking at high pressures promotes slower cooking times (10 minutes) than cooking performed at atmospheric pressure as well as smaller losses of antioxidant substances (phenolic compounds) to the cooking broth.

Results different from those found in the present study were observed by Marathe *et al.* (2011), who analyzed cowpea varieties with red and brown seed coats. A high antioxidant capacity was assessed using the DPPH (values greater than 400 $\mu\text{mol DPPH}/\text{g}$ sample) and ABTS (values greater than 12.0 $\mu\text{mol TEAC}/\text{g}$ sample) methods. The studied varieties exhibited high levels of phenolic compounds due to the color of the seed coat, which was reflected in the antioxidant capacity. This may explain the differences observed in the present study because the analyzed beans had light-colored seed coats (white and green).

However, the levels measured in the present study were higher than the values assessed by Oboh (2006), who evaluated the ability of raw cowpea samples (two cultivars with white seed coats and three with brown) to scavenge the free radical DPPH, recording percentages of free radical inhibition in the range of 5.5-29.9%. High percentages of inhibition of the DPPH radical were observed in the present study, which ranged from 40-50% in the raw cultivars and from 25-40% after cooking.

Table 4 - Antioxidant activity in raw and cowpea cooked grains and in the cooking broths according to the DPPH and ABTS methods

Method	Cultivars	Processing ($\mu\text{mol TEAC}^*/100\text{ g}$)		Cooking broth ($\mu\text{mol TEAC}^*/100\text{ g}$)
		Raw	Cooked	
		Mean \pm SD	Mean \pm SD	Mean \pm SD
DPPH	BRS Milênio	566.0 \pm 9.67 aA	349.7 \pm 5.87 bA	286.6 \pm 3.76 cA
	BRS Aracê	614.7 \pm 5.43 aB	336.1 \pm 4.99 bB	167.9 \pm 2.98 cB
	BRS Tumucumaque	551.5 \pm 4.89 aC	278.4 \pm 5.23 bC	140.2 \pm 2.09 cC
	BRS Xiquexique	575.4 \pm 7.98 aD	419.8 \pm 6.80 bD	225.8 \pm 2.56 cD
ABTS	BRS Milênio	655.6 \pm 5.87 aA	552.1 \pm 4.78 bA	335.9 \pm 3.56 cA
	BRS Aracê	660.1 \pm 7.98 aBA	523.4 \pm 7.32 bB	174.4 \pm 8.65 cB
	BRS Tumucumaque	556.7 \pm 8.65 aC	420.6 \pm 9.43 bC	154.8 \pm 4.85 cC
	BRS Xiquexique	608.5 \pm 9.09 aD	494.6 \pm 1.43 bD	204.5 \pm 4.12 cD

*Trolox equivalent antioxidant capacity (TEAC). Mean of three replicates \pm standard deviation (SD); Lowercase letters compare the type of processing applied and uppercase letters compare between cultivars within each processing. Means followed by different lowercase letters (in columns) and different uppercase letters (in lines) are statistically different ($p < 0.05$)

The reduced antioxidant action recorded in the present study may have occurred because thermal processing promotes the destruction of bioactive compounds, leading to their reduction and/or the formation of new compounds with pro-oxidant action.

Among the analyzed bioactive compounds, a strong correlation ($R^2 = 0.98$) was observed between the levels of phenolic compounds (particularly the total flavonoids) in the grains cowpea extracts and the antioxidant activity evaluated using the two methods (data not shown).

Thus, the phenolic compounds (specifically the flavonoids) contributed to the high antioxidant activity of the analyzed cultivars, as the raw cultivar BRS Aracê exhibited the highest levels of phenolic compounds, which was reflected in its high DPPH and ABTS free radical-scavenging capacities.

The correlations of the total phenolic compounds and flavonoids with the results of the antioxidant activity assessment tests were high, corroborating the reports of other researchers, including Marathe *et al.* (2011) and Xu and Chang (2012), when evaluating legumes.

Hassimoto *et al.* (2005) emphasized that antioxidant activity does not result from one specific antioxidant compound alone, but rather from the synergism among such compounds, resulting in the total antioxidant activity of foods. The present study suggests that the antioxidant activity of the analyzed cowpea cultivars basically results from the total phenolic compound class of bioactive compounds, among which the total flavonoids stood out in particular.

The grains cowpea studied both before and after cooking mostly showed high levels of bioactive compounds and antioxidant activity, corroborating several studies and reinforcing the role of beans as a functional food. The consumption of cooked grains together with the cooking broth contributes to retaining substances with antioxidant properties, including phenolic compounds and flavonoids. This is a key health-promoting aspect, and dietary supplementation with cowpea and/or cowpea flour in food products is recommended to help reduce the risk of no communicable chronic diseases, including cardiovascular disease, diabetes and cancer.

CONCLUSIONS

1. The cowpea grains studied before and after cooking showed high contents of bioactive compounds and antioxidant activity, which is consistent with several studies, reinforcing the role of beans as functional food, highlighting cultivar BRS Aracê;

2. Flavonoids were the main bioactive compounds contribute to the antioxidant activity of grain cowpea cultivars, confirmed by the high correlation observed;
3. For all bioactive compounds evaluated, thermal processing applied promoted a significant decrease in the content of these, however, grains remained relevant nutritional and functional characteristics, it is recommended to cowpea consumption with the cooking broth for retaining compounds with antioxidant properties.

ACKNOWLEDGMENTS

We thank the National Council for Scientific and Technological Development (CNPq) for funding the study through Public Notice no. 482292/2011-3, the National Program for Academic Cooperation/Collaboration Project Process no. 552239/2011-9 and the Project with Process no. 301939/20128. Additionally, we thank the Coordination for the Improvement of Higher Education Personnel (CAPES) for the graduate scholarship granted, and the Brazilian Agricultural Research Corporation - Mid North (Empresa Brasileira de Pesquisa Agropecuária, Embrapa - Meio Norte) for providing the cowpea cultivars used in the study.

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