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Effect of thermal processing on total polyphenol content in the grain of cowpea cultivars[1]

Efeito do processamento térmico no teor de polifenóis totais em grãos de cultivares de feijão-caupi

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ABSTRACT - Cowpea grain is noteworthy due to its functional properties, which are probably a result of the synergistic action of its nutrients and bioactive compounds. Since these compounds undergo the effects of thermal processing, the aim of the present study was to verify the influence of cooking on the total polyphenol content of the grain of cowpea cultivars. Samples were obtained from the experimental area of Embrapa Meio-Norte, located at 5°5' S and 42°48' W, at an altitude of 72 metres. Meal (0.5 mesh) resulting from the raw grain being ground in a cyclone rotor mill (Tecnal model TE-651/2-T), and cooked beans (with no maceration, at a bean to water ratio of 1:5 (w/v), cooked in a domestic pressure cooker (2 L) for 780 seconds) were stored in airtight containers under refrigeration (8 °C) for analysis, which was carried out in triplicate. Total phenolic content was determined by spectrophotometry (λ = 765 nm), using Folin-Ciocalteau reagent. The total phenolic content, mg GAE (gallic acid equivalent)/100g, in the raw grain of the BRS Marataoã, BR 17-Gurguéia, BRS Itaim, BRS Cauamé and BRS Guariba cultivars was 295.23, 255.67, 132.32, 98.14 and 89.43 respectively. After cooking, the content was 405.87, 255.4, 161.71, 127.79 and 111.92 respectively, considering both grain and broth. This rise may have been the result of increased solubilisation and extraction of the compounds, due to the softening or rupture of the plant cells. It was concluded that, considering both grain and broth, the total phenolic content increased after cooking; the BRS Marataoã and BR 17-Gurguéia cultivars showed the highest levels.

Key words: *Vigna unguiculata* (L.) Walp. Cooking. Bioactive compounds.

RESUMO - Os grãos de feijão-caupi se destacam devido suas propriedades funcionais, provavelmente, pelas ações sinérgicas dos seus nutrientes e compostos bioativos. Visto que estes compostos sofrem efeitos do processamento térmico, o presente trabalho objetivou verificar a influência da cocção no conteúdo de polifenóis totais em grãos de cultivares de feijão-caupi. As amostras foram provenientes do campo experimental da Embrapa Meio-Norte, que está a 72 metros de altitude, 5° 5' de Latitude Sul e 42° 48' de Longitude Oeste. As farinhas (0,5 *mesh*) resultantes dos grãos crus moídos em moinho rotor tipo ciclone (Tecnal modelo TE-651/2-T) e os grãos cozidos (sem maceração em uma proporção feijão:água de 1:5 (p/v) em panela de pressão doméstica (2 L), durante 780 segundos) foram armazenados em embalagens herméticas sob refrigeração (8 °C) até o momento das análises, realizadas em triplicata. O conteúdo de fenólicos totais foi determinado espectrofotometricamente (λ= 765 nm), utilizando-se reagente *Folin-Ciocalteau.* Os teores de fenólicos totais, mg GAE (equivalente a ácido gálico)/100g, nos grãos crus das cultivares BRS Marataoã, BR 17 - Gurguéia, BRS Itaim, BRS Cauamé e BRS Guariba foram 295,23; 255,67; 132,32; 98,14 e 89,43, respectivamente. Após a cocção, esses teores foram 405,87; 255,4; 161,71; 127,79 e 111,92, respectivamente, considerando-se grãos mais caldo. Este incremento pode ser resultado do aumento na solubilização e extração desses compostos, em virtude do amolecimento ou rompimento das células vegetais. Concluiu-se que após o cozimento os teores dos fenólicos totais aumentaram considerando-se grãos mais caldo; as cultivares BRS Marataoã e BR 17 - Gurguéia apresentaram os maiores teores.

Palavras-chave: *Vigna unguiculata* (L.) Walp. Cocção. Compostos bioativos.

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INTRODUCTION

Cowpea grain (*Vigna unguiculata* (L.) Walp) is an important component in the diet of developing countries in Africa, Latin America and Asia, and is a valuable source of low-cost protein. In the north and northeast of Brazil, the grain is one of the foods which are most consumed by the low-income population who suffer from deficiency diseases such as malnutrition and micronutrient deficiency (FROTA *et al*., 2008; PEREIRA *et al*., 2014).

Of African origin, the cowpea was introduced to Brazil in the second half of the 16th century by Portuguese settlers in the State of Bahia. The states of Ceará, Piauí, Mato Grosso, Pernambuco, Bahia and Paraíba rank as the largest producers in the country. Consumption has been spreading more intensively to the mid-west and southeast of Brazil (FREIRE FILHO *et al*., 2011).

In addition to being a significant source of carbohydrates, proteins, vitamins, minerals and dietary fibre, bioactive compounds are found in the grain; these compounds, due to their antioxidant action, help reduce the risk of diseases such as cancer, diabetes and cardiovascular disorders (RAMÍREZ-CÁRDENAS; LEONEL; COSTA, 2008).

Phenolic compounds are chemical structures that present hydroxyl and aromatic rings which confer antioxidant ability. In simple form or as polymers, free or complexed with sugars and proteins, these compounds originate in the secondary metabolism of plants, being essential for their growth and reproduction, and are also produced under such stress conditions as infection, injury and ultraviolet radiation (UV), among others. The structural diversity of phenolic compounds is due to the great variety of combinations that occur in nature; the resulting compounds are called polyphenols, which in food are responsible for colour, astringency, aroma and oxidative stability (ANGELO; JORGE, 2007).

Data which refer to beans after thermal processing are pertinent, since this food is usually consumed when cooked. The methods of preparing beans influence the sensory quality and nutritional value of the food. As the cooked form of these legumes is the most consumed, heating allows the grains to be utilised, improving digestion, nutrient absorption and sensorial characteristics (appearance, aroma, taste and texture). In general, thermal processing affects the nutritional quality of grain, with changes seen in the levels of macronutrients, vitamins, minerals and phytochemicals (FERNANDES; CALVO; PROENÇA, 2012; TOLEDO; CANNIATTI-BRAZACA, 2008).

The aim of this study therefore, was to evaluate the effect of the domestic cooking process on the total polyphenol content of the grain of five cowpea cultivars.

MATERIAL AND METHODS

The analysis was carried out from March to December 2014 in the Food Science Laboratory (LABROMBIOQ) of the Department of Nutrition (DN), at the Centre for Health Science (CCS) on the Ministro Petrônio Portella Campus of the Federal University of Piauí - UFPI.

The cowpea samples came from the experimental area of Embrapa Meio-Norte in Teresina, in the State of Piauí, located at 5°5’ S and 42°48’ W, at an altitude of 72 metres. The samples were kept in sealed containers at a temperature of 8 °C for analysis.

The grain from five cultivars were analysed: BRS Marataoã, BR 17-Gurguéia, BRS Itaim, BRS Cauamé and BRS Guariba.

The cowpea grain was manually selected to remove dirt and any grains that did not look to be intact. Intact grains from each cultivar were submitted to two different procedures to carry out the analysis:

*** RAW GRAIN:** ground in a cyclone rotor mill (Tecnal model TE-651/2-T) until a meal was obtained (0.5 mesh), that was stored in low-density polyethylene bags, 0.08 mm thick, under refrigeration at 8 °C for analysis, which was carried out in triplicate.

*** COOKED GRAIN:** cooked with no maceration, at a bean to water ratio of 1:5 (w/v) in a 2 L domestic pressure cooker for 780 seconds from the start of a constant flow of steam through the pressure valve. The cooked grain was then separated from the broth with the aid of plastic sieves, and homogenised with a pestle and mortar for later analysis.

The cooked grain was also stored in low-density polyethylene bags of 0.08 mm thick film, and the broths stored in plastic Falcon tubes (0.05 L), both were kept under refrigeration (8 °C) for analysis, which was carried out in triplicate.

The extracts were obtained following a procedure adapted from Rufino *et al*. (2007), with tests using several solvents (water, 80% acetone and ethanol), as the solubility of phenolic compounds varies according to the polarity of the solvent, the degree of polymerisation of the compounds and their interaction. However, an extractant of 50% methanol - 70% acetone - water (2:2:1) resulted in better extraction yields.

A 0.05 L Falcon tube was used to weigh 0.003 kg of the sample, with the addition of 0.01 L of 50% methanol. The sample was homogenised and extracted for 1,800 seconds with ultrasound. The sample was then centrifuged at 4000 rpm for 900 seconds, and the supernatant transferred to a 0.025 L volumetric flask.

The residue of the first extraction was taken and 0.01 L of 70% acetone was added, followed by homogenisation and extraction for 1,800 seconds with ultrasound. This was centrifuged at 4000 rpm for 900 seconds, and the supernatant transferred to the same flask. The volume was topped up using Milli-Q water.

The total phenolic content was determined by spectrophotometry, using Folin-Ciocalteau reagent (ROSSI; SINGLETON, 1965). The methodology was characterised by adding 0.002 L of deionised water to a 0.01 L volumetric flask, followed by 100 µL of the extract from the sample (deionised water was used for the blank). Subsequently, 0.0005 L of pure Folin-Ciocalteau reactant was added, while vigorously shaking to homogenise the mixture. After 30 seconds and before 480 seconds, 0.0015 L of 20% (w/v) sodium carbonate solution was incorporated into the flask. The volume in the flask (0.01 L) was topped up with deionised water, and the mixture kept in the dark for 7,200 seconds at 24 °C. At the end of this time, the absorbance at a wavelength of 765 nm was measured with a spectrophotometer (BEL 1102, Monza, Milan, Italy).

The phenolic compounds were quantified by interpolating the absorbances in a previously-constructed standard curve of gallic acid, and the values expressed in mg of gallic acid equivalent per 100 g fresh weight (mg GAE/100g).

Data analysis was carried out using the Statistical Package for the Social Sciences (SPSS) 17.0 software (SPSS, 2006). The results were presented with mean value and standard deviation. By means of the One-Way ANOVA post hoc method, Tukey's test was applied at a level of 5% probability ($p<0.05$) and a confidence level of 95% respectively, to analyse differences between the mean values for the cultivars, and between the raw grain, cooked grain and the broth (HILBE, 2013).

RESULTS AND DISCUSSION

According to Marathe *et al*. (2011) legumes are classified according to their phenolic content, as low (<100 mg GAE/100 g), medium (100-200 mg GAE/100 g), or high (>200 mg GAE/100 g). Among the cultivars under analysis, the raw grain of BRS Marataoã and BR 17-Gurguéia showed a high total polyphenol content, followed by the grain of BRS Itaim with a medium content, and BRS Cauamé and Guariba with a low content.

In this study, the total polyphenol content of the raw grain from the five cultivars was within the range obtained by Cai, Hettiarachchy and Jalaluddin (2003), of 34.6 to 376.6 mg/100 g^{-1} in 17 varieties of the legume. Also in this study, the presence of phenolic acids was identified, where the major component was protocatechic acid, followed by p-hydroxybenzoic, caffeic, p-coumaric, ferulic and cinnamic acids.

After thermal processing, there was a decrease in total polyphenol content in the grain from the five cowpea cultivars, as seen by Delfini and Canniatti-Brazaca (2008) and Siddhuraju and Becker (2007) in genotypes of the cowpea and the common bean, however the presence of these compounds was found in the broth. According to Afonso (2010), Avanza *et al*. (2013) and Granito, Paolini and Pérez (2008), this transfer can be explained by the water solubility of the phenolic compounds; the literature emphasises that during maceration and cooking these are released into the water used respectively in the maceration and cooking process.

The BRS Marataoã cultivar also stood out among the cooked grains and the broths, with values of 131.59 and 274.28 mg GAE/100g respectively (Table 1); BR 17-Gurguéia displayed the second-highest levels; this can be explained by these cultivars having grains with coloured integuments.

Table 1 - Total polyphenols in the grain of cowpea cultivars before and after thermal processing, and in the cooking broth

Total Polyphenols (mg GAE/100g)				
Cultivar	Raw Grain	Cooked Grain (CdG)	Cooking Broth (CBr)	CdG + CBr
	Mean ± SD	Mean ± SD	Mean ± SD	
BRS Marataoã	295.23 ± 24.80 aA	131.59 ± 22.29 aB	274.28 ± 21.18 aC	405.87
BR 17-Gurguéia	255.67 ± 10.65 bA	91.74 ± 3.26 bB	163.65 ± 52.49 bC	255.39
BRS Itaim	132.32 ± 11.42 cA	66.50 ± 6.96 cB	95.21 ± 21.84 cC	161.71
BRS Cauamé	98.14 ± 9.05 dA	55.00 ± 10.00 dB	72.79 ± 7.14 dC	127.79
BRS Guariba	89.43 ± 11.08 eA	48.97 ± 8.03 eB	62.95 ± 12.18 eC	111.92

For the same superscript letters in a row, and uppercase letters in a column, there is no significant difference between mean values at a level of 5%. Tukey's test was applied in the columns and rows

The integument is recognisably the principal source of polyphenols in legume grain, as expected due to its protective function. In turn, coloured integuments display the highest levels. Mesquita *et al*. (2007) obtained higher levels of phenolic compounds in lines of *Phaseolus vulgaris* L. with integuments that were red, beige with brown streaks and beige with pink streaks. The line with the lowest content was "Small White", which has a white integument. According to Marathe *et al*. (2011) and Silva, Rocha and Canniatti Brazaca (2009), this variation in the levels of phenolic compounds is known to be due to genetic factors, the degree of maturity and environmental conditions.

When the total polyphenol content was determined in the broths, a high level of these compounds was found in the broth from the BRS Marataoã grain, which probably displays the highest levels due to being differentiated because of its technological and genetic characteristics.

In a study with three genotypes of the common bean, IAPAR 81, Uirapuru and BAF 55, Valdés (2010) found that the broth showed the highest mean value for total phenolic content, of 655.6 mg GAE/100 g, followed by the raw grain with 450.57 mg GAE/100g and cooked grain with a mean value of 232.43 mg GAE/100g. The higher polyphenol content of the broth may be explained by a decrease in protein-bound polyphenols as a result of the cooking process.

In the present study, it was found that after cooking, the level of phenolic compounds tends to increase when summing the content for both the cooked grains and their respective broths. In a study by Sultana, Anwar and Iqbal (2008), the total phenolic content of pea samples also increased after thermal processing (microwave cooking). The authors explain this rise by a possible increase in the solubilisation and extraction of these compounds, which results from the softening or rupture of the plant cell walls and disruption of the structure of complex phenolic compounds.

The presence of phenolic compounds in the grain of cowpea cultivars, as well as the antioxidant activity they carry out, make this legume a food that can help reduce the risk of diseases such as cancer, cardiovascular disorders, atherosclerosis and diabetes.

CONCLUSION

With the domestic cooking process, the presence of total polyphenols is found in the broth. There is an increase in the content of these phytochemicals when considering both grain and broth; after cooking, BRS Marataoã and BR 17-Gurguéia display a high phenolic content, and BRS Itaim, BRS Cauamé and BRS Guariba, a moderate content.

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