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Chemical characterization of green grain before and after thermal processing in biofortified cowpea cultivars[1]

Caracterização química de grãos verdes crus e após processamento térmico em cultivares biofortificadas de feijão-caupi

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ABSTRACT - The cowpea (*Vigna unguiculata* (L.) Walp.) is one of the most widely consumed legumes in the North and Northeast of Brazil, representing an important source of protein, energy, fibre and minerals, in addition to generating employment and income. The aim of this study was to determine the chemical composition, and the effect of thermal processing on the green grain from biofortified cowpea cultivars. Samples of green grain from four cowpea cultivars were analysed, only one not being biofortified (standard). Centesimal composition and total energy value (TEV) were determined before and after thermal processing. The mean values of the characteristics under evaluation were compared by Student's t-test and Tukey's test (p<0.05). Moisture and TEV content in the raw green grain ranged from 58.32 to 60.66% and from 159 to 170 Kcal/100 g (wet basis) respectively. The ash, protein, lipid and carbohydrate (dry basis) content of the raw green grain varied from 1.58 to 1.68%, 11.03 to 13.25%, 1.31 to 2.23%, and 36.11 to 38.13% respectively. After heat treatment, there was an increase in lipid content (1.57 to 2.36%) and a reduction in the levels of ash (0.83 to 1.09%), protein (10.25 to 13.13%) and carbohydrates (25.22 to 28.50%), except for the BRS Tumucumaque cultivar, which had a reduction in lipid content (2.04%) and an increase in protein content (12.94%). It was concluded that the grain from the cowpea cultivars was affected by thermal processing, reducing the levels of ash, protein and carbohydrates, and increasing the lipid content in three of the four cowpea cultivars under study. BRS Tumucumaque was the only cultivar that did not behave in a similar way to the others, with an increase in protein content and a reduction in lipids after heat treatment.

Key words: *Vigna unguiculata*. Nutritive value. Cooking.

RESUMO - O feijão-caupi (*Vigna unguiculata* (L.) Walp.) é uma das leguminosas mais consumidas no Norte e Nordeste do Brasil, representando importante fonte de proteínas, energia, fibras e minerais, além de gerar emprego e renda. O objetivo deste trabalho foi determinar a composição química e o efeito do processamento térmico em grãos verdes de cultivares de feijão-caupi biofortificadas. Foram analisadas amostras de grãos verdes de quatro cultivares de feijão-caupi, sendo apenas uma não biofortificada (padrão). Determinou-se a composição centesimal e o valor energético total (VET), antes e após o processamento térmico. As médias das características avaliadas foram comparadas pelos testes *t* de *Student* e *Tukey* (p<0,05). Os teores de umidade e VET dos grãos verdes crus variaram, respectivamente, de 58,32 a 60,66% e 159 a 170 Kcal/100 g (base úmida). Os teores de cinzas, proteínas, lipídeos e carboidratos (base seca) dos grãos verdes crus variaram, respectivamente, de 1,58 a 1,68%; 11,03 a 13,25%; 1,31 a 2,23%; 36,11 a 38,13%. Após o tratamento térmico houve aumento no conteúdo de lipídios (1,57 - 2,36%) e redução nos conteúdos de cinzas (0,83 - 1,09%), proteínas (10,25 - 13,13%) e carboidratos (25,22 - 28,50%), exceto na cultivar BRS Tumucumaque que teve redução no conteúdo de lipídios (2,04%) e aumento no conteúdo de proteínas (12,94%). Concluiu-se que os grãos das cultivares de feijão-caupi foram afetados pelo processamento térmico, reduzindo os teores de cinzas, proteínas, carboidratos; e aumentando o conteúdo de lipídeos em três das quatro cultivares de feijão-caupi estudadas. A única cultivar que não se comportou de maneira similar às demais foi a BRS Tumucumaque, que teve um aumento no conteúdo de proteínas e redução de lipídeos, após o tratamento térmico.

Palavras-chave: *Vigna unguiculata.* Valor nutritivo. Cocção.

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INTRODUCTION

The cowpea (*Vigna unguiculata* (L.) Walp.) adapts to a wide range of environments in tropical and subtropical regions of the world, and faced with the prospects of climate change, is a crop with great potential. In Brazil, it is one of the most consumed legumes in the North and Northeast, representing an important source of protein, energy, fibre and minerals, in addition to generating employment and income (FREIRE FILHO, 2011; FROTA; SOARES; ARÊAS, 2008).

Cowpea is used for various purposes and in different production systems, and can be marketed as dry grain (main market), immature grain (green beans), seeds, and flour for use in local dishes. The cowpea market mainly revolves around the production of dry or immature grain. Widely appreciated for its taste and easy preparation, the immature grain is used in several dishes typical of the Northeast, the so-called '*baião-de-dois*' being the most popular, where cowpea and rice are cooked together (ANDRADE *et al*., 2011).

Green grain, so called because they are close to the stage of physiological maturation, have a high percentage of water and, as a consequence, are highly perishable, requiring the use of costly methods of conservation when compared to dry grain (LIMA *et al*., 2000; LIMA *et al*., 2003). At the time of harvest, the grains display around 60 to 70% humidity, i.e. a little before or after the stage in which they stop accumulating photosynthates, and begin the process of natural dehydration. This is easy to recognise, as the pods are fairly swollen, and begin to undergo a slight change in colour (FREIRE FILHO *et al*., 2005).

The production and consumption of immature or green cowpea grain represents a highly promising market, making it a good choice of income for family farmers (ROCHA *et al*., 2007). For this reason, it has become an important regional source of employment and income. The production of green grain shows great potential for an increase in consumption, as well as industrial processing, especially when produced in the off-season, a time when the product reaches high prices in the market (FREIRE FILHO *et al*., 2007).

Biofortification consists of the improvement of plants of the same species, which are crossed, selecting cultivars with higher levels of micronutrients. In the cowpea, activities for biofortification include the evaluation of genotypes from the breeding program and active germplasm bank of Embrapa Meio-Norte, the selection of parents with high concentrations of iron and zinc in the grain, the carrying out of crossing and backcrossing, the selection of biofortified cultivars, and the multiplication of seeds (EMBRAPA MEIO-NORTE, 2010).

The chemical composition and nutritional properties of the cowpea vary considerably according to the cultivar. Vasconcelos *et al*. (2010) reported the importance of the chemical and nutritional monitoring of new cowpea cultivars.

Research has been carried out in order to characterize chemically lines and cultivars of dry and green grain in the cowpea, mainly in relation to levels of protein, carbohydrates, fibre, vitamins and minerals, and more recently, bioactive compounds (ANDRADE *et al.*, 2011; BARROS, 2014; CARVALHO *et al.*, 2012; DINIZ *et al.*, 2001; ELHARDALLOU *et al.*, 2015; FAMATA *et al.*, 2013; KALPANADEVI; MOHAN, 2013; LIMA *et al.*, 2000; LIMA *et al.*, 2003; NDERITU *et al.*, 2013; NUNES *et al.*, 2006; PINHEIRO, 2013; SALGADO *et al.*, 2005). However, in relation to the chemical composition of the green grain, studies are rare (FREIRE FILHO *et al.*, 2011; NUNES *et al.*, 2006).

Due to the small amount of knowledge on the nutritive characteristics of green grain from biofortified cowpea cultivars, the present study was carried out with the aim of evaluating the chemical composition and effect of thermal processing on green grain from biofortified cultivars of the cowpea, thus aiding in the selection and genetic breeding of genotypes with high nutrient concentrations.

MATERIAL AND METHODS

In this study, samples of green grain from three cowpea cultivars biofortified with iron and zinc were used (BRS Aracê, BRS Tumucumaque and BRS Xiquexique), with an unbiofortified cultivar (BRS Guariba) used as standard.

Genetically improved cowpea cultivars (biofortified and standard) were supplied in a single batch from the Cowpea Genetic Breeding Program of Embrapa Meio-Norte. The green grain from the cowpea cultivars were received shortly after harvesting, in August 2013.

The analysis of centesimal composition was performed in the Laboratory of Food Science and Food Biochemistry of the Department of Nutrition, Centre for Health Sciences, Federal University of Piauí (UFPI), in Teresina, in the State of Piauí, Brazil. All the analyses were carried out from September 2013 to March 2014.

The grain was manually selected to remove dirt and non-standard quality grain. The raw and cooked grain from the cowpea cultivars was submitted to different treatments for analysis.

For the analysis of the raw grain from the cowpea cultivars, the samples were washed with distilled water to remove dirt, homogenised with a mortar and pestle, packed in polyethylene bags and then stored at -18 ºC for analysis.

The cooking time for the grain from the cowpea cultivars was determined by cooking with moist heat in an unpressurised saucepan; the grain samples were placed to cook in water at a ratio of 1:3 (w/v), and the time measured immediately after the start of boiling, as per Diniz *et al*. (2001).

To analyse the cooked grain from the cowpea cultivars, they were previously boiled in a standard, 2 L saucepan, according to the amount of cooking time (minutes) determined earlier: BRS Aracê (28 ± 2.08); BRS Tumucumaque (30 ± 3.21); BRS Xiquexique (26 ± 2.08) and BRS Guariba (22 ± 2.08).

The grain was then separated from the cooking broth with the help of a plastic sieve, and the cooked grain macerated with a mortar and pestle as per a methodology described by Pinheiro (2013), adapted in the present study, where the grain was not macerated together with the cooking broth. All the cooked samples were packed in polyethylene bags and stored under freezing for analysis.

Moisture was determined gravimetrically in a FANEM model 315 SE oven at 105 ºC. The ashes were determined by incineration in a Marconi model MA385/2 muffler at 55 °C. The lipid fraction was determined by solvent extraction in a Marconi model MA 491 Soxhlet intermittent extractor, using Hexane P.A. (SYNTH) as solvent. Protein analysis was based on the Kjeldahl method of nitrogen determination, using a conversion factor of 6.25. The carbohydrate content was found by difference from the other constituents of the centesimal composition (moisture, ashes, proteins and lipids). All the nutrients were determined according to AOAC methodology (2005).

Total energy value was calculated as per Watt and Merrill (1963), using the Atwater conversion factors (carbohydrates = 4.0, lipids = 9.0, proteins = 4.0).

All the chemical analyses were carried out in triplicate.

Statistical analysis of the data was performed using the SAS software (SAS INSTITUTE, 2002). The results were expressed in tables of mean values and standard deviation. The mean values for the raw and cooked treatments of the cowpea cultivars were compared by Student's t-test and Tukey's test. In all tests, a significance level of 5% was adopted.

RESULTS AND DISCUSSION

The results of the centesimal composition and TEV on a wet-basis, of the samples of raw and cooked green grain from the cowpea cultivars under evaluation are shown in Table 1.

Table 1 - Centesimal composition and total energy value (TEV) of the samples of raw and cooked green grain from the four cowpea cultivars (wet basis)

Nutrient/TEV	Process	Cultivar (Mean ± SD)			
		BRS Aracê	BRS Tumucumaque	BRS Xiquexique	BRS Guariba
Moisture (%)	Raw	58.32 ± 0.20 bB	58.57 ± 0.23 bB	60.66 ± 0.31 bA	58.87 ± 0.26 bB
	Cooked	66.42 ± 0.36 aA	66.27 ± 0.20 aA	66.25 ± 0.08 aA	64.31 ± 0.23 aB
Ash (%)	Raw	1.57 ± 0.02 aCB	1.56 ± 0.0 aC	1.66 ± 0.01 aA	1.61 ± 0.02 aB
	Cooked	0.89 ± 0.0 bC	0.82 ± 0.01 bD	0.98 ± 0.02 bB	1.08 ± 0.01 bA
Protein (%)	Raw	10.99 ± 0.39 aBA	10.83 ± 0.37 aB	9.93 ± 0.22 aC	11.70 ± 0.11 aAB
	Cooked	9.36 ± 0.16 bB	11.45 ± 0.38 aA	9.30 ± 0.18 bB	11.61 ± 0.29 aA
Lipids (%)	Raw	1.87 ± 0.14 bB	2.18 ± 0.05 aA	1.59 ± 0.01 bBC	1.30 ± 0.08 bB
	Cooked	2.31 ± 0.21 aA	2.00 ± 0.02 bA	1.94 ± 0.04 aAB	1.55 ± 0.03 aB
Carbohydrates (%)	Raw	27.25 ± 0.36 aA	27.60 ± 1.12 aA	29.69 ± 0.80 aA	26.53 ± 0.33 aA
	Cooked	21.32 ± 0.38 bA	20.12 ± 1.46 bA	22.17 ± 0.98 bA	21.45 ± 0.58 bA
TEV (Kcal/100g)	Raw	169.80 ± 1.39 aA	170.91 ± 0.84 aAAAAA	159.33 ± 0.59 aC	164.59 ± 0.57 aB
	Cooked	143.51 ± 2.52 bA	141.40 ± 0.80 bAA	140.73 ± 0.36 bB	146.19 ± 0.98 bA

Average of three replications ± standard deviation (SD). Uppercase letters in the same row and lower case letters in the same column between each double-row of raw and cooked values, show no significant difference (p<0.05) between mean values by Tukey's test and Student's t-test respectively

The moisture content of the samples of raw green grain from the cowpea cultivars ranged from 58.32 to 60.66%, showing similarity between the biofortified cultivars BRS Aracê, BRS Tumucumaque and BRS Guariba (Table 1). The samples of green grain from the cowpea cultivars after cooking showed an increase in moisture content when compared to the raw grain, with a statistically significant difference between the raw and cooked samples. This increase can be explained by imbibition of the green grain during cooking.

The samples of raw grain from the cowpea cultivars showed a total energy value (TEV) of 159 to 170 kcal 100 g^{-1}, with a significant difference (p<0.05) between the cultivars BRS Xiquexique and BRS Guariba (Table 1). The samples of cooked grain from the cultivars showed a reduction in TEV; there was a significant difference (p <0.05) between the raw and cooked grain, with values of 143.51 kcal 100 g^{-1}, 141.40 kcal 100 g^{-1}, 140.73 kcal 100 g^{-1} and 146.19 kcal 100 g^{-1} for the cultivars, BRS Aracê, BRS Tumucumaque, BRS Xiquexique and BRS Guariba respectively.

The BRS Guariba cultivar (standard) displayed the highest value for TEV after cooking. This happened because the values for ash and protein after thermal processing were also higher when compared to the other cultivars, which demonstrates that, despite being more calorific, the standard cultivar maintains nutritionally relevant characteristics after thermal processing.

The reduction in TEV after cooking can be attributed to a reduction in the level of some macronutrients, such as carbohydrates and proteins. The TEV found in the raw and cooked cowpea cultivars was inferior to those presented in studies by Pinheiro (2013), TACO-UNICAMP (2011), and Frota, Soares and Arêas (2008), who found values of 200 to 300 kcal 100g^{-1} in dry grains, demonstrating that the grain from the cultivars evaluated in the present study are less calorific. In the researched literature on the cowpea, no data were found relating to centesimal composition of green grain after cooking.

The results of the chemical composition on a dry basis, of the samples of raw and cooked green grain from the cowpea cultivars are shown in Table 2.

A variation of from 1.58 to 1.68% was seen in the ash content of the samples of raw green grain from the cowpea cultivars, with a statistically significant difference between some of the cultivars, and BRS Xiquexique showing the greatest content, as seen in Table 2. These values agree with the work of Salgado *et al.* (2005), who found an average value of 1.63% when analysing green grain from cowpea cultivars.

The samples of green grain from the cowpea cultivars after cooking showed a reduction in ash content, with a statistically significant difference between the raw and cooked treatments (Table 2). This reduction in ash content can be attributed to the loss of minerals through diffusion in the water used in the thermal treatment. The ash content of the cooked grains ranged from 0.83 to 1.09%, with the cultivar BRS Guariba showing the highest levels, and a significant difference (p<0.05) between the cultivars (Table 2).

The protein content of the raw green grain from the cowpea cultivars varied from 11.03 to 13.25%, with no statistically significant difference between the BRS Tumucumaque and BRS Aracê cultivars (Table 2). The BRS Guariba cultivar was noteworthy for its levels of this nutrient. Analysing green grain from cowpea cultivars, Salgado *et al*. (2005) found a mean value for protein content of 9.65%, and Nunes *et al.* (2006), a mean value of 10.28%. However, Diniz *et al*. (2001) presented somewhat

Table 2 - Chemical composition of the samples of raw and cooked green grain from the four cowpea cultivars (dry basis)

Nutrient	Process	Cultivar (Mean±SD)			
		BRS Aracê	BRS Tumucumaque	BRS Xiquexique	BRS Guariba
Ash (%)	Raw	1.60 ± 0.02 aBC	1.58 ± 0.00 aC	1.68 ± 0.01 aA	1.63 ± 0.02 aB
	Cooked	0.90 ± 0.01 bCD	0.83 ± 0.01 bD	0.98 ± 0.04 bB	1.09 ± 0.01 bA
Protein (%)	Raw	12.35 ± 0.49 aB	12.14 ± 0.46 aB	11.03 ± 0.27 aC	13.25 ± 0.13 aA
	Cooked	10.33 ± 0.19 bB	12.94 ± 0.49 aA	10.25 ± 0.22 bB	13.13 ± 0.37 aA
Lipids (%)	Raw	1.91 ± 0.14 bAB	2.23 ± 0.06 aA	1.62 ± 0.01 bBC	1.31 ± 0.08 bC
	Cooked	2.36 ± 0.22 aAB	2.04 ± 0.02 bAB	1.98 ± 0.04 aB	1.57 ± 0.03 aC
Carbohydrates (%)	Raw	37.46 ± 0.68 aA	38.13 ± 2.16 aA	36.42 ± 1.49 aA	36.11 ± 0.61 aA
	Cooked	27.08 ± 0.63 bA	25.22 ± 2.29 bA	28.50 ± 1.62 bA	27.32 ± 0.94 bA

Average of three replications ± standard deviation (SD). Uppercase letters in the same row and lower case letters in the same column between each double-row of raw and cooked values, show no significant difference (p<0.05) between mean values by Tukey's test and Student's t-test respectively lower values for the protein content of green grain from the cowpea (7.48 to 9.12%). The cultivars analysed in the present study therefore showed slightly higher values than the above-mentioned studies.

The green grain from the cowpea cultivars after thermal processing showed a reduction in protein content for the BRS Aracê, BRS Guariba and BRS Xiquexique cultivars, and an increase for BRS Tumucumaque, with no statistically significant difference between the raw and cooked treatments for BRS Tumucumaque or BRS Guariba (Table 2). This reduction in protein content may be due to loss when cooking, as a small amount of amino acids may have become solubilised in the cooking water, causing a decrease in the protein content of the grain. The protein content of the samples of cooked grain from the cultivars ranged from 10.25 to 13.13%.

Other studies carried out with the common bean also found a reduction in protein content in dry grain after cooking. Brigide and Canniatti-Brazaca (2011) found a reduction in protein content of 27.4 (raw) to 23.9% (cooked) respectively, and in the study by Ramírez-Cárdenas, Leonel and Costa (2008), a reduction of 24.42 to 23.07% was found in one of the cultivars under study. The data presented in TACO - UNICAMP (2011) showed a greater reduction in protein content, from 20.2 to 5.1%.

The cowpea cultivar, BRS Tumucumaque, was noteworthy for the lipid content (2.23%) of the raw green grain (Table 2). The lipid content increased after cooking in the grain samples from the BRS Aracê, BRS Xiquexique and BRS Guariba cultivars, and decreased in the BRS Tumucumaque cultivar, with a significant difference ($p<0.05$) between the raw and cooked grain samples. However, it should be emphasised that no oil was added when cooking the cultivars. The increase in lipid content can be attributed to a reduction in the levels of ash, protein and carbohydrates after cooking, with the decrease being a result of the increase in protein in the grain from the BRS Tumucumaque after thermal processing.

An increase in lipid content was also seen in the grain after thermal processing in the studies of Pinheiro (2013) and Ramírez-Cárdenas, Leonel and Costa (2008). Barros (2014) only found an increase in lipid content in the BRS Tumucumaque cultivar.

A variation of 36.11 to 38.13% was seen in the carbohydrate content of the samples of raw green grain from the cowpea cultivars, with no statistically significant difference between cultivars, and BRS Tumucumaque displaying the greatest content, as shown in Table 2.

The carbohydrate content decreased after cooking, with a significant difference ($p<0.05$) between the raw and cooked grain samples (Table 2). The cooked grain from the cultivars showed a content of 25.22 to 28.50%, but exhibited similarity for this nutrient in the samples of cooked grain.

The studies of Pinheiro (2013) and Barros (2014) also found a reduction in carbohydrate content in the grain after thermal processing, the results seen by Pinheiro (2013) varying from 21.68 to 26.72%, similar to the values of the present study.

CONCLUSION

In general, thermal treatment reduced the levels of ash, protein, and carbohydrates, and increased the lipid content in the cowpea cultivars after cooking. The only cultivar that did not show similar behaviour to the others was BRS Tumucumaque, which showed an increase in protein content and a reduction in lipids after the thermal treatment.

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