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EFFECT OF DRYING TEMPERATURE ON THE NUTRITIONAL AND ANTIOXIDANT QUALITIES OF CUMARI PEPPERS FROM PARÁ (*Capsicum chinense* Jacqui)

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Abstract - This study evaluated the proximate components, concentration of total polyphenols, antioxidant activity, and capsaicin and dihydrocapsaicin in the Cumari chili pepper from Pará, Brazil (*Capsicum chinense Jacqui*) both fresh and after subjected to three different drying temperatures. The results showed that the contents of ash and vitamin C for the dried pepper differed significantly ($P < 0.05$) compared with the fresh pepper. There was a significant difference in concentrations of total phenolics, antioxidant activity and capsaicinoids between the fresh pepper and those submitted to the drying treatments. It was concluded that higher temperatures increase shelf life and decrease the volume of the product, preserve macronutrients and degrade micronutrients, antioxidants and the spicy hotness of the Cumari pepper.
Keywords: Antioxidants; Capsaicinoids; Drying.

INTRODUCTION

Peppers are one of the vegetable spices widely used in cooking; they are grown in tropical and temperate regions around the world and are basically divided into processed and ornamental natural products (Luz *et al*., 2006).

A number of properties are attributed to peppers, including therapeutical, nutritional, and they are optimizers of flavor, aroma and color of foods. They present high concentrations of vitamins A, C and E (Kuda *et al*., 2004) and recent studies indicate significant antioxidant activity and phenolic content in some species of the genus *Capsicum* (Costa *et al*. 2010; Materska and Perucka, 2005).

The use and search for natural antioxidants has increased significantly, both due to increasing industrial interest and the presence of numerous epidemiological studies that associate antioxidant activity with the prevention of several diseases. Antioxidant properties of spices are due to a variety of active phytochemicals, including vitamins, carote-

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noids, terpenes, alkaloids, lignanes, and phenolic compounds (Chatterjee *et al*., 2007).

The hotness of peppers is due to the presence of alkaloids in the seeds, including capsaicin, found in the placenta and, to a lesser extent, in the pericarp of the fruit. Their therapeutic properties are due in large part to the presence of these alkaloids and capsaicin is currently employed as an analgesic, cicatrizant and anticoagulant in the treatment of alopecia, skin allergies, mucositis, skin rash and skin tumors, and also aids in digestion by increasing the release of gastric juice (Chatterjee *et al*. 2007; Surh, 2002).

The Cumari chili pepper species from Pará, Brazil (*Capsicum chinense* Jacqui) presents a yellow fruit when ripe, with a strong smell and high hotness, measuring about 2 to 3 cm in length and 1 cm wide, and is sometimes confused with the Cumari Verdadeira chili pepper (Emater, 2006).

Drying is one of the most traditional processes for food preservation, which promotes the concentration of the macronutrient content, eliminating the use of additives. It allows alteration of the original organoleptic properties, giving rise to new products and allowing their addition in different formulations, improving the sensorial aspect and quality of other foods (Vilela and Arthur, 2008). Drying is a complex process involving simultaneous coupled transient heat, mass and momentum transport (Haghi and. Amanifard, 2008).

The objective of the present study was to compare the nutritional components and antioxidant activity, as well as quantify total phenols and the different fractions of capsaicinoids of Cumari chili peppers from Pará (*C. chinense* Jacqui), both fresh and dried at temperatures of 45, 55 and 65 °C in order to verify whether there was degradation of chemical and antioxidant components in dried peppers.

MATERIAL AND METHODS

Drying of the Peppers

Drying of the Cumari pepper from Pará (*C. chinense* Jacqui) was conducted in a fixed bed dryer equipped with three circular trays consisting of a meshed base to allow air to pass through the product and an adjustable diaphragm before the fan. Drying temperatures used were 45, 55 and 65 °C. The air was heated by a set of electrical resistances with total power of 5 kw. Temperature was controlled using a PID controller (N480 D) coupled to one of the resistances. To control the drying air velocity at the top of the tray, a fan anemometer was used to maintain the air velocity at approximately 1 m s^{-1}. During the drying process the trays were weighed on a digital scale accurate to 10^{-2} kg until reaching constant weight.

After drying, both the fresh pepper (control treatment) and the dried peppers were ground in a cyclone-type micro mill and immediately stored in plastic bags for determination of the chemical composition.

Determination of the Chemical Composition

For chemical characterization all analyses were performed in triplicate to determinate the concentrations of water, ash (muffle 550 °C), ether extract (Soxhlet), vitamin C, pH, titratable acidity, crude protein (macro Kjeldahl) and crude fiber as described by the Association of Official Analytical Chemists (AOAC, 2000).

Preparation of the Extracts for Determination of Phenols and Antioxidant Activity

All samples were extracted with 50% methanol (v/v) followed by extraction with 70% acetone (v/v) according to the methodology described by Rufino *et al*. (2007) to obtain the crude extract, which would later be used in determining the content of phenolic compounds and in the antioxidant activity tests.

A 2 x 10^{-3} kg sample of each product was transferred to a 10^{-1} L beaker and 4 x 10^{-2} L of 50% methanol was added. A mixer with a steel shaft was used to homogenize the mixture, which was then left at rest for 1 hour at room temperature. The supernatant was transferred to a 10^{-1} L volumetric flask. To the residue from the first extraction, 4 x 10^{-2} L of 70% acetone was added, homogenized and left at rest for more than 1 hour at room temperature. After this time the new supernatant was transferred to the same volumetric flask containing the first supernatant and the volume was completed to 10^{-1} L with distilled water. All extractions were performed in triplicate.

Determination of the Content of Phenolic Compounds

The content of phenolic compounds in the crude extract was determined considering the colorimetric method of Folin-Denis. This method is based on reduction of the molybdic and tungstic reagent (Folin-Denis) to a blue colored complex in alkaline solution by phenolic compounds.

The values of total phenolics were expressed as gallic acid equivalents (mg gallic acid equivalent

(GAE) per 100 g of sample). All treatments were analyzed in triplicate.

Evaluation of Antioxidant Activity by the DPPH Method (2,2-Diphenyl-1-Picrylhydrazyl)

The DPPH method is based on the reduction in absorbance of the free radical DPPH (1,1-diphenyl - 2-picrylhydrazyl) by antioxidants at the visible wavelength of 517 nm.

This method considers the transfer of electrons from an antioxidant compound to the free radical, DPPH, which losses its purple color when reduced. Thus, it evaluates only the reducing power of the antioxidant, which undergoes one electron oxidation and for this reason does not detect pro-oxidant substances.

The methods described by Duarte-Almeida (2006), Andrade (2007) and Meda (2005) were used to evaluate the scavenging activity of the free radical DPPH, with a reaction time of 30 minutes and absorbance measured at 517 nm. As a control, an ethanolic solution of 1 mM DPPH was prepared. To assess the radical capture activity, the percent inhibition was obtained in accordance with Equation (1):

$$\% \text{ Inhibition} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \text{x}100 \qquad (1)$$

All treatments were analyzed in triplicate.

Quantification of Capsaicinoids

Determination of the capsaicinoids capsaicin and dihydrocapsaicin was performed by High Performance Liquid Chromatography (HPLC) according to the methodology proposed by Maynard and George (2007). Samples of 2 x 10^{-7} L of the extract were injected onto the HPLC, equipped with a UV detector at 280 nm and a C18 column. The mobile phase for analysis and separation of the capsaicinoids was composed of methanol: water:acetic acid (70:29:1, v/v/v) at a flow rate of 1.0 mL/min and 20 ºC. Identification and quantification of capsaicinoids were performed using the calibration curve and compared with capsaicin and dihydrocapsaicin standards. Data were expressed as milligrams per gram of dry fruit (mg g^{-1}). All treatments were analyzed in triplicate.

Statistical Analysis

All analyses were performed in triplicate. Analysis of variance (ANOVA) and the Tukey test were used to identify significant differences between means, utilizing the SAS - Statistical Analysis System, Version 9.1 (SAS, 1999) licensed to the Universidade Federal de Viçosa - 2006. Differences between means at 5% ($P < 0.05$) were considered significant.

RESULTS AND DISCUSSION

Proximate Composition

Table 1 is presented to verify the influence of drying temperature on components of the pepper. The means of the chemical composition of fresh and dried pepper at temperatures of 45, 55 and 65 °C are shown in Table 2.

During the drying process, moisture loss occurs due to the difference in water vapor pressure between the product and the air surrounding it. This process increases the shelf life due to the lower availability of water for activity of microorganisms and enzymes, also resulting in fewer nutritional and sensorial alterations (Martinazzo *et al*., 2007).

The ash content in fresh peppers differed significantly ($P \geq 0.05$) from peppers subjected to drying, which may have resulted from the temperatures applied, which degrade the micronutrients represented in the analysis of the ashes. Peiró *et al*. (2006) and Peiró-Mena *et al*. (2006) analyzed grapefruit and pineapple and encountered percent losses of the minerals Na, K, Ca and Mg.

Table 1: Summary of the analysis of variance for the chemical components studied.

Component	DF	QM	F
Ashes (g kg^{-1})	3	13.17	1729.12^{*}
Lipids(g kg^{-1})	3	0.05	1.97^{ns}
Proteins (g kg^{-1})	3	0.09	3.07^{ns}
Crude Fiber (g kg^{-1})	3	$0.01\ 10^{-2}$	0.84^{ns}
Vitamin C (mg kg^{-1})	3	2608.55	1043.42^{*}

*F-test significant at 5% probability; ^{ns} F-test non-significant at 5% probability.

Table 2: Means ± standard deviation of the results of chemical composition on a dry basis (db) for the fresh pepper and those dried at the temperatures of 45, 55 and 65 ºC.

Proximate Composition on a dry basis (db)[1]	Cumari Pepper from Pará			
	Fresh pepper	45 °C	55 °C	65 °C
Ashes (g kg^{-1})	$5.18^{a} \pm 0.02$	$1.03^{b} \pm 0.03$	$0.98^{b} \pm 0.10$	$0.96^{b} \pm 0.09$
Lipids(g kg^{-1})	$5.20^{a} \pm 0.02$	$5.19^{a} \pm 0.01$	$5.15^{a} \pm 0.30$	$4.92^{a} \pm 0.24$
Proteins (g kg^{-1})	$4.34^{a} \pm 0.06$	$4.05^{a} \pm 0.03$	$3.98^{a} \pm 0.05$	$3.98^{a} \pm 0.02$
Crude Fiber (g kg^{-1})	$0.13^{a} \pm 0.09$	$0.13^{a} \pm 0.01$	$0.12^{a} \pm 0.10$	$0.11^{a} \pm 0.02$
Vitamin C (mg kg^{-1})	$232.33^{a} \pm 0.10$	$175.67^{b} \pm 0.08$	$172.67^{b} \pm 0.07$	$172.20^{b} \pm 0.70$

*Means followed by same superscript letters, in the same row, do not differ by Tukey test at 5% probability.

Regarding the lipid content, no treatment differed by the Tukey test ($P \geq 0.05$). This indicates the absence of fat degradation during drying. According to Maynard and George (2007), the amount of lipids found in edible raw peppers is 2 g kg^{-1} d.b., lower than that found in this study for all treatments. This difference may be linked to the variation of pepper species studied.

The protein contents also did not differ between treatments. According to Morris *et al*. (2004), heating generally improves the digestibility of foods, making some nutrients more available as in the case of proteins in legumes, which become more digestible after heating because of the inactivation of anti-nutrients such as trypsin inhibitors.

The same was true for crude fiber, where there was no difference between treatments ($P \geq 0.05$). Maynard and George (2007) indicated that the amount of fiber found in edible raw peppers is 18 g kg^{-1}, much greater than that encountered in the present study, which may be attributed to interferences of climate, season and planting techniques, degree of ripeness and other factors (Chitarra and Chitarra, 2005).

Vitamin C contents found in peppers surpassed those of oranges and are equal to those of the fruits guava and acerola (Malpighia emarginata) (Hassimotto *et al*., 2005). Regarding the average content of vitamin C, only the fresh pepper differed from the treatments with dehydration ($P < 0.05$). Queiroz and Vieira (2008) analyzed the content of vitamin C in fresh guava fruits and those dehydrated by different pre-treatment techniques. Convective drying promoted vitamin C losses of 32 to 68% in all treatments. The samples not subjected to osmosis showed less retention of ascorbic acid in this stage than those subjected to osmotic pre-treatments. Veras *et al*. (2012) studied *dedo-de-moça* pepper (*Capsicum baccatum*) during convective and freeze drying and found that in terms of ascorbic acid content, lyophilized samples were superior to convectively dried ones. Vitamin C degradation increased with temperature in the convective drying.

Determination of the Content of Phenolic Compounds, Antioxidant Activity, Capsaicin and Dihydrocapsaicin

Table 3 presents the analysis of variance for phenolic compounds, antioxidant activity and levels of capsaicin and dihydrocapsaicin. Averages of the phenolic compounds, antioxidant activity and levels of capsaicin and dihydrocapsaicin are expressed in Table 4.

The content of phenolic compounds obtained from the fresh pepper was significantly higher than those found in several studies of other species of *Capsicum* (Hassimotto *et al*., 2005; Deepa, 2007). The work of Hassimotto *et al*. (2005), which analyzed the ethanolic extract of ten species of *C. annuum* L., showed total phenolic concentrations in green peppers between 1860 and 11220 mg kg^{-1} and from 323 to 852 mg kg^{-1} in red peppers. Deepa (2007), also using the Folin Ciocalteu reagent and methanol extracts of *Capsicum* peppers, found between 2840 and 5700 mg kg^{-1} of total phenolics in ripe peppers of four species of *C. annuum* and from 2560 to 3540 mg.kg^{-1} in immature peppers.

Table 3: Summary of the analysis of variance for the total phenolic compounds, antioxidant activity, capsaicin and dihydrocapsaicin.

Component	GL	QM	F
Phenolic compounds (mg GAE kg^{-1})	3	51662492.90	1488.34*
Antioxidant Activity (%)	3	1051.95	7442.78*
Capsaicin (mg g^{-1})	3	0.75	51.79*
Dihydrocapsaicin (mg g^{-1})	3	0.04	180.87*

*F-test significant at 5% probability; ns F-test non-significant at 5% probability.

Table 4: Average contents of phenolic compounds, antioxidant activity, capsaicin and dihydrocapsaicin on a dry basis (db) of peppers *in natura* and dried at temperatures of 45, 55 and 65 °C

Drying (°C)	Phenolic compounds (mg GAE kg^{-1})	Antioxidant Activity (%)	Capsaicin (mg g^{-1})*	Dihydrocapsaicin (mg g^{-1})*
in natura	$9748.22^{a} \pm 0.91$	$95.41^{a} \pm 1.02$	$3.40^{a} \pm 0.02$	$0.44^{a} \pm 0.02$
45	$1480.25^{b} \pm 1.09$	$58.65^{b} \pm 2.34$	$2.42^{bc} \pm 0.06$	$0.40^{a} \pm 0.05$
55	$1450.70^{b} \pm 0.67$	$58.10^{bc} \pm 3.32$	$2.73^{b} \pm 0.06$	$0.24^{b} \pm 0.06$
65	$1415.44^{b} \pm 0.88$	$57.19^{c} \pm 1.00$	$2.26^{c} \pm 0.09$	$0.21^{b} \pm 0.08$

*Means followed by same superscript letters, in the same column, do not differ by Tukey test at 5% probability.

Expressing the data in this study on a 1 g basis, closer to the real consumption, a value of 97.48 mg of GAE per 1g of the *C. chinense Jacqui* pepper is obtained. This value is higher than that reported for fresh apples (mean of 2.96 ± 6.40 mg $GAE.g^{-1}$), considered to be a good source of phenolic compounds (Howard, 2000).

With respect to kiln dried peppers, they presented lower total phenolic contents when compared to fresh peppers. There was no statistical difference ($P > 0.05$) when analyzing the effect of different temperatures in relation to the content of phenolic compounds. Therefore, the highest temperature can be considered to be the most viable, since it reduces the time and consequently the costs of processing, resulting in amounts of phenols statistically equal to the other temperatures. This fact is interesting because the use of high temperatures during extraction, pasteurization and storage of food can cause losses of phenolic compounds, especially due to the degradation of anthocyanins; however, processes that utilize short treatment periods at high temperatures have been recommended for retention of pigments. In the case of red fruit juices, anthocyanin losses were shown to be negligible for heat treatments lasting less than 12 minutes at 100 °C (Sun *et al*., 2002).

In addition to temperature and light, the content of phenols is also affected by hydrolysis and oxidation reactions (enzymatic or not), as well as complexation. Enzymatic oxidation of phenolic compounds is mainly caused by polyphenol oxidases. Injury to the cell membrane liberates and therefore activates these enzymes, which in turn oxidize phenolic compounds to quinones (Markakis, 1982).

Results of the quantitative evaluation of antioxidant activity for the extracts, at the concentration of 2000 μg/mL, determined by the DPPH test, presented radical scavenging activities with a significant difference ($P \geq 0.05$) between the fresh pepper and the drying treatments. Significant differences were verified between the treatments at different temperatures, where the use of lower temperatures caused a slower loss of antioxidant activity.

Using the classification of Hassimotto *et al*. (2005) as a parameter, in which inhibition values > 70% indicated good activity, with intermediate for values of 40 to 70% inhibition and low for < 40% inhibition, it was observed that: the extracts from the fresh pepper presented excellent antioxidant action, while the peppers subjected to dehydration showed intermediate antioxidant activity.

Among the capsaicinoids, capsaicin is the alkaloid found in highest quantities in the peppers (33 to 77%), followed by dihydrocapsaicin (22 to 51%), which together account for about 90% of total capsaicinoids. Other types of capsaicinoids are nordihydrocapsaicin (7-15%), homocapsaicin (≈ 1%) and homodihydrocapsaicin (≈ 1%) (Markakis, 1984).

Lannes (2007) evaluated the species *C. chinense* and encountered capsaicin concentrations of 4.0 mg. g^{-1} and dihydrocapsaicin concentrations of 2.4 mg. g^{-1} for samples dried at 60 ºC for 72h. These values are greater than those found in the present study.

Materska and Perucka (2005), in evaluating the antioxidant activity of capsaicin and dihydrocapsaicin extracted from *C. annuum* L., verified by the DPPH method that the antioxidant action of capsaicin was greater than that of dihydrocapsaicin, indicating that the double bond in the lipid chain of capsaicin influenced antioxidant activity. However, Daood *et al*. (2006) found that capsaicinoids have a different impact on the stability of carotenoids depending on maturation and processing conditions.

Thus, the higher level of capsaicin in the fresh pepper justifies its higher antioxidant activity compared to peppers subjected to heat treatments.

CONCLUSION

It can be concluded from the present study that the macronutrients were not degraded when subjected to temperatures of 65 °C. However, the micronutrients (ash and vitamin C), together with the

phenolic compounds, capsaicin and antioxidant activity, were degraded by the use of high temperatures. The content of phenolic compounds with antioxidant activity can be associated with the percentage of capsaicin found after treatment of different types of peppers. Dehydration treatments increase the shelf life and reduce the volume of the product, but do not retain the nutritional and antioxidant characteristics of fresh Cumari chili peppers obtained from Pará.

NOMENCLATURE

V/V	Volume/volume
GAE	Gallic acid equivalent
DPPH	1,1- diphenyl – 2 picrylhydrazyl
HPLC	High performance liquid chromatography
ANOVA	Analysis of variance
SAS	Statistical analysis system

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