Evaluation of antioxidant potential of pepper sauce (Capsicum frutescens L.)

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Abstract: Functional properties of substances present in in natura foods such as fruits and vegetables are well documented; however, the activity that remains after processing needs more research. The present study aimed to evaluate the antioxidant potential in fruit processed as sauce and quantify the compounds able to contribute to such activity. Three different treatments were developed varying only the concentration of pepper Capsicum frutescens L., with treatment ratios (fruit: water: vinegar: salt) being: treatment 1 (0.5: 1: 0.5: 0.33), 2 (1: 1: 0.5: 0.33), and 3 (2: 1: 0.5: 0.33). By the DPPH method, the values found for EC₅₀ (g g DPPH⁻¹) from 3726.9 to 5425.9 for the alcoholic extract were the most significant. The content of total phenols did not vary between the three treatments. While the content of carotenoids found was significantly different in the treatment with lower content of the fruit in natura, when compared to the treatment with higher content (44.02 and 56.09 μg of β-carotene 100 g⁻¹, respectively) and the content of ascorbic acid varied between 10.95 and 21.59 mg 100⁻¹ g. Therefore, the pepper sauce was presented as an alternative to the consumption of bioactive compounds that may have antioxidant potential.

Subjects: Food Analysis; Fruit & Vegetables; Nutraceuticals & Functional Foods

Keywords: pepper sauce; antioxidant potential; Capsicum frutescens L.

1. Introduction
The genus Capsicum, which originates from tropical and humid zones of Central and Southern America, belongs to the Solanaceae family and includes peppers of important economic value where
three of the several widely spread Capsicum species are Capsicum annuum, Capsicum frutescens, and Capsicum chinense (Giuffrida et al., 2013). These are also the main domesticated species of peppers (Capsicum sp.) which are consumed in Brazil (Aguiar, Coutinho, Barbero, Godoy, & Martínez, in press).

There is a growing search for foods that benefit health, such as foods containing antioxidants since many of the health problems are associated with the action of toxic forms of oxygen responsible for oxidation processes. Peppers contain a number of these substances with antioxidant properties that can have a significant impact on diseases and are essential to human health (Carvalho et al., 2015), so they are an important nutrient for human diet since they are a good source of different phytochemical compounds, including vitamin C, phenolic compounds, flavonoids, and carotenoids (Aguiar et al., in press; Korkutata & Kavaz, 2015; Zhuang, Chen, Sun, & Cao, 2012). In addition, peppers are heavily consumed throughout the world and valued for their sensory character as colorants, flavors, and pungency (Conforti, Statti, & Menichini, 2007).

The pepper market is segmented and diverse due to the variety of products and byproducts, uses, and forms of consumption and is basically divided into fresh products and processed as colorants, paste, paprika, oleoresin, preserves, and sauces (Junior, Tavares, Filho, Zini, & Godoy, 2012; Topuz & Ozdemir, 2007). Although there are several researches conducted on fresh peppers, mainly to quantify bioactive compounds and/or antioxidant activity (Carvalho et al., 2015; Deepa, Kaur, George, Singh, & Kapoor, 2007; Topuz & Ozdemir, 2007; Zhuang et al., 2012), however, researches with peppers of the Capsicum genus are needed to evaluate its bioactive compounds after processing and their use in the preparation of sauces, since the content and antioxidant capacity of vegetables can be altered due to processing such as in the case of processing using cooking temperatures.

Thus, the present study aimed to evaluate and verify the maintenance of antioxidant potential, after processing in the form of sauce. In addition, the content of total phenolic compounds which are substances with declared antioxidant activity present in the sauce was also evaluated, as well as the content of total carotenoids and ascorbic acid.

2. Materials and methods

2.1. Material

The in natura chili peppers (C. frutescens L.) were obtained in an open market in the city of Barra do Garças (MT). The fruits were purchased with sizes between 1.5 and 3.0 cm long and 0.4–0.5 cm wide, selected by the degree of completely red color, firm, and free from damage or injuries. These were sanitized and packaged in the Food Analysis Laboratory of the Federal University of Mato Grosso until the time of sauce preparation. Wine vinegar, water, and sodium chloride were used for the treatment of the sauce.

2.1.1. Preparing the sauce

For the preparation of sauces, about 2 kg of in natura chili peppers were crushed in a blender with water. Three treatments were prepared, of which only the pulp concentration varied. For the first treatment, pulp was mixed with water, vinegar, and salt, yielding a sauce at a ratio (0.5:1:0.5:0.33), for Treatments 2 and 3, the sauce ratios were (1:1:0.5:0.33) and (2:1:0.5:0.33), respectively. The extracts were subjected to cooking for about 10 min and were then packaged at a temperature of 85 °C in properly cleaned and labeled 1 l glass bottles.

2.1.2. Extracts

To carry out the total phenolic and antioxidant potential analysis, chili sauce extracts were used obtained by mixing about 15 g of sauce to 100 ml of ethyl alcohol PA and subjecting the mixture to magnetic stirring for 60 min, followed by centrifugation, and then storing the supernatant amber vial.
For the aqueous extract, the same procedure was followed, except the solvent, which was now 100 ml of ultrapure water.

2.2. Methods

2.2.1. Antioxidant activity
The antioxidant activity was determined by the method proposed by Rufino et al. (2007), which consists of a decrease in the absorbance as the organic radical DPPH (2,2-diphenyl-1-picryl-hydrazyl) is exposed to an antioxidant. The results were expressed as EC50 (g g of DPPH⁻¹).

2.2.2. Determination of total phenols
Total phenolic compounds were determined using the methodology elucidated by Badiale-Furlong, Colla, Bortolato, Baisch, and Souza-Soares (2003) with some modifications. Twenty-five-milliliter aliquots of extract were removed and clarified with 5 ml of barium hydroxide 0.3 M and 5 ml of 5% zinc sulfate, leaving at rest for 20 min and then proceeding to centrifugation.

Two milliliters of clarified extract was used, to which 2 ml of 2% calcium carbonate in NaOH was added. This was left in water bath for 10 min at 37 °C and then 1 ml of Folin Ciocateau (1:2) was added in ultrapure water. The measurement was made in a spectrophotometer (SP-2000) at 565 nm, using ethanol as white to make reading of the ethanol and ultrapure water extract as white to the aqueous extract. As a standard, gallic acid concentrations ranging from 10 to 100 mg l⁻¹ was used to construct the standard curve. Through this curve, the total phenol content in mg GAE 100 g⁻¹ fruit.

2.2.3. Determination of total carotenoids
The quantification of carotenoids was performed according to the method described by Rodriguez-Amaya (1999). Quantitation was conducted spectrophotometrically (SP-2000) at a range of 450 nm using petroleum ether as white. The content of carotenoids was expressed in μg of β-carotene g⁻¹ sample.

2.2.4. Determination of ascorbic acid content
For the analysis of ascorbic acid, the methodology proposed by Benassi and Antunes (1998), which is based on reducing the DCFI indicator (2,6-dichlorobenzenoindofenol) by ascorbic acid was used. The results were expressed in mg of ascorbic acid 100 pulp g⁻¹.

2.2.5. Physicochemical determinations
The titratable acidity was determined by the volumetric method described by Adolfo Lutz Institute (2008), at 0.1 M. The pH was measured using direct determination with a pH meter (DLA-PH model) (Adolfo Lutz Institute, 2008). The determination of soluble solids was performed according to the methodology of the Adolfo Lutz (2008), whose reading was performed on a WYA-Abbé refractometer (2WA-J) and the results were expressed as °Brix.

2.2.6. Statistical analysis
The experiment had a completely randomized design. Data were subjected to analysis of variance (p < 0.05) and Tukey test. The results were expressed as mean ± standard deviation. To process the data, the Assistat 7.7 software was used.

3. Results and discussion
Pepper is an important vegetable in human nutrition, thus it is important to understand the contributions of some of its bioactive compounds for one of the processed forms, and that is most widely consumed around the world, the chili sauce. The antioxidant activity results of aqueous and alcoholic extracts for pepper sauce C. frutescens L. are shown in Figure 1. The values were expressed as EC50 (concentration of antioxidants present in extracts, capable of reacting with 50% of the radical in DPPH solution).
The lower the EC$_{50}$ value, the higher the antioxidant activity of the extract, since less extract is needed to achieve such an effect. Therefore, the alcoholic extract showed the highest antioxidant activity compared to the aqueous extract. These results suggest that the antioxidant compounds present in pepper sauce may have a greater chemical affinity for the ethanol used in the extraction, than with water, which explains the greater antioxidant activity in this extract.

Comparing the alcoholic extract of *in natura* *C. frutescens* L. with alcohols extracts obtained from the different treatments, it can be seen that the antioxidant property suffers a loss of about 29.13%, on the most important alcoholic extract (treatment 3). The same comparison made for the aqueous extract suggests that although the antioxidant activity is lower than in the alcoholic extract, the loss of this activity was lower, representing about 14.55%.

Evaluating different varieties of *Capsicum*, Carvalho et al. (2015) found values between 1745.18 and 4905.06 g g DPPH$^{-1}$ for different varieties of *Capsicum*, with results similar to those obtained in this study for the alcoholic extract, but not for the aqueous extract.

The results of the total phenolic content, total carotenoids, and vitamin C of pepper sauce (*C. frutescens* L.) can be found in Table 1.

Phenolic compounds have received the attention of researchers because of their biological activity, mainly antioxidant activity and the ability to act in the human body as free radicals, developing certain role in preventing certain diseases such as cardiovascular, neurodegenerative disorders, and cancer (Aguiar et al., in press; Zhuang et al., 2012). The content of phenolic compounds among the treatments studied did not differ significantly (p < 0.05), suggesting that independent of the pepper

| Treatment | Total phenols (mg GAE 100$^{-1}$ g fruit) | Total carotenoids (μg g$^{-1}$ fruit)* | Ascorbic acid (mg 100$^{-1}$ g fruit) |
|-----------|-----------------------------------------|--------------------------------------|-------------------------------------|
| 1         | 37.29±0.08                              | 44.02±3.77                           | 10.95±1.74                          |
| 2         | 37.58±0.85                              | 51.87±3.37                           | 15.59±2.25                          |
| 3         | 41.75±5.14                              | 56.09±1.27                           | 21.59±0.95                          |

Notes: Means followed by different lowercase letters in the column differ by Tukey test (p ≤ 0.05).
*μg equivalent of β-carotene g$^{-1}$ of fruit.
concentration used in the treatment of the sauce in the present study, the phenolic content does not vary. For *in natura* Capsicum annum L. peppers, Nagy et al. (2015) found a total phenolic content of 95.5 mg GAE 100 g⁻¹, and this result was superior to that found in present sauce treatments evaluated. The results obtained by Aguiar et al. (in press) and Zhuang et al. (2012) for *C. frutescens* peppers also contradict the values obtained for the pepper sauce; this may be due to the effect of thermal processing to prepare the product.

According to Conforti et al. (2007), peppers are often a good source of carotenoids, which may vary in composition or content depending on aging and genetic differences. The content of carotenoids found for treatment 3 (56.09 μg g⁻¹) was the one that had the highest result, not differing significantly from treatment 2 (51.87 μg g⁻¹). In comparison with the content of carotenoids evaluated by Carvalho et al. (2015) for different species of red peppers *Capsicum* sp; *C. annuum* L. and *C. baccatum* L. var. *Umbilicatum*, being 1349.97, 1064.35, and 580.98 μg g⁻¹, respectively. Zhuang et al. (2012) also reported a total carotenoid content of 1112.94 μg g⁻¹ for *C. frutescens* L. peppers, results that were much higher than those found in this study for the sauces.

Similar to the content of carotenoids, ascorbic acid content was higher for Treatment 3 (21.59 mg 100⁻¹), which in turn did not differ significantly from Treatment 2 (15.59 mg 100⁻¹), thus by analyzing the pepper sauce for its content of bioactive compounds, Treatments 2 and 3 are the best, since they showed the largest values and do not differ significantly as a function of the three bioactive compounds studied.

Rebouças, Valverde, and Teixeira (2013) showed a content of 121.5 g mg 100⁻¹ for *in natura* *C. frutescens* pepper and 14.5 g mg 100⁻¹ for the same pepper processed in a canned form, a value within the range obtained in this study for the sauce. According to Costa, Moura, Marangoni, Mendes, and Teixeira (2010), the content of ascorbic acid present in peppers is influenced as a stage of maturation, storage, and conservation; however, besides these factors, Rebouças et al. (2013) point out that vitamins such as ascorbic acid are sensitive and can be degraded by the action of temperature, presence of oxygen, light, moisture, pH, and duration of treatment which the food was submitted. Ascorbic acid content found in this study may probably have suffered the action of sauce processing, mainly due to temperature, which explains the low levels when compared to the literature data.

The results of physicochemical parameters evaluated for *C. frutescens* L. pepper sauce are shown in Table 2.

With respect to the values obtained for the soluble solids, the three treatments differed significantly (*p < 0.05*) from each other, with Treatment 1 being of the highest value (9.3 °Brix) and Treatment 3 the lowest (6.3 °Brix). As for the results of titratable acidity and pH, Rebouças et al. (2013) obtained values of 0.0353% TA and pH 5.48 for *in natura* chili pepper and 0.2046% TA and pH of 3.76 for the same pepper processed in the form of preserves, and demonstrated an inverse relation between pH and acidity values. The results found here confer with this inverse relation. The low pH found for the pepper sauce can be explained by the incorporation of vinegar in different treatments.

### Table 2. Physicochemical parameters evaluated for the *C. frutescens* L. hot sauce

| Treatments | Soluble solids * | Titratable acidity ** | pH       |
|------------|-----------------|-----------------------|----------|
| 1          | 9.3±0           | 0.69±0.001            | 3.87±0.01|
| 2          | 6.5±0           | 0.52±0.01             | 4.08±0.01|
| 3          | 6.3±0           | 0.41±0.01             | 4.05±0.07|

Notes: Means followed by different lowercase letters in the column differ by Tukey test (*p ≤ 0.05*).

*Expressed in °Brix.

**Expressed as percentage citric acid.
4. Conclusions

The antioxidant potential for chili sauce was considerable and is probably due to the synergistic contribution of the bioactive compounds present in the sauce constituents. However, more studies are needed for a more detailed characterization of the antioxidant activity of this product.

The variation in the composition of the sauce influenced the content of total carotenoids and vitamin C content, with the best found both in Treatments 2 and 3, since they did not differ. The chili pepper (C. frutescens L.) sauce was presented as a major source of phenolic compounds, which in turn did not change significantly depending on the composition of the studied sauces.

Thus, the C. frutescens L. hot sauce is presented as an alternative for the use of bioactive compounds that may have antioxidant potential and may contribute to the prevention of the negative effects of free radicals.

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The authors declare no competing interest.

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