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Non-enzymatic and enzymatic antioxidant components of the mature Cambuí metabolism

Componentes antioxidantes no enzimáticos y enzimáticos del metabolismo de Cambuí maduro

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Abstract

The objective of this work was to identify and evaluate the bioactive compounds, as well as the antioxidant potential, present in mature fruits of cambuizeiro (*Myrcia multiflora*) from the Chapada do Araripe, Brazil. In the fruits were evaluated: vitamin C, total anthocyanins, yellow flavonoids, total polyphenols, total antioxidant activity by the FRAP, DPPH and ABTS methods, and the antioxidant superoxide dismutase (SOD) and catalase (CAT) activity. Cambui had an average vitamin C content of 56.40 mg/100 g, 2869.44 mg/100 g total anthocyanins 2858.50 mg/100 g yellow flavonoids and 1007.64 mg EAG/100 g of total polyphenols. While the total antioxidant activity according to FRAP method was 123.94 µM ferrous sulphate/g, DPPH was 1805.66 g/g EC50, and ABTS was 32.62 µM trolox/g. The enzymatic activity of SOD was 846,203 AU/g, while CAT did not read. These results indicate that the mature fruits of cambuizeiro present high antioxidant potential, corroborating to the idea that their consumption may result in benefits to human health.

Key words: antioxidant activity; bioactive compounds; plant extractivism; *Myrcia multiflora*; Myrtacea.

Resumen

El objetivo de este trabajo fue identificar y evaluar los compuestos bioactivos, así como el potencial antioxidante, presentes en frutos maduros de cambuizeiro (*Myrcia multiflora*) procedente de la Chapada del Araripe, Brasil. En los frutos fueron evaluados: vitamina C, antocianinas totales, flavonoides amarillos, polifenoles totales, actividad antioxidante total por los métodos de FRAP, DPPH y ABTS y actividad de la enzima antioxidante superóxido de la dismutasa (SOD) y catalasa (CAT). En los frutos de cambuí se encontró un contenido medio de vitamina C de 56,40 mg/100 g; 2869,44 mg/100g de antocianinas totales 2858,50 mg/100g de flavonoides amarillos y 1007,64 mg EAG/100 g de polifenoles totales. Mientras que la actividad antioxidante total según método FRAP fue de 123,94 µM de sulfato ferroso/ g, el DPPH fue 1805,66 g/g EC50, y el ABTS fue 32,62 µM trolox/g. La actividad enzimática de la SOD fue 846,203 UA/g, mientras que no se registró presencia de CAT. Estos resultados indican que los frutos maduros del cambuizeiro presentan elevado potencial antioxidante lo que sugiere que su consumo puede resultar en beneficios a la salud humana.

Palabras-clave: actividad antioxidante; compuestos bioactivos; extractivismo vegetal; *Myrcia multiflora*; Myrtacea.

Introduction

The Caatinga is an exclusively Brazilian biome, characteristic of having a wide diversity. Extractivist resources stand out because of their significant social, economic and environmental importance, which is often attributed to smallholdings, making up a significant part of the income of the families involved (Gariglio, Sampaio, Cestaro and Kageyama, 2010).

The Chapada do Araripe is a great wall that divides the Brazilian states: Ceará, Pernambuco and Piauí. Its vegetation contributes to local characterization, as well as a direct influence on the availability of local water resources, making possible the presence of numerous water sources, even during periods of irregular rains and/or periods of drought (Araújo et al., 2013).

In the Chapada do Araripe it is possible to find the cambuizeiro (Cambuí) native, an arboreal mirtácea, much appreciated by birds and sought by the local population, where it is extracted by family farmers who make sweets, such as ice cream and jellies, produce flour from the bark, use leaves for medicinal purposes, and produce organic wine (Gama et al., 2017).

Cambuí (*Myrcia multiflora*) is a globose fruit, with juicy and edible pulp. They present fruits with colors ranging from dark red to purple when ripe, containing one, two or three seeds, with a bitter taste. They occur mainly on highland plots, poor soils, sandy or non-floodable field margins (Lorenzi, 2009).

Although the cambuí is characterized as one of the main fruits responsible for generating income to the family farmers of the region of Chapada do Araripe, as well as empirically it is used in the treatment of some diseases, in some regions of Brazil a decrease is occurring in the extraction of these fruits.

Today, the search for healthy eating has become more and more frequent. People are concerned with introducing into their nutritional diet foods produced from nutrient-rich raw materials, especially those substances that cannot be synthesized by humans, such as vitamin C (Santos and Oliveira, 2014).

Studies addressing food sources with levels of bioactive compounds are showing an increasing interest not only in scientific society but also in consumers. Particularly noteworthy is the antioxidant activity, since it presents beneficial actions against chronic and degenerative diseases. According to Oliveira, Pinto and Rezende (2017), vitamin C is an antioxidant that is directly involved in the formation of connective tissue, antibodies and production of hormones, being essential for human health. Other substances such as phenolic compounds are also associated with the antioxidant potential, reducing the risks of cardiovascular diseases, as well as acting on the oxidative stress that is directly related to a series of health problems (Pessoa et al., 2015).

In this context, new studies of physical, chemical and biochemical properties of fruits from regions with great biodiversity still unknown are becoming more relevant. The lack of research work or studies available in the specialized literature related to the chemical composition and nutritional quality of the cambuí can lead to the devaluation in the commercialization of products and by-products from the fruits. The evaluation of the potential of the fruit becomes an alternative to add value to the product and by-products produced in the region of Chapada do Araripe, and consequently the increase in the income generation of extractive families. In this way, the objective of the present work was to identify and evaluate the bioactive compounds, as well as the antioxidant potential, present in mature fruits of Cambuizeiro from the Chapada do Araripe.

Material and methods

Collection and preparation of the sample

In the present study, the fruits of the red variety Cambui, from the Araripe Plateau, were analyzed in order to evaluate the secondary metabolites and enzymatic and antioxidant activity. About 800 g of the fruits were purchased by donation in April 2017 by residents of the Cariri region, Ceará, Brazil.

The experiment was conducted at the Laboratory of Biochemistry and Post-Harvest Physiology of Fruits, in the Department of Biochemistry and Molecular Biology, Federal University of Ceará - UFC. The fruits containing whole seeds, shells and pulp were crushed in the Philips Walita Juicer multiprocessor (RI 1858) and filtered through a sieve to facilitate homogenization. After obtaining, the pulps were stored in eleven airtight containers, for better handling at the time of analysis, thus avoiding the loss of the material by external agents (temperature, luminosity, etc.) and kept in freezer (-20 °C). The analysis were performed in triplicates.

Non-enzymatic antioxidant components

For quantification of ascorbic acid, the method described by Chen and Wang (2002) was used, using the concentration of 0.1 g of cambium pulp in 5 ml of 5% trichloroacetic acid (TCA).

The absorbance of the solution was recorded at 525 nm and the results expressed in mg/100 g fresh sample. Total anthocyanins and yellow flavonoids, determined spectrophotometer according to Francis (1982), with absorbance of 535 nm for total anthocyanins and 374 nm for yellow flavonoids.

The quantification of polyphenols was started by obtaining the extract, where it was also used to determine the antioxidant activity by the FRAP, ABTS and DPPH methods. The extraction of polyphenols was performed according to the methodology of Larrauri, Rupérez and Saura-Calixto (1997), using the Folin-Ciocalteu reagent, with adaptations made by Rufino et al. (2006a). Polyphenol quantification was performed spectrophotometrically at 700 nm of absorbance, using a standard curve prepared with gallic acid (gallic acid equivalent). The assays were performed in triplicate and in dark environment; the readings were performed at 30 minutes after addition of the reagents. The results were expressed in mg EAG/100 g fresh sample.

In the evaluation of the total antioxidant capacity by the FRAP method (Ferric Reducing Antioxidant Power) was adopted the methodology of Rufino et al. (2006b). The reading was performed with absorbance of 595 nm calibrated with the FRAP reagent after 30 minutes of the preparation of the mixture. The results were expressed in µM ferrous sulfate/g fresh sample. In the DPPH (2,2-diphenyl-1-picryl-hydrazyl) method, the methodology of Rufino et al. (2007a) was adopted. Absorbance was monitored every minute, where its reduction stabilized at 40 minutes. The final absorbance reading for the calculation was performed after absorbance stabilization. The results were expressed as g/g EC 50. For ABTS + (Trolox Equivalent Antioxidant Capacity) the methodology of Rufino et al. (2007b) was followed, in which the absorbance reading of 734 nm was taken after 6 minutes of the mixture, using ethyl alcohol, as white, to calibrate the spectrophotometer. The results were expressed in µM trolox/g fresh sample.

Enzymatic antioxidant components

In the determination of the activity of the enzyme superoxide dismutase (SOD) the methodology of Giannopolitis and Ries (1997) was used, which evaluated SOD due to the ability of the enzyme to inhibit nitrotetrazolium (NBT) blue photoreduction. The reading was made at 560 nm, where one unit of SOD (U) activity was defined as the amount of enzyme required to inhibit 50% reduction of NBT and activity was expressed in UA/g. The determination of the activity of catalase (CAT) enzymes was performed by the methodology of Beers Júnior and Sizer (1952), where the activity was determined by the degradation of H2O2 in the interval of 1 minute, at 240 nm. The quantification was done by adopting the molar extinction coefficient of 36 M-1 cm-1 and expressed in µmol H2O2 g-1 min-1 P.

Statistical analysis

The results of the analyzes were expressed as mean ± standard deviation, using the R® program, version 3.4.3. The Pearson correlation analysis was performed using the Statistica program, version 10, and its accuracy was evaluated by the correlation coefficient.

Results

The samples analyzed for non-enzymatic antioxidant content presented significant amounts of vitamin C, registering 56.40 mg/100 g. The total anthocyanins presented 2869.44 mg/100 g, while the yellow flavonoid levels registered 2858.50 mg/100 g. Regarding the total polyphenol content (PET), cambuí samples presented 1007.64 mg EAG/100 g. The total antioxidant activity (ATT) determined by the FRAP, DPPH and ABTS methods presented respectively: 123.94 µM ferrous sulphate/g, 1805.66 g/g EC50 and 32.62 µM trolox/g (Table 1).

Table 1. Quantification of the non-enzymatic antioxidants of cambuizeiro fruits. Chapada do Araripe, Brazil.

Non-enzymatic antioxidants	Results*
Vitamin C	56.40 ± 10.73**
Total anthocyanins	2869.44 ± 80.86
Yellow flavonoids	2858.50 ± 84.23
Total polyphenols	1007.64 ± 60.92
Total Antioxidant Activity (ATT):	
FRAP	123.94 ± 13.71
DPPH	1805.66 ± 259.17
ABTS	32.62 ± 7.59

* Vitamin C, total anthocyanins and yellow flavonoids (mg/100g); Total polyphenols (mg EAG/100g); FRAP (µM ferrous sulphate/g fresh sample); DPPH (g/g EC 50); ABTS (µM trolox/g fresh sample). ** Mean values ± standard deviation of the mean determination in triplicate.

As for the quantification of the enzymatic antioxidant content, the fruits presented 846,203 AU/g for SOD. However, the enzymatic activity of CAT was characterized at 0, 15, 30, 45, 60, 90, 120 minutes, but did not present reading (Table 2). The antioxidant activity of the cambuí fruits showed a significant correlation with the content of polyphenols (R^2 = 0.405). The other correlations were direct and significant, however, too weak.

Table 2. Quantification of the enzymatic antioxidants of cambuizeiro fruits. Chapada do Araripe, Brazil.

Antioxidants enzyme	Results
SOD	846.203 ± 30.51*
CAT	ND

SOD (UA/g); CAT (µmol H_2O_2 mg^{-1} min^{-1} P). ND = Not detected. * Mean values ± standard deviation of the mean determination in triplicate.

Discussion

The content of vitamin C found is similar to that found in other fruits, such as orange (62.50 mg/100g), recognized for its source of vitamin C, mandarin (41.8 mg/100g), guava (71.4 mg/100g), papaya (Table 1), which is the most common source of fruit consumption in Brazil, and is the main source of fruit consumption in Brazil (Couto and Canniatti-Brazaca, 2010; Oliveira, Aquino, Ribeiro, Proença and Pinheiro-Sant'Ana, 2011).

Ramful, Tarnus, Aruoma, Bourdon and Bahorun (2011) studied citrus fruits in relation to ascorbic acid content in three categories: low (<30mg/100g-1), medium (30-50mg/100g -1) and high (> 50mg/100g-1). According to this classification, the cambuí samples evaluated can be classified with high ascorbic acid content.

The natural vitamin C content of the fruit undergoes changes that depend on many factors, including maturation stage, cultural treatments, soil type, climatic conditions, and the time they were harvested (Santos, Abreu, Freire and Corrêa, 2013). The conditions and period of post-harvest storage may also directly influence the amount of this constituent.

The anthocyanins result can be justified by the strong presence of pigmentation in the pulp, besides the bark. When compared to those found by Ribeiro et al. (2011), where they analyzed the amount of anthocyanins in juçara and açaí pulps, reaching values of 235.8 mg/100 g for juçara and 32.32 mg 100 g for açaí, the expressed value of anthocyanins becomes high. This value is relevant, considering that the presence of anthocyanins acts in the biological system against oxidative damages. The highest amount of anthocyanins in the mature stage can be justified by the synthesis of these pigments that occurs during their maturation, reaching the maximum value in the fully mature fruit (Taiz and Zeiger, 2012).

The high amount of yellow flavonoids found, reaffirms the benefits of the insertion of cambuí to the nutritional diet, considering that the flavonoids exhibit various biological activities, such as antioxidant, anti-inflammatory and antitumor activity, besides the inhibition of collagen destruction platelet aggregation (Araújo, 2008).

The result of polyphenols was superior when compared to other fruits. In research with papaya formosa, fruit commercialized in large scale in Ceasa of Minas Gerais, Oliveira et al. (2011) quantified it with 88.1mg EAG/100g. While in commercial and native Pythia species it was possible to obtain an average value of 23.08 mg EAG/100 g in studies from Lima, Faleiro, Junqueira, Cohen and Guimarães, (2013). Rufino et al. (2010), when studying the content of total extractable polyphenols in eighteen fruit species, found a variation of 23.8 mg EAG/100 g for bacuri at 1176.0 mg/100 g for camu-camu. In the same experiment, Rufino et al. (2010) quantified the jabuticaba, belonging to the same Cambui family with 440.0 mg/100 g, thus demonstrating the variation of polyphenols in fruits of the same family.

The content of polyphenols in food varies by several factors, such as the geographic region of planting, variation to the sun exposure, method of cultivation adopted, use of fertilization or not, variety chosen, and others (Faller and Fialho, 2009). Regarding the iron reduction capacity (FRAP), the cambuízeiro fruit exhibited moderate activity, if comparable to the açaí studied by Infante et al. (2013), which presented 220 µmol Fe_2SO_4/g. Through this result obtained with the analyzes, it was verified that the fruit presents considerable antioxidant activity by the FRAP test, being even larger than that of some vegetables commonly consumed in Brazil. However, the DPPH result demonstrates a greater affinity of the sample for the radical, thus demonstrating that cambuí exhibits oxidizing power.

Rufino et al. (2010) studying 18 non-traditional tropical species, quantified some species of climatic conditions similar to Cambui, as well as umbu and mangaba, and achieved 7074.0 g/g EC50 and 3385.0 g/g EC50, respectively. Thus, demonstrating that the fruits coming from the Caatinga, have great antioxidant activity.

The results by the ABTS method were like those found by Rufino et al. (2010) when studying the bioactive compounds and antioxidant capacity of eighteen Brazilian non-traditional tropical fruits by ABTS method obtained values from 6.3µM trolox/g of pulp to the umbu to 152.7µM trolox/g of pulp for camu-camu, confirming the high variation existing for antioxidant capacity in different fruits.

Cunha Neto, Rabelo, Bertini, Marques and Miranda (2012) studied the agronomic characterization and the antioxidant potential of fruits of clays of acerola, obtained SOD variation from 57.48 to 688.21 AU/g, among the fruits of the different clones. The result found in cambui was superior and can be directly related to the senescence process of the fruit.

The results obtained in the cambuí samples indicate that the SOD among the enzymes evaluated is the most present, since it presented considerable values. In the correlation test, it was possible to identify that the higher the antioxidant activity of the fruits the higher the correlation of the antioxidant capacity with the cambui polyphenols. Almeida et al. (2011) verified positive correlations between PET content and antioxidant activity in exotic fruits of the northeast of Brazil, using ABTS and DPPH methods.

Conclusions

The phenolic compounds contributed significantly to the antioxidant activity of the cambuí fruits evaluated. The fruit presented higher antioxidant activity by the free radical sequestration method DPPH.

In view of the data presented, Cambui is a fruit rich in bioactive compounds, especially non-enzymatic antioxidants (vitamin C, total anthocyanins, yellow flavonoids and total polyphenols), lacking in more studies about the profile of these compounds, especially its health benefits.

The presence of antioxidant activity in this fruit, derived from extractivism, contributes to the perspective of a better use, from the nutritional and technological point of view, adding value to the fruit and its products. In addition, it may be a source of study for pharmacological and nutraceutical use.

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