EVALUATION OF CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF *AÇAÍ* PULP IN THREE RIPENESS STAGES

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Abstract.

Açaí (*Euterpe oleracea* Mart.) is a palm tree native of the Amazon region and of great social, economical and nutritional relevance for its population. From its fruit, a thick dark-purple pulp is obtained, of creamy texture and energetic value due to its high lipid content (20 g/100g dm). However, available data on *açaí* composition is scarce and mainly for ripe fruits. The objective of this work was to evaluate the chemical composition and antioxidant activity of *açaí* pulp obtained in three different ripeness stages. *Açaí* fruits were collected at the Experimental Station of the Federal Rural University of Amazon in Castanhal, PA, Brazil, and processed for pulp extraction under controlled operational conditions. Proximal composition, pH, soluble solids, acidity, total phenolics, anthocyanins and antioxidant activity (ABTS) were measured in pulp samples obtained at the three ripeness degrees. Lipid, protein and carbohydrate content, ash, acidity, soluble and total solids were higher for the ripe fruit pulp. Anthocyanins content was very low for the unripe and half-ripe fruit pulps when compared to those mature suggesting that these flavonoid components are formed during maturation. Two major monomeric anthocyanins were identified by HPLC in ripe fruit pulp, cianydin-3-ruthinoside and cyanidin-3-glycoside, with a higher content of the former. Total phenolics decreased (31%) from the unripe to the half-ripe pulp but increased (189%) in the ripe pulp. A similar pattern of change with ripeness degree was observed for the antioxidant activity suggesting that total phenolic compounds are major determinants of antioxidant activity in *açaí* pulp.

Keywords. Tropical fruits, bioactive compounds, anthocyanins, phenolic compounds, antioxidant activity.

1.Introduction

Açaí is the fruit of *açaí* tree (*Euterpe oleracea* Mart), a tropical palm tree native of the Amazon region that naturally occurs in the Northern States of Brazil. Fruit grows in bunches that can produce 2.5kg of fruit each year. Fruits are round, small, with 1.5 cm diameter, and dark purple color.

Açaí production in Brazil has been around 200 thousand tons per year, being the greater production concentrated in Pará State, where about 80% of the production is extractive (Nogueira et al., 2005).

From the fruit it is obtained a thick dark pulp with creamy texture that is largely consumed in the production region of Brazil as part of the population daily dietary habits, being normally used in main meals as lunch and dinner. In the other country regions, *açaí* consumption has been continuously growing due to the claim as an energetic and, recently, as a functional product. This last factor also aroused the interest on *açaí* by researchers and markets all over the world, and *açaí* pulp has been exported to countries like USA, Australia, Japan, Germany, the Netherlands, among others (Frutas e Derivados, 2006).

The great interest on *açaí* is due to its composition on phenolic compounds that are responsible for its relevant antioxidant activity being the anthocyanins the main class of *açaí* phenolics. Some studies have been done aiming at the extraction of compounds of interest while others aiming at the preservation of such compounds. Constant (2003), for example, studied the *açaí* anthocyanins extraction for use as a color pigment in foods, obtaining good results when applying it in *petit-Suisse* cheese, while Cordova-Fraga et al. (2004) evaluated the use of *açaí* pulp as an alternative oral contrast in magnetic resonance imaging for the gastrointestinal system, based on its mineral composition. Palacio (2008), on the other side, studied the concentration of a clarified *açaí* juice, aiming at the preservation of the phenolic compounds and the antioxidant activity during the concentration process.

The chemical composition and physical characteristics of the fruits vary considerably as function of many factors, some related to the production region, such as soil, temperature, rainfall, humidity and harvest period, and others as intrinsic factors, such as genetic variability and maturity stage. In this sense, the desirable characteristics of the fruit will depend on its final destination: for consumption as fresh fruit, for processing as juice or fruit syrup, or for use as raw material for compounds extraction, and in each case different properties will be required.

For acerola fruits, for example, ripeness and harvest period have a significant effect on its main property that is the vitamin C content. In the dry season and in the green fruits the vitamin C content is higher than in humid seasons and in mature fruit (Nogueira *et al.*, 2002). When evaluating the phenolic content of a specific *açaí* variety, harvested in different periods, Cohen *et al.* (2007) verified that the lower values were determined in fruits harvested in the month of higher rainfall.

The objective of this work was, therefore, to evaluate the effect of ripeness on *açaí* composition and on its *in vitro* antioxidant activity.

2. Material and Methods

The *açaí* fruits, unripe, half-ripe and mature were collected at the Experimental Station of the Federal Rural University of Amazon in

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Castanhal, PA, Brazil. They were processed separately following the common steps used in industry. After their reception, the fruits were selected, washed, weighted, sanitized by immersion in chlorinated water and submitted to the maceration stage before proceeding to extraction. The maceration step consisted in immersing the fruits in hot water (50ºC) during 30 minutes to facilitate extraction. It was followed by the extraction step in an *açaí* specific extractor with water addition in the proportion of 0.6 liters for each kilogram of fruit. The pulp yield in each maturation stage was determined as the percentage of the obtained pulp (in mass) in relation to the mass of fruit.

The obtained pulps were characterized physically and chemically in order to determine the effect of the maturation degree on the contents of bioactive compounds and antioxidant activity of the fruit. Soluble solids content, pH, acidity and proximal composition were determined in the pulps at the three different maturity stages (AOAC, 2000).

Total phenolic compounds were determined by the Folin-Ciocalteu assay proposed by Singleton & Rossi (1965) and modified by Georgé *et al.* (2005). The results were expressed in mg of gallic acid/100g of sample.

The quantification of the total and monomeric anthocyanins was accomplished according to the differential pH methodology proposed by Giusti & Wrolstad (2001). Samples were filtered to remove the suspension solids and the extracts were obtained with two different buffers, having their absorbance measured at 510 and 700nm. The results were expressed in mg of cyaniding-3-glycoside/100g.

The monomeric anthocyanins were identified by high performance liquid chromatography (Brito *et al*, 2007). The identification was accomplished by comparison of the sample chromatograms obtained in this study with a standard chromatogram of *açaí* obtained under the same conditions (extraction, mobile phase, column type, flow rate and detection at 530 nm), using an HPLC-MS system (model HP serialize 1100 MSD with a diode-array detector).

Antioxidant activity of the pulps were determined after extraction in methanol/acetone solution (Rufino *et al.* (2007) and quantification with ABTS (acid 2,2 azinobis (3-etilbenzotiazolin-6-sulfonic) diamonion) using Trolox® (acid 6-hidroxi-2-5-7-8-tetrametiicrom2-carboxilic) as standard (Re *et al.*, 1999). The results were expressed in µmoles of Trolox equivalent per g of sample (TEAC).

The statistical analysis of data was accomplished by one-way analysis of variance followed by Tukey test, at 95% of probability, using the XLSTAT 7.5 software.

3. Results and Discussion

The proximal composition and physico-chemical parameters of the *açaí* pulp in the three ripeness degrees are presented in Table 1.

Table 1. Proximal composition and physico-chemical parameters of *açaí* pulp in three ripeness degrees

Parameters	*Açaí* pulp		
	Unripe fruit	Half-ripe fruit	Ripe fruit
Ash (g/100g)	0.2 ± 0.0[c]	0.3 ± 0.0[b]	0.4 ± 0.0[a]
Lipid (g/100g)	0.1 ± 0.0[c]	1.2 ± 0.0[b]	4.8 ± 0.4[a]
Total protein (g/100)	0.3 ± 0.0[c]	0.5 ± 0.0[b]	1.2 ± 0.0[a]
Carbohidrates (g/100g)	0.8 ± 0.2[c]	1.9 ± 0.1[b]	3.5 ± 0.4[a]
Total solids (g/100g)	1.8 ± 0.1[c]	5.3 ± 0.0[b]	10.7 ± 0.4[a]
Pulp content (g/100g)	6.4 ± 0.1[c]	20.9 ± 0.8[b]	38.8 ± 1.1[a]
pH	5.2 ± 0.0[a]	5.1 ± 0.0[ab]	5.1 ± 0.0[b]
Soluble solids (°Brix)	1.0 ± 0.0[c]	1.4 ± 0.2[b]	3.0 ± 0.0[a]
Total acidity[1]	0.04 ± 0.00[c]	0.06 ± 0.00[b]	0.12 ± 0.00[a]

[1] *expressed in g of malic acid/100g. Different letters in the same line indicate significant difference at 95% probability.*

The variations among the samples were expected, since maturation can be defined as a sequence of changes in color, flavor and texture of the fruits and vegetables, which makes them adequate for consumption or industrialization. The main changes that occur during maturation include the development of the seeds, change in color, variations in breathing rate and in ethylene production, changes in tissues permeability and in texture, and chemical alterations in the compounds (carbohydrates, organic acids, pectins, pigments, phenolic compounds, etc.), besides the production of volatile substances and formation of waxes in the peels (Chitarra & Chitarra, 2005).

The values of soluble solids, acidity, carbohydrates, total protein and lipids increased with maturation, generating the expected alterations in flavor and texture. The total solids also increased with maturation, which can be partially attributed to the difficulty of pulp extraction in the unripe and half-ripe fruits, when the edible part of *açaí* (about 15% of the fruit weight) is much adhered to the seed, making difficult the mechanical extraction even after the maceration step.

The *açaí* pulp obtained with the ripe fruit presented an average content of total lipids of 4.8g/100g in wet basis, which represents 44.8g/100g in dry basis, very close to the value found by Sanabria & Sangronis (2007) and by Menezes (2005), between 42.0 and 49.4g/100g. These values are in agreement with the Brazilian legislation (Brazil, 2000) that establishes a range of 20 to 60g/100g of total lipids in *açaí*, depending on its type (fine, medium or thick).

In terms of total carbohydrates, the pulp of the ripe *açaí* presented an average value of 3.5g/100g, or 33.0g/100g in dry basis, in the same range (31.6g/100g) of the value reported by Sanabria & Sangronis (2007) for *açaí* pulp also obtained from fruits harvested in the month of February, being both values lower than the value of the pulp obtained with the fruits harvested in July (48.0g/100g) and to the values determined by Menezes (2005) in commercial *açaí* pulp (41.2g/100g).

The bioactive compounds, total phenolics and anthocyanins, and the antioxidant activity of the *açaí* pulps in the three ripeness degrees, unripe, half-ripe and ripe are presented in Table 2.

Table 2 – Bioactive compounds and in vitro antioxidant activity of açaí pulp in three ripeness degrees.

Parameters	*Açaí* pulp		
	Unripe fruit	Half-ripe fruit	Ripe fruit
Total phenolics[1]	172.5 ± 3.7[b]	118.7 ± 5.8[c]	343.7 ± 15.4[a]
Total anthocyanins[2]	2.5 ± 0.2[c]	9.5 ± 0.4[b]	80.4 ± 1.6[a]
Monomeric anthocyanins[2]	0.0[c]	3.1 ± 0.4[b]	52.7 ± 1.2[a]
Antioxidant activity[3] (ABTS)	23.8 ± 1.0[b]	15.8 ± 0.2[c]	27.8± 1.0[a]

[1]expressed in mg of gallic acid/100g; [2]expressed in mg of cianidyne-3-glycoside/100g; [3]expressed in µmol Trolox/g; different letters in the same line indicate significant difference at 95% probability.

The *açaí* anthocyanins presented a behavior of increasing concentration with the maturation stage, in agreement with the studies of Castrejón *et al.* (2008) on four cultivars of blueberry, and of Cheng & Breen (1991), on strawberries during the period of ripening after antithesis. The authors verified that although flavonoids were synthesized along all the fruit development period, the anthocyanins accumulation only occurred during the maturation stage. Cheng & Breen (1991) also observed that in strawberries the reduction in tannins occur at the same time of the second peak of activity of the enzyme phenylalanine ammonia-lyase (PAL), the key enzyme involved in the phenylpropanoids biosynthesis.

The total content of anthocyanins in the pulp of ripe *açaí* (80.4mg/100g) was close to the value (78.8mg/100g) determined by Menezes (2005) in thick *açaí* and higher than the anthocyanins contents of grape pulp (30.9mg/100g) and mulberry pulp (41.8mg/100g), reported by Kuskosky *et al.* (2007). Therefore, anthocyanins content in *açaí* is twice or more than the content found in other fruits indicating that *açaí* is a good source of these pigments.

Cyanidin-3-rutinoside and cyanidin-3-glycoside were identified in the pulp obtained with ripe fruits of *açaí* (Figure 1), in agreement to the literature (Galori *et al.*, 2004; Brito *et al.* 2007). Cyanidin-3-rutinoside was predominant, with 67% of the total, followed by cyanidin-3-glycoside with 26%, being this profile similar to the one determined by Schauss *et al.* (2006), with approximately 60% of cyanidin-3-ruthinoside and 30% of cyanidin-3-glycoside.

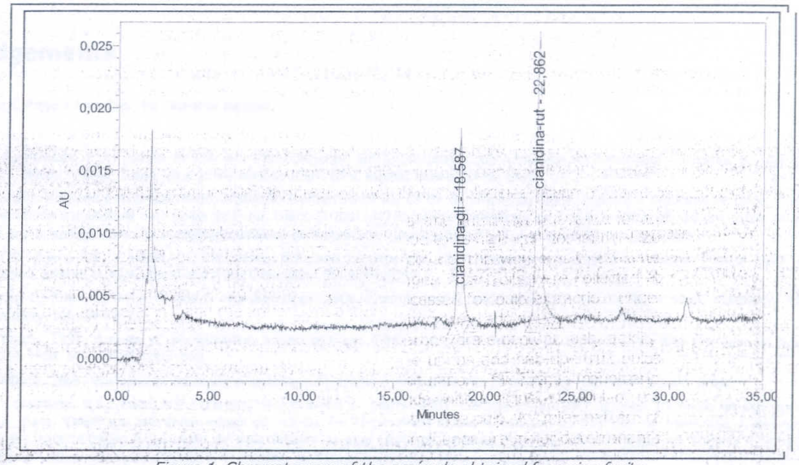

Figure 1. Chromatogram of the açaí pulp obtained from ripe fruits.

The total phenolics of the *açaí* pulps in the three ripeness degrees (Table 2) cultivated in Castanhal, Pará, Brazil presented a profile of smaller concentration in the pulp from half-ripe fruit when compared to the pulp from unripe fruit, followed by an increase in the ripe fruit pulp. Castrejón *et al.* (2008) studied four blueberry cultivars and verified a similar behavior although the higher value in the ripe fruit was observed for only three cultivars.

The behavior of the antioxidant activity with maturation (Figure 2) was similar to that of the total phenolics compounds although the unripe pulp presented about 50% of the phenolics content of the ripe pulp and the antioxidant activity represented 85% of that of the ripe pulp. The ratio between the antioxidant activity and phenolics was similar in the pulps from unripe and half-ripe fruit (about 13 µmol Trolox per mg of phenolic) and smaller in the pulp from ripe fruits (8µmol Trolox for mg of phenolic), suggesting a different phenolic composition and a higher reduction capacity in the pulps from unripe and half-ripe fruits. The antioxidant activity of the unripe pulp is probably due mainly to non-anthocyanic phenolics.

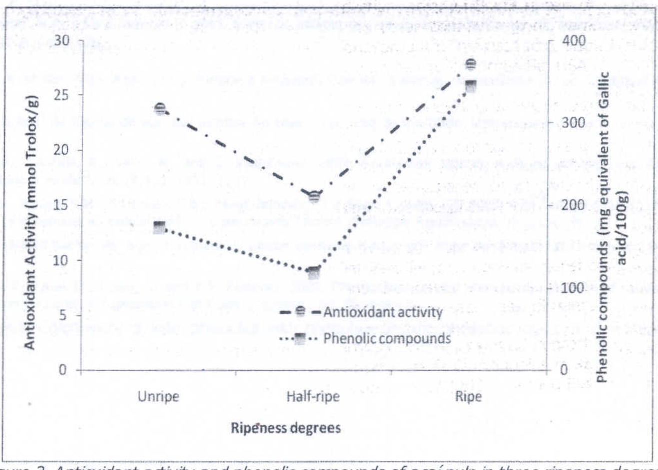

Figure 2. Antioxidant activity and phenolic compounds of açaí pulp in three ripeness degrees.

4. Conclusions

Almost all the characteristics of açaí pulp were higher for the ripe fruit pulp. Anthocyanins were formed in the end of maturation and its identification showed the presence of cianydin-3-ruthinoside and cyanidin-3-glycoside, with a higher content of the former. Total phenolics decreased (31%) from the unripe to the half-ripe pulp but increased (189%) in the ripe pulp. A similar pattern of change with ripeness degree was observed for the antioxidant activity suggesting that total phenolic compounds are major determinants of antioxidant activity in *açaí* pulp.

5. Acknowledgements

To CAPES and to FP6 PAVUC Project Inco-Dev, for financial support.

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