INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES

Volume-1, Issue-3 Sept-Nov-2011 www.ijpaes.com ISSN 2231-4490

THE INFLUENCE OF SEASONALITY ON THE ANTHOCYANIN CONCENTRATIONS IN THE AÇAÍ FRUIT (*Euterpe oleracea* Mart.) FROM THE BRAZILIAN AMAZON

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ABSTRACT: The açaí fruit (*Euterpe oleracea* Mart.) is widely consumed in Brazil in various forms. We assessed the influence of seasonality on the total anthocyanin concentration in fruits from three production sites in the state of Amapá, Brazil, during the summer (April to October) and the winter (December to March) harvests for native, managed, and cultivated açaí. We quantified total anthocyanin concentrations for 12 months. The results indicate that açaí fruits exhibited high anthocyanin levels that ranged from 363.72 to 590.23 mg/100 g in native fruit, 175.63 to 748.39 mg/100 g in managed fruit, and 312.28 to 743.18 mg/100 g in cultivated fruit. The correlation between seasonality and anthocyanin content was significant for açaí fruits from all production sites. The highest anthocyanin levels were observed in the summer crop, from July to October, and were significant. From January to May, we observed a small, non-significant variation on anthocyanin levels in the winter crop. Our results indicate that seasonality crucially affects anthocyanin levels, which are considered functional markers of the açaí fruit. Therefore, seasonality is an important environmental factor in using açaí as a functional food.

Keywords: anthocyanin, *Euterpe oleracea*, seasonality

INTRODUCTION

In Brazil, the Amazon River estuary is considered to be the origin of the açaí palm, *Euterpe oleracea* Mart..[1] The açaí palm grows naturally in the Amazon region in moist soils and, more frequently, in floodplains. In the state of Amapá, the açaí palm can be found along rivers, streams, lowlands, and wetlands. Collecting fruits and cutting palm are traditional activities in floodplains that positively impact the local economy.[2] Açaí wine, a beverage widely consumed by the Amazonian population as a food supplement or even as the main meal, can be produced by softening the fruit in hot water.

Açaí fruits come from different production sites in the state of Amapá. During harvest time, the fruit comes from Pará Island, whereas inter-harvest fruits come from other regions in the state of Pará. The two periods of fruit production at Pará Island consist of a small harvest season from December to March, termed "safrinha", and a large harvest season from June to August. Fruit harvested in the remaining months is predominantly consumed by local producers. Harvest season in the city of Mazagão, in the state of Amapá, occurs in the second half of the year, during which July, August, and September are the optimal months for harvesting.[3]

Epidemiological studies have shown an inverse correlation between diets rich in polyphenolic compounds and cardiovascular disease. Phenolics are a broad and complex group of phytochemical compounds that originate from the secondary metabolism of plants and harbor an aromatic hydrocarbon ring with one or more hydroxyl groups in their structure, enabling them to act as reducing agents, which protect the body from oxidative stress.[4]

Flavonoids are the predominant phenolic compounds in fruits and vegetables. Their beneficial properties are attributed to their ability to sequester free radicals, inhibiting oxidation processes in some systems. However, they are not effective in protecting cells and tissues from all types of oxidative damage.[5]

The typical reddish-purple color of the açaí fruit is caused by the presence of anthocyanins, which are flavonoids. Anthocyanins are natural pigments responsible for the color of a large variety of vegetables, many of which are consumed by people. Evidence for a correlation between the harvest season and anthocyanin and flavonoid content in the açaí fruit, and their variation throughout the year, remains weak.

This study aimed to assess the influence of harvest seasonality on the anthocyanin concentrations in the açaí fruit from three production sites (Mazagão Novo, Pará Island, and Maracá) in the state of Amapá, Brazil.

MATERIALS AND METHODS

Sampling of açaí fruits

Fruits were sampled from three different production sites in the state of Amapá: Mazagão Novo (00.090.77 S and 051.317.42 W), Francisco Luiz (Pará Island, 00.324.08 S and 051.283.38 W), and Maracá (city of Mazagão, 00.085.16 S and 051.302.13 W). At each site, 10 native, 10 managed, and 10 cultivated açaí trees were selected. Fruits were sampled for 12 months and were always collected during the same week of each month.

Quantification of anthocyanin concentrations and extract preparation were standardized at 4 hours post-harvest at Mazagão Novo, 6 hrs at Pará Island, and 12 hrs at Maracá.

Anthocyanin extraction

We used the method described by Francis[5] to extract 1 g of sample in an ethanol-1.5 N HCl solution (85%:15%) for 12 hrs, with a subsequent reading at a 535 nm wavelength.

Statistical analyses

Results are expressed as the mean ± standard error. To test for differences between variables, we used an analysis of variance (ANOVA) followed by the Tukey's test. Results with a p value < 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

The anthocyanin concentrations in native açaí from the Mazagão Novo community ranged from 319.92 ± 3.2 mg/100 g in October, the end of the harvest period, to 565.99 ± 4.6 mg/100 g in September (Table 1). For managed açaí, mean values ranged from 202.74 ± 2.65 mg/100 g in December, the month with the lowest production, to 805.99 ± 1.15 mg/100 g in September. The harvest period for cultivated açaí started in March, and the concentration of anthocyanins ranged from 233.15 ± 5.91 mg/100 g in May to 594.91 ± 6.31 mg/100 g in September. The period from July to September yields the highest açaí production for the city of Mazagão.

In the Mazagão Novo community, trading of fruits commences in April; however, peak production and commercialization occur from July to September.

Mean anthocyanin concentration values were not statistically different between native and cultivated açaí in the Mazagão Novo community but differed for managed açaí ($p < 0.05$).

We observed an increase in anthocyanin levels in native açaí from July to September, followed by a decrease in October and a subsequent increase in November (Table 1). After November, we were not able to obtain native açaí fruits for sampling. After November, fruits were not traded but were consumed internally in the community. The decrease in anthocyanin levels observed in October may be associated with the different maturation rates of fruits during the harvest period. Local açaí producers rank fruits for harvesting as green, "paiol" (in season), and ripe. A fruit maturation peak in the trees selected for sampling was observed in September, which may explain why the highest anthocyanin levels were observed in September.

The phenolic content of plants may vary according to the species, cultivar, environmental stresses, growth and maturation stages, and biological stresses, such as the prolonged exposure to ultraviolet rays[7] experienced in the state of Amapá from July to October.[7]

The harvest period of açaí fruits in the city of Mazagão extends from April to November, but the peak of production occurs in the second half of the year, from July to September.[3]

Managed açaí were sampled from January to December. In the Mazagão Novo production site, producers used the minimum impact management technique. This management technique increases fruit production up to five times, enabling nearly full-year commercialization. According to Malcher et al.[3], 57% of the açaí producers from the city have a management area, and açaí tree management has existed for over 10 years, which may explain why managed açaí trees were harvested year-round. Anthocyanin levels for managed açaí increased from January to March, although non-significantly (Table 1), but significantly peaked in September. Notedly, anthocyanin levels recorded for managed açaí in September were also higher than for native and cultivated açaí.

Table-1: Mean anthocyanin concentration values, expressed as mg/100 g, from native, managed, and cultivated açaí from the Mazagão Novo community (Vila Nova).

Month	Native Açaí	Managed Açaí	Cultivated Açaí
January	-	265.56 ± 5.04	-
February	-	280.68 ± 3.01	-
March	-	288.08 ± 7.21	334.39 ± 4.56
April	-	253.02 ± 2.18	518.92 ± 7.03
May	-	261.12 ± 3.11	233.15 ± 5.91
June	-	375.64 ± 6.29	358.22 ± 1.56
July	412.92 ± 6.5	375.84 ± 4.27	556.94 ± 9.12
August	538.12 ± 4.23	550.26 ± 2.18	571.85 ± 8.72
September	565.99 ± 4.6	805.99 ± 1.15*	594.91 ± 6.31
October	319.93 ± 3.2	437.68 ± 1.03	479.45 ± 3.15
November	438.93 ± 1.7	466.72 ± 2.78	-
December	-	202.74 ± 2.65	-

Numbers represent the mean ± standard deviation of $n = 10$ per analysis. * $p < 0.05$, Student's t-test.

Harvesting of cultivated açaí began in March. To replace removed tussocks, cultivated açaí is re-cultivated in areas of açaí management. Anthocyanin levels for cultivated açaí were higher than for native açaí in all samples; however, differences were not statistically significant.

Anthocyanin levels in cultivated açaí were higher than in managed açaí, except in May, June, and September; however, differences were also not statistically significant. Managed açaí fruits exhibited a gradual increase in anthocyanin concentrations associated with the increasing maturation stage of fruits, whereas the cultivated açaí harvested in April were riper than when harvested in March. This period concludes the small harvest, the "safrinha", which occurs from January to April primarily in managed and cultivated açaí. Fruits undergo a first stage of maturation during the winter harvest (January to April) and a subsequent stage from April to October. Managed açaí are harvested during both periods.

Mean anthocyanin concentration values for monthly samples collected during summer and winter harvests in Maracá are presented in Table 2. Anthocyanin concentrations ranged from 332.09 ± 1.18 mg/100 g in June to 640.04 ± 1.03 mg/100 g in September in native açaí, from 206.45 ± 4.12 mg/100 g in December to 834.84 ± 2.21 mg/100 g in October in managed açaí, and from 434.71 ± 2.33 mg/100 g in November to 846.94 ± 1.85 mg/100 g in October in cultivated açaí.

In the city of Maracá, fruit commercialization intensifies in April until October, although peak production and commercialization occur from July to September, as in Mazagão Novo. Both Mazagão Novo and Maracá belong to the city of Mazagão.

We observed an increase in anthocyanin levels for native açaí from June to September, a period during which the highest quantified value of 640.04 ± 1.03 mg/100 g was recorded. Anthocyanin levels began decreasing in October, and native açaí were sampled through November.

Managed açaí fruit were sampled at the beginning of April. In Maracá, as in Mazagão Novo, the minimum impact management technique was used by producers. Anthocyanin concentrations increased from April to July, followed by a small decrease in September and a concentration peak in October. There were no fruits from January to March, and all fruit were collected at a proper maturation stage from April onwards, which may explain the high anthocyanin levels observed.

Because cultivated açaí harvested in April were more mature, the anthocyanin levels were higher in April than in May and June. From May onwards, anthocyanin levels increased until October, when the highest levels were observed.

Mean anthocyanin concentration values for native açaí from Maracá were statistically different from managed and cultivated açaí ($p < 0.05$, ANOVA with Tukey's post-hoc test; Table 2).

Table-2: Mean anthocyanin concentration values, expressed as mg/100 g, for native, managed, and cultivated açaí sampled from the city of Maracá.

Month	Native Açaí	Managed Açaí	Cultivated Açaí
April	-	640.66 ± 4.19	508.65 ± 2.1
May	-	647.10 ± 5.34	464.43 ± 4.7
June	332.09 ± 1.18	666.84 ± 1.19	470.75 ± 1.55
July	547.73 ± 2.76	738.66 ± 0.12	639.99 ± 1.6
August	570.21 ± 4.91	720.66 ± 0.81	640.6 ± 1.03
September	640.04 ± 1.03	708.12 ± 1.61	643.18 ± 2.05
October	441.46 ± 4.17	834.84 ± 2.21*	846.94 ± 1.85*
November	431.17 ± 3.61	445.12 ± 2.33	434.71 ± 2.33
December	-	206.45 ± 4.12	

Numbers represent the mean ± standard deviation of n = 10 per analysis. * $p < 0.05$, Student's t- test.

Anthocyanin levels for native açaí from the Francisco Luiz community, Pará Island, ranged from 346.15 mg/100 g in June, which corresponds to the onset of the harvesting period, to 564.66 mg/100 g in August (Table 3), when peak production was observed. Managed açaí anthocyanin levels ranged from 117.69 mg/100 g in January, a period uncommon for açaí in the region, to 604.35 mg/100 g in September, a period of peak production. Cultivated açaí anthocyanin levels ranged from 167.74 mg/100 g in January to 787.69 mg/100 g in September. There are two harvesting periods in Pará Island.[3] A smaller winter harvesting period occurs from January to April, and a larger summer harvesting period occurs from June to October. Fruit harvested in the remaining months is consumed predominantly by local producers.

Native açaí anthocyanin levels increased from July to September from Francisco Luiz (Pará Island), as in Mazagão Novo, and sampling continued until October. The increase in anthocyanin levels was due to the maturation of fruits during the harvesting period, until September/October.

Managed and cultivated açaí were sampled in two maturation stages, from January to April (winter harvest) and from May to October (summer harvest), which may explain the gradual increase in anthocyanin levels in both harvesting periods (Table 3). Even though the highest anthocyanin levels were recorded in September for each of the three types of fruit, there were no statistical differences between them ($p > 0.05$, ANOVA with Tukey's post-hoc test). The time between fruit harvesting and processing was standardized for the analyses of anthocyanin quantification. In Maracá, where the highest anthocyanin levels were recorded, all analyses were performed 12 hrs post-harvest. Thus, time of processing did not influence anthocyanin levels. Native açaí were harvested at all three production sites through November.

Table-3 : Mean anthocyanin concentration values, expressed as mg/100 g, for native, managed, and cultivated açaí sampled from the Francisco Luiz community, Pará Island, Brazil.

Month	Native Açaí	Managed Açaí	Cultivated Açaí
January	-	117.69 ± 1.26	167.74 ± 3.75
February	-	197.91 ± 3.78	251.8 ± 4.08
March	-	271.88 ± 5.21	278.78 ± 5.01
April	-	250.01 ± 4.19	283.18 ± 2.24
May	-	259.21 ± 1.25	273.96 ± 2.16
June	-	325.42 ± 1.28	475.81 ± 4.48
July	346.15 ± 4.42	372.24 ± 2.21	572.01 ± 6.23
August	441.19 ± 2.15	503.38 ± 3.05	586.38 ± 1.18
September	564.66 ± 3.6	604.35 ± 3.19	787.69 ± 5.21
October	436.01 ± 2.14	417.63 ± 2.15	386.64 ± 4.98
November	-	464.86 ± 2.15	-
December	-	199.71 ± 2.05	-

Numbers represent the mean ± standard deviation of $n = 10$ per analysis. * $p < 0.05$, Student's t-test.

Anthocyanin levels varied significantly (Student's t-test, $p < 0.05$) for native açaí from the three production sites (Mazagão Novo, Pará Island, and Maracá) from July to November (Table 4). In July, August, and October, differences were observed between Mazagão Novo and Maracá, Mazagão Novo and Pará Island, and Pará Island and Maracá. All anthocyanin levels recorded from the Francisco Luiz community (Pará Island) were lower than the levels from Mazagão Novo and Maracá. In September, differences were observed only between Mazagão Novo and Maracá and Pará Island and Maracá. Anthocyanin levels from Pará Island were lower than levels from Mazagão Novo and Maracá. For all samples analyzed, native açaí from Maracá exhibited the highest anthocyanin levels. The harvesting period in Maracá began in June, whereas native açaí are first harvested in July in the other two production sites.

Table-4 Mean anthocyanin concentration values for native açaí, expressed as mg/100 g, from three production sites in the state of Amapá, Brazil.

Month	Native Açaí Mazagão Novo	Native Açaí Pará Island	Native Açaí Maracá
June	-	-	332.09 ± 1.18
July	412.92 ± 6.5	346.15 ± 4.42	[a]547.73 ± 2.76
August	*[a]538.12 ± 4.23	441.19 ± 2.15	[a]570.21 ± 4.91
September	*565.99 ± 4.6	*564.66 ± 3.6	*[a]640.04 ± 1.03
October	319.93 ± 3.2	[a]436.01 ± 2.14	[a]441.46 ± 4.17
November	438.93 ± 1.7	-	431.17 ± 3.61

Numbers represent the mean ± standard deviation of n = 30 per analysis. * $p < 0.05$, same type of Student's t-test, and [a] $p < 0.05$ different types of analysis.

Anthocyanin levels varied significantly (Student's t-test, $p < 0.05$) for managed açaí in all three production sites from April to December (Table 5).

Table-5: Mean anthocyanin concentration values for managed açaí, expressed as mg/100 g, from three production sites in the state of Amapá, Brazil.

Month	Managed Açaí Mazagão Novo	Managed Açaí Pará Island	Managed Açaí Maracá
January	265.56 ± 5.04	117.69 ± 1.26	-
February	280.68 ± 3.01	197.91 ± 3.78	-
March	288.08 ± 7.21	271.88 ± 5.21	-
April	253.02 ± 2.18	250.01 ± 4.19	640.66 ± 4.19
May	261.12 ± 3.11	259.21 ± 1.25	647.1 ± 5.34
June	375.64 ± 6.29	325.42 ± 1.28	666.84 ± 1.19
July	375.84 ± 4.27	372.24 ± 2.21	738.66 ± 9.12
August	550.26 ± 2.18	*503.38 ± 3.05	720.66 ± 5.81
September	[a]*805.99 ± 1.15	*604.35 ± 3.19	708.12 ± 3.61
October	437.68 ± 1.03	417.63 ± 2.15	*834.84 ± 3.21
November	466.72 ± 2.78	464.86 ± 2.15	445.12 ± 6.33
December	202.74 ± 2.65	199.71 ± 2.05	206.45 ± 4.12

Numbers represent the mean ± standard deviation of n = 30 per analysis. * $p < 0.05$ Student's t-test, and [a] $p < 0.05$ for sites.

In May and June, anthocyanin levels were considerably higher in Maracá, and differences were observed only between Mazagão Novo and Maracá. From July to December, significant differences were observed between Mazagão Novo and Maracá, Mazagão Novo and Pará Island, and Pará Island and Maracá, and again anthocyanin levels for Pará Island were lower than in Mazagão Novo and Maracá. Anthocyanin levels were higher in Maracá than in Mazagão Novo, except during September and November.

Cultivated açaí anthocyanin levels varied significantly (Student's t-test, $p < 0.05$) from all three production sites from March to October (Table 6). In March, differences were observed only between Mazagão Novo and Pará Island, and anthocyanin levels were higher for fruit from Maracá than from Mazagão Novo. From June to October, statistical differences were observed between Mazagão Novo and Maracá, Mazagão Novo and Pará Island, and Pará Island and Maracá. Anthocyanin levels were also lower for fruit from Pará Island than from Mazagão Novo. In Maracá, anthocyanin levels were considerably higher than in the other two production sites during all harvesting periods.

Table-6: Mean anthocyanin concentration values for cultivated açaí, expressed as mg/100 g, from three production sites in the state of Amapá, Brazil.

Month	Cultivated Açaí Mazagão Novo	Cultivated Açaí Pará Island	Cultivated Açaí Maracá
January	-	167.74 ± 3.75	-
February	-	251.8 ± 4.08	-
March	334.39 ± 4.56	278.78 ± 5.01	-
April	518.92 ± 7.03	283.18 ± 2.24	508.65 ± 2.1
May	233.15 ± 5.91	273.96 ± 2.16	464.43 ± 4.7
June	358.22 ± 1.56	475.81 ± 4.48	470.75 ± 1.55*
July	556.94 ± 9.12	572.01 ± 6.23	639.99 ± 1.6*
August	571.85 ± 8.72	586.38 ± 1.18	640.6 ± 1.03*
September	594.91 ± 6.31	787.69 ± 5.21	643.18 ± 2.05*
October	479.45 ± 3.15	386.64 ± 4.98	846.94 ± 1.85*
November	-	-	434.71 ± 2.33

Numbers represent the mean ± standard deviation of n = 30 per analysis. * $p < 0.05$ Student's t-test.

Similar results for the açaí fruit were found by Ozela,[8] who reported anthocyanin concentrations of fruit from the harvest and inter-harvest periods, expressed as mg/ 100 g of fruit, of 926.1 and 356.7 mg /100 g, respectively. Souza's[9] analysis of different progenies of açaí palm trees (*E. oleracea*) revealed anthocyanin levels ranging from 73.52 to 145.62 mg/100 g. Roger[10] documented average levels of 440 mg/kg in fruit from the state of Pará, and Bobbio[11] observed a level of 50 ± 5 mg/100 g in manually peeled fruit.

Anthocyanin levels for the açaí fruit documented in this study are within the range observed by Oliveira[1], who analyzed anthocyanins from Seibel grapes. Anthocyanin levels ranged from 500.5 mg/100 g in the skin to 112.72 mg/100 g in the seed-free pulp. Thus, our results are similar to (during the inter-harvest period, from January to May) or even higher than (e.g., for cultivated açaí in May) those observed for Seibel grapes.

Several factors that define anthocyanin stability may have attributed to the discrepancies in our observations. In fact, according to Março and Poppi (2008)[12], the presence of sugars, acids, co-pigments, methoxy groups, and variations in temperature, oxygen, and pH levels play important roles in determining the color and stability of anthocyanins.

Factors such as time between fruit harvesting and processing, storage conditions, temperature, genetic variability, and prolonged exposure to adverse conditions during shipping may influence the different results reported in the literature. For example, the time between the harvesting of the fruit and anthocyanin quantification ranged from 4 to 12 hrs in our study.

Lichtenthaler et al.[13] and Schaus et al.[14] identified two major anthocyanins from anthocyanins in açaí fruit and pulp: cyanidin-3-glucoside and cyanidin-3-rutinoside. Additionally, Del Pozo-Insfran et al.[16] (2004) not only identified both compounds described above but also observed significant amounts of pelargonidin-3-glucoside.

CONCLUSION

We observed significant anthocyanin levels in açaí fruit from three production sites. During the summer harvest at the second half of the year, the variation was significant, specifically from July to October, when the highest values were recorded. The correlation between anthocyanin levels and seasonality was significant, and during the winter harvest, from January to May, the variation was small and non-significant.

Anthocyanins are considered to be functional markers for açaí fruit. Therefore, seasonality is an important environmental factor in assessing açaí as a functional food.

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