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Original Article

Microbiological, parasitic, microscopic, physical and chemical characterization of processed acai (*Euterpe oleracea* Mart.) fruits

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Acai (*Euterpe oleracea* Mart.) fruit consumption has been increasing in recent years. The increasing demand for this fruit increased concerns about its quality. Thus, the objective of this work was to assess the microbiological, parasitical, microscopic, physical and chemical characteristics of processed acai. Processed acai of 12 commercial establishments were subjected to microbiological analyses (aerobic mesophilic bacteria, molds, yeasts and total and thermotolerant coliform quantifications, *Staphylococcus* catalase positive test and search for *Salmonella* ssp.), search for parasites, microscopic analysis (search for light and heavy dirt) and physical and chemical characterization (titratable acidity, pH, total soluble solids °Brix, moisture content, ash, color). The samples had high amounts of aerobic mesophilic bacteria; 75% had total coliforms, molds and yeasts; 58.3% had *Staphylococcus* (catalase positive); and 8.33% had *Salmonella spp*. Moreover, the samples were not within the recommended microscopic standards, presenting foreign matter (sand and plastic). However, parasites were not found in the samples evaluated. The physical and chemical characteristics of the samples was within the criteria established by the Brazilian Ministry of Agriculture, Livestock and Food Supply (Regulation No 01 of January 7, 2000). The hygienic-sanitary conditions of the processed acai evaluated were unsatisfactory, compromising the harmlessness of this food and consequently, its safety for human health.

INTRODUCTION

The Brazilian fruit production sector has been standing out in the domestic and foreign markets (MENEZES; TORRES; SRUR, 2008). Acai, a fruit of the acai palm (*Euterpe oleracea* Mart.), is an important native fruit in this sector (GARZÓN et al., 2017) due to its nutritional value (MENEZES; TORRES; SRUR, 2008), high nutrient contents, such as protein, lipids and minerals (NEVES et al., 2015), and high anthocyanin contents, which are phenolic substances with coloring and antioxidant properties (BORGES et al., 2016; GARZÓN et al., 2017;

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KUSKOSKI et al., 2006). These properties qualify the acai as a functional food (PORTINHO; ZIMMERMANN; BRUCK, 2012).

Acai presents fast deterioration (ETO et al., 2010) and short shelf life (TONON; BRABET; HUBINGER, 2013) due to several factors, such as the presence of microorganisms, filamentous fungi and yeasts, normally found in the superficial microbiota of this fruit, and coliforms, which indicate inadequate handling (COHEN; ALVES, 2006). Acai is usually frozen for marketing and transporting to increase its shelf life. However, undesirable growth of microorganisms may occur during handling and transporting due to temperature variation (PEREIRA et al., 2006), as well as occurrence of typical changes of freezing, such as coloration, physical and chemical parameters that modify its original properties (COUTINHO et al., 2017).

Deteriorating, pathogen and indicator microorganisms stand out as the most important groups of microorganisms in acai. Researches have reported high amounts of microbes in acai fruits (COHEN; ALVES, 2006; FARIA et al., 2012; JONES; LEMES, 2014), which is very concerning for public health, especially when pathogens, such as *Salmonella*, *Escherichia coli* and *Staphylococcus aureus*, are found. Evaluation of aerobic mesophilic bacteria is useful for assessing the contamination level of acai (FARIA et al., 2012) and therefore, to indicate its hygienic-sanitary quality (ETO et al., 2010).

Parasites cause diverse pathologies, with significant persistence in several countries, especially protozoa and helminths, which spread due to food globalization and international travels (DORNY et al., 2009). *Trypanosoma cruzi* stands out among the protozoa species that can be carried by food, such as the acai pulp (BARBOSA et al, 2016). Although the acai tree is not the ecotype of this vector, its fruit can be contaminated with it due to the lack of hygiene in harvesting, processing to commercialization.

According to Fregonesi et al. (2010) contamination by foreign matter can occur during the acai processing, and studies on quality of derivatives of this fruit can assist in standardize its quality and in the adoption of sanitary measures. Therefore, microscopic evaluation for searching and identification of foreign matter, such as dirt, is important to characterize the quality of this food. In this context, the objective of this work was to conduct a microbiological, parasitic, microscopic, physical and chemical characterization of processed acai from commercial establishments in Mossoró, State of Rio Grande do Norte, Brazil.

MATERIAL AND METHODS

Experimental design and sample collection

Twelve samples were collected in randomly selected commercial establishments that commercialize processed acai (acai bowl) at the night time, considering only one establishment per neighborhood of Mossoró, State of Rio Grande do Norte.

Samples were collected at the same time from January to March 2016. The samples were taken with the container provided by each establishment, their temperature was measured, using a digital thermometer, to verify if they were properly frozen. The samples were then conditioned in isothermal ice boxes to minimize the risk of external interference and thawing and immediately taken for analysis.

Microbiological analyses

The microbiological characterization of acai samples was carried out according to the methodology described by Silva et al. (2007). Thus, the samples were subjected to aerobic mesophilic bacteria, molds, yeasts and total and thermotolerant coliform quantifications, *Staphylococcus* catalase positive test and search for *Salmonella* ssp. The analyses were performed in duplicate, aseptically, in a laminar flow hood using previously sterilized materials and equipment.

First, 25 g of each sample was homogenized in 225 mL of previously-sterilized buffered peptone saline solution to obtain the first dilution (10^{-1}), followed by dilutions until the fourth dilution (10^{-4}).

A bacteriological culture on Plate Count Agar (PCA) was conducted for quantification of aerobic mesophilic bacteria. The plates were incubated inverted in a bacteriological oven at 36±1 °C for 48 hours. A surface plating was carried out in Potato Dextrose Agar (PDA), with subsequent cultivation in a BOD oven at 28 °C for five days, for quantification of molds and yeasts (SILVA et al., 2007).

A surface culture in Agar Baird Parker supplemented with egg yolk and potassium tellurite was conducted for *Staphylococcus* bacteria quantification. The plates were incubated inverted for 48 hours in a bacteriological oven and Gram staining and catalase proof were performed as confirmatory biochemical tests (SILVA et al., 2007).

The most probable number (MPN) method was used for coliform bacteria quantification, with results expressed in MPN g^{-1}. Thus, 1-mL aliquots of the 10^{-1} to 10^{-3} dilutions of each sample were inoculated into a lauryl sulfate broth for presumptive testing, with incubation in a water bath at 36 ± 0.5 °C for 48 hours. The presence of total coliforms in the broths of positive tubes (gas formation) was confirmed by subsampling and incubate them in brilliant-green bile lactose broth at the same temperature and time. Subsamples of these positive

tubes were then incubated in an *Escherichia coli* broth at 45±0.5 °C for the thermotolerant coliform quantification.

The subsampling was performed using a platinum loop (SILVA et al., 2007; APHA, 1998).

Twenty-five grams of each sample, weighed in a precision analytical balance, was homogenized in 225 mL of buffered peptone water and placed in a sterile Erlenmeyer flask for searching of *Salmonella* spp. bacteria. This content was incubated at 36 °C for 24 hours in a bacteriological oven. Subsequently, 1-mL aliquots were transferred to three different broths (Rappaport Vassiliadis, Selenite Cystine and Tetrathionate) for selective enrichment and incubated for 24 hours at 41 °C. Then, they were plated on petri dishes with *Salmonella*, *Shigella* and Rambach agar. The plates were incubated inverted at 36 °C. After 24 hours, the formation of typical colonies was verified. The biochemical tests performed were lysine decarboxylation, lactose and/or sucrose fermentation and H_2S production, on lysine iron agar and triple sugar iron agar and urease test, respectively (SILVA et al., 2007; APHA, 1998).

Parasitological analyses

The spontaneous sedimentation technique, adapted from the Hoffmann's method (HOFFMAN; PONS; JANER, 1934) was used for parasitological analysis. This technique consists of weighing five grams of each sample, diluting them in 30 ml of distilled water, homogenize them with a glass rod and leave this solution to sedimentation for 30 minutes, with subsequent removal of the supernatant and visualization of the pellet under an optical microscope. Subsequently, the samples are analyzed by the Faust's flotation method, based on the flotation of some eggs on the surface of the hypersaturated saline solutions (FAUST, 1939).

Microscopic analyses

The amounts of light and heavy dirt were assessed through the methodology described by the Association of Official Analytical Chemists (AOAC, 2005), with some adaptations. Thus, 50 g of the sample, 200 mL of distilled water and 10 mL of mineral oil were mixed and shaken vigorously for 30 seconds. Then, the solution was placed in a separation funnel to the formation of 3 phases (oil, water and heavy dirt), which was then separated in different containers. This procedure was repeated for the heavy dirt until the liquid was translucent. This liquid was then passed through a filter paper, and with the aid of a vacuum pump, all the water from the sample was removed. The material contained in the oil container was put on a filter paper and a vacuum pump was used to remove all the oil from the sample. Subsequently, they were observed under an optical microscope with a 40x objective.

Physical and chemical analyses

Physical and chemical parameters (titratable acidity, pH, total soluble solids, moisture content, ash and color) were evaluated in triplicates.

Titratable acidity was evaluated by titration with sodium hydroxide and expressed as grams of citric acid per 100 mL of the sample (INSTITUTO ADOLFO LUTZ, 2008). A digital potentiometer was used to evaluate the pH. Total soluble solids were evaluated by the refractometer method and the results expressed as °Brix. Moisture and ash content (%) was evaluated by the difference between the wet weight and the dry weight of the samples after subjected to drying in a cabin dryer. These analyzes followed the recommendations of the Instituto Adolfo Lutz (2008).

Color was characterized by the method described by Alves et al. (2008). A portable digital colorimeter was used to measure the L* (luminosity, 0 to 100), a* (yellow and red) and b* (green and blue) coordinates (ALVES et al., 2008).

RESULTS AND DISCUSSION

Microbiological, parasitological and microscopic analyses

The amount of aerobic mesophilic bacteria ranged from 3.362 to 4.615 Log_{10} UFC mL^{-1} (Table 1). These results were high compared with those reported by Faria et al. (2012), who found amounts ranging from 1.477 to 4.518 Log_{10} UFC mL^{-1}. However, they were lower than those reported by Jones; Lemes (2014), who found amounts of up to 7.47 Log_{10} UFC mL^{-1} in acai pulps marketed in Itajubá, State of Minas Gerais, Brazil.

The Brazilian Regulation (BRASIL, 2000) does not account for standard amounts of aerobic mesophilic bacteria, however, these microorganisms are important indicators of hygienic-sanitary conditions of food products (ETO et al., 2010). Moreover, food with high amount of aerobic mesophilic bacteria can easily deteriorate (CARVALHO, 2010).

According to Franco; Landgraf (2008), the maximum amount of aerobic mesophilic bacteria allowed in food is 6 Log_{10} UFC mL^{-1}, in this sense, the aerobic mesophilic bacteria in the acai evaluated in the present work was below this maximum.

Table 1. Quantification of aerobic mesophilic bacteria, molds and yeasts and total and thermotolerant coliforms in processed acai marketed in Mossoró, State of Rio Grande do Norte, Brazil.

Acai sample	Aerobic mesophilic bacteria	Molds and yeasts	Total coliforms	Thermotolerant coliforms
	Log_{10} UFC mL^{-1}	Log_{10} UFC mL^{-1}	MPN g^{-1}	MPN g^{-1}
1	3.362	3.462	23	<3.0
2	4.230	3.633	93	<3.0
3	4.182	3.079	23	<3.0
4	3.908	4.029*	23	<3.0
5	4.223	4.013*	<3.0	<3.0
6	4.276	4.281*	<3.0	<3.0
7	4.360	4.107*	3.6	<3.0
8	4.068	4.417*	75	<3.0
9	4.615	4.594*	43	<3.0
10	4.272	4.480*	3.6	<3.0
11	4.100	4.617*	<3.0	<3.0
12	4.422	3.833*	43	<3.0
Standard*	-	3.6989	-	1

*Standards established by the Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA) in the Regulation No 01 of January 7, 2000 (Brasil, 2000).

The amounts of molds and yeasts ranged from 3.079 to 4.617 Log_{10} UFC mL^{-1}, with 25% of the samples within the standards recommended by the Brazilian Regulation for frozen and not frozen acai fruit products (less than 3.698 Log_{10} UFC mL^{-1}) (BRASIL, 2000). Molds and yeasts in fruit pulps at higher levels than these may represent a risk to human health, because of mycotoxins that can be carried to the consumer (FARIA et al., 2016).

A possible explanation for this result is the lack of hygienic measures and/or failures during processing and handling operations from the harvest to the final packaging of the product, such as temperature variations, which may decrease the conservation time of this food by favoring the development of microorganisms (PEREIRA et al., 2006). Filamentous fungi and yeasts may be part of the surface microbiota of acai, and their incorporation into the fruit usually occurs during harvesting operations (COHEN; ALVES, 2006). High amounts of microorganisms of this group may decrease the fruit sensory quality, making them unsuitable for consumption (FORSYTHE, 2013; FRANCO; LANDGRAF, 2008; JAY, 2005).

In a study conducted by Faria et al. (2012), 8.33% of the acai samples evaluated showed high amounts of molds and yeasts.

Although 75% of the samples showed positivity for total coliforms, no thermotolerant coliforms were found. The MPN of total coliforms ranged from 23 to 93 MPN g^{-1} (Table 1).

The Brazilian Regulation establishes a maximum amount of 1 thermotolerant coliform per gram of fruit pulp (BRASIL, 2000). Thus, all the acai evaluated in the present work were within these standards. Faria et al. (2012) found positivity for total coliforms in 75% of their acai samples, and presence of thermotolerant coliforms in 16.7%. However, Freitas et al. (2015) and Eto et al. (2010) did not find total coliforms in acai pulps.

Salmonella was detected in 8.33% of the acai samples and the amount *of Staphylococcus* ranged from 1.477 to 3.348 log UFC mL^{-1}, with 58.3% reacting positively on the catalase test, and all characterized as Gram positive in the Gram staining test (Table 2).

Table 2. *Salmonella*, S*taphylococcus*, catalase test and microscopic analyses of processed acai marketed in Mossoró, State of Rio Grande do Norte, Brazil.

Acai sample	*Salmonella*	*Staphylococcus* (Log UFC mL^{-1})	Catalase test	Dirt (g in 50 g)	Dirt type
A1	Absence	1.477	Negative	0.1802	Sand
A2	Absence	2.462	Positive	0.279	Sand and cuticle
A3	Absence	2.230	Positive	0.1577	Sand
A4	Absence	2.114	Positive	0.5927	Sand
A5	Absence	2.000	Negative	0.2092	Sand
A6	Absence	2.415	Positive	0.0747	Sand
A7	Absence	2.079	Negative	0.3735	Sand
A8	Presence	2.544	Positive	0.502	Sand
A9	Absence	3.049	Positive	0.2073	Sand
A10	Absence	3.348	Positive	0.2495	Sand and plastic
A11	Absence	2.114	Negative	0.2393	Sand
A12	Absence	1.477	Negative	0.0717	Sand and plastic

Sousa; Melo; Almeida (1999), evaluated acai in Macapá AP, Brazil, and found presence of *S. aureus* in 33.3% of the samples. Santos et al. (2016) found *Staphylococcus* spp. in 50% of their acai samples in São Paulo SP, Brazil. The genus *Staphylococcus* is frequently found in mucous membranes of the respiratory tract and epidermis of humans, thus, the high amounts of these microorganisms may be related to incorrect handling, and the handler is a potential source of transmission of these pathogens to the food. Moreover, inadequate storage and food-inherent factors also contribute to the proliferation of these bacteria, favoring the incidence of food-borne diseases (AMSON; HARACEMIV; MASSON, 2006; JAY, 2005).

Salmonella is a genus of the family Enterobacteriaceae, whose presence in food suggests fecal contamination (AMSON; HARACEMIV; MASSON, 2006). Occurrence of *Salmonella* in acai makes it inappropriate for consumption, according to the Brazilian Regulation (BRASIL, 2000), since this bacterium is pathogenic, frequently associated with cases of food infection (D'AOUST et al., 2001), and reported as a significant cause of morbidity, mortality and economic losses (FORSYTHE, 2013).

Jones; Lemes (2014) found absence of S*almonella* in their acai samples in Itajubá MG, Brazil. This result was also reported by Eto et al. (2010) in acai pulp and mixes stored in a freezer, confirming that the management of processing can improve the safety of the product. Souza et al. (2016) evaluated the microbiology of various fruit pulps marketed in Juazeiro do Norte CE, Brazil, and found *Salmonella* in 5% of their samples.

Contamination of food can occur during various stages of processing (PARISSENTI et al., 2013). Thus, careful handling throughout the production chain and control tools, such as microbiological analyzes, are essential to ensure the quality and safe consumption of acai.

Parasitological analyzes showed no parasites in the 12 samples evaluated, probably because the samples were frozen, inactivating the parasites that could contaminate the samples. Passos et al. (2012) confirmed the effect of temperature on *T. cruzi* on acai, with absence of this parasite in the eluate of the fresh fruit pulp mixes after frozen at -20 ^{o}C; however, 100% of the trypomastigotes were active when it was refrigerated at 4 ^{o}C. In this context, some acai products can provide a suitable environment to the parasite survival. These data are very relevant, since acai is consumed in various forms of preparation (OLIVEIRA et al., 2002).

Dirt was found in the 12 samples evaluated. All samples contained sand and 25% (3) contained also other residues, such as plastic (2) and cuticle (1). According to the Brazilian Regulation, fruit pulp cannot contain dirt, foreign matter, parasites, insect fragments or inedible parts of the fruit or plant (BRASIL, 2000). Thus, the samples A2, A10 and A12 were classified as unsuitable for consumption, since fragments of plastic and cuticle were found in them. The presence of foreign matter, such as fragments of insects, mites, sand crystals and human hair shows failures in fruit production, processing, handling and marketing, i.e., a lack of adoption or maintenance of good production practices (FREGONESI et al., 2010).

Fregonesi et al. (2010) evaluated frozen acai pulp and found non-appropriate results of microscopic parameters in 53.33% of the samples, with a sample (3.33%) considered unsuitable for consumption, presenting rodent hair and foreign matter, which are harmful to human health. On the other hand, Sousa; Melo; Almeida (1999) evaluated the quality of acai marketed in Macapá AP, Brazil, and found no dirt or parasites in their samples. Pereira et al. (2006) evaluated physical, chemical, microbiological and microscopic quality of frozen fruit pulps marketed in Viçosa MG, Brazil, and found fragments of insects (larvae) in cashew, guava and graviola pulps, classifying them as unsuitable for consumption. This results confirm the need for implementation and monitoring of good production practices throughout the acai production chain.

Physical and chemical analyses

The results of the analyzes of total soluble solids (TSS), expressed in °Brix, pH, moisture, total titratable acidity (TTA) and color are presented in Table 3.

Evaluation of TSS (°Brix) are important for fruits intended for fresh consumption and for processed products, since they indicate the total sugars of the product (CHITARRA; CHITARRA, 2005). The TSS of the samples used were within the range recommended by the current legislation (40.0 to 60.0 g 100 g^{-1}) (BRASIL, 2000). Eto et al. (2010) evaluated acai mixes and found TSS of 29 to 30 (g 100 g^{-1}), similar to those found in the present work. However, they differed from those found by Alexandre; Cunha; Hubinger (2004), who found TSS close to 3.2 ºBrix.

The high TSS (°Brix) found may be related to the addition of guarana syrup to the pulp. Another factor that confirms a possible presence of syrups in the acai preparation is the high moisture content found (80.19% to 73.8%). Fregonesi et al. (2010) found mean moisture content in acai of 89.50% (type essential) and 90.01 (type C), and explained this high moisture by the amount of water in the production of these types of products.

Table 3. Physical and chemical characteristics of processed acai marketed in Mossoró, State of Rio Grande do Norte, Brazil.

Acai Sample	TSS (°Brix)	pH	Moisture	TTA	Color		
					L*	a*	b*
1	18.1	5.06	80.19	0.51	22.29	8.95	1.27
2	21.1	5.06	74.4	0.56	17.64	4.54	0.31
3	24.1	4.36	76.47	0.95	28.80	8.25	1.43
4	27.6	5.0	73.8	0.69	17.23	4.73	0.31
5	29	4.5	72.6	0.78	17.40	5.17	-0.38
6	32.6	4.5	75.22	0.47	25.43	10.45	1.16
7	33.3	4.6	75.84	0.52	22.58	4.28	-0.06
8	29.6	4.8	74.85	0.48	79.58	11.85	2.17
9	23.3	4.5	75.8	0.40	20.36	5.32	0.03
10	23.6	4.5	76.38	0.72	25.44	6.07	-0.14
11	19.3	4.4	77.32	0.44	22.60	4.65	-0.33
12	20	4.6	80.81	0.32	25.60	9.43	1.82

TSS = total soluble solids; TTA = total titratable acidity; L* = luminosity; a* = yellow and red; b* = green and blue.

The TSS (°Brix) and moisture found indicate a probable preparation of the food with addition of syrups in all samples evaluated, since the TSS and moisture of acai in pure form are lower than those (ALEXANDRE; CUNHA; HUBINGER, 2004).

According to the Brazilian Regulation, acai pulps intended for marketing must have 0.27 to 0.45% of citric acid (ATT) and pH of 4.0 to 6.0 (BRASIL, 2000). Thus, the ATT (0.32 to 0.95) and pH (4.4 to 5.6) of all samples were within the recommended range, with most of the samples presenting pH of 4.5, denoting that none of the possible additives added, such as syrups, altered these characteristics.

Regarding the color analysis, the Brazilian Regulation for acai established that the fruit must have purple coloration even after being heated to 80 °C (BRASIL, 2000). The color is measured in the coordinates L* a* and b*. The L* coordinate represents the luminosity of the food, varying from 0 to 100; the a* coordinate represents the yellow and red; and the b* coordinate represents the green and blue. The most important coordinates for acai are L* and b* (ALVES et al., 2008).

The L* coordinate of the samples had maximum of 79.58 and minimum of 17.23, with most of the samples with L* close to 22, indicating the dark coloration of the acai samples evaluated. The b* coordinate ranged from -0.38 to 1.82, indicating a trend of blue color. Visually, the samples had a purplish color, indicating that the samples collected had the characteristics of the fruit that originated them (BRASIL, 2000).

CONCLUSIONS

The microbiological characterization of the processed acai evaluated showed that some samples were not within the recommended microbiological and microscopic standards, presenting deteriorating and pathogenic microorganisms and foreign matter. However, no parasites were found and the physical and chemical characterization of the acai samples were in accordance with the Brazilian regulation.

Thus, the adoption of adequate hygienic-sanitary measures and appropriate conservation methods are necessary to ensure the harmlessness of this food and consequently, improve its safety for human consumption.

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