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Chemical and microbiological evaluations of *Platonia insignis* Mart and *Theobroma grandiflorum* Schum pulps, native fruits from Brazilian Amazon

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Abstract

The aim of the present study was to evaluate chemical and microbiological parameters of industrialized and artisanal bacuri (*Platonia insignis* Mart) and cupuaçu (*Theobroma grandiflorum* Schum) pulps, native Brazilian Amazon fruits. Several fruit pulp brands were selected as follows: industrialized pulps (IP), brands A and B and artisanal pulps (AP), brands C and D. The chemical analyses were based on the determination of initial pH, total titratable acidity (TTA), total soluble solids (TSS) and their ratio (TSS/TTA). Microbiological analyzes quantified mold, yeast, *Staphyloccus aureus* and *Salmonella* spp. Their contamination were also determined. Chemical characterization demonstrated that bacuri pulps had lower level of TTA and TSS than cupuaçu pulp. However, ration between TSS and TAA showed higher level in bacuri pulp/brand AP/D. The microbiological analyses showed a ranging from $2.83x10^5$ to $9.35x10^7$ and $7.32x10^6$ to $7.06x10^7$ mold and yeast colony forming units (CFU/g) for bacuri and cupuaçu pulps, respectively. All pulps presented high amounts of *Staphylococcus aureus*. The presence of *Salmonella* was verified in industrialized pulp for both bacuri and cupuaçu fruits. We concluded that both industrialized and artisanal bacuri and cupuaçu pulps have poor microbiological food quality, indicating a health threat to the consumers.

Keywords: bacuri, cupuaçu, native fruit, pulp quality.
Abbreviations: IP_Industrialized Pulp, AP_Artisanal Pulp, TTA_Total Titratable Acidity, TSS_Total Soluble SolidsRatio (TSS/TTA) _ Relation between TTA and TSS, pH _ Potential hydrogen.

Introduction

Brazil is the third largest fruit producer in the world, producing about 45 million tons in recent years, behind only China and India. Fruit production in Brazil stands out as an important agriculture sector. However, the production of Amazonian fruits represents less than 0.2% of this total (Romero, 2009). Currently, native fruits and derivatives from this region have become increasingly popular, attracting international interest (Azevedo et al., 2019). This growing interest in native Brazilian fruits, such as açaí, cupuaçu, pupunha, bacuri, among others, is attributed to their high economic potential, as well as their high functional potential, due to the presence of bioactive compounds that may present therapeutic properties (Azevedo et al., 2019; Avila-Sosa et al., 2019). The Bacuri (*Platonia insignis* Mart) is a native Brazilian Amazon fruit, due to its peculiar flavor and aroma. It has been widely used for producing juices, ice creams, sweets, jams or even in natura consumption by Amazon and part of Northeastern Brazil populations. (Nascimento et al., 2007). The Cupuaçu (*Theobroma grandiflorum* Schum) is of significant importance for the Amazon region, mainly due to the high commercialization value in natura or processed pulp. Cupuaçu pulp sustains the production, industrialization and commercialization of this fruit, consumed in several forms, including juice, pie, ice cream, cream, jelly, biscuit, jams, nectar and other sweets which, for the most part, are processed and marketed artisanally (Mesquita et al., 2014).

Cupuaçu and bacuri are large fruits, collected on the ground, with thick skin, bulky seeds and low to medium pulp yield, with pulp being the usual form of consumption. The high perishability of the fruits, associated with the storage problems contribute to postharvest losses. Thus, obtaining a storable product, from these highly perishable foods, makes the production of frozen fruit pulps an important part of the production chain, favoring the full use of fruits, also in the off-season (Santos, 2012). In search of a healthier diet in recent years, it is notable that the intake of processed fruits and their derivatives has increased considerably. Despite considerable increases in production, pulps marketed with altered physical, chemical, microbiological and organoleptic characteristics have been reported, probably due to problems associated with inadequate processing and/or inadequate storage, since the production of these pulps was mostly carried out from artisanally (Caldas et al., 2010).

Bacuri and cupuaçu pulps are handmade. In other words, they do not receive heat treatments, which would reduce the likely contamination. Likewise, under unsuitable Good Manufacturing Practices (GMP), industrially processed pulps can also pose health risks to consumers that are unaware of the quality standards required by the law. Inadequate product preparation can pose risks to consumer health, as the food products may be exposed to bacteria, mold and yeast, which are the microbial agents that generally display the most impact on fruit deterioration, with the potential to cause illnesses. The presence of fungi and yeasts is of particular concern, due to their ability to produce mycotoxins, some mutagenic and carcinogenic (Jay, 2005). Although fruit products are more susceptible to mold and yeast contamination, enteric disease outbreaks caused by bacteria, parasites and viruses have also been documented (Beuchat, 2006), while some *salmonell*as is outbreaks have been linked certain varieties of fruits and vegetables (Forsythe, 2013). Among the most important parameters that determine the quality of a food according to Franco and Landgraf (1996), are those that define its microbiological characteristics, which allows evaluating it regarding the conditions of processing, storage, distribution for consumption, shelf-life and health risks to the population. The pulps of fruit processed by hand are very popular in Brazil, mainly because they do not use chemical preservatives. It has huge attraction for consumers, but they show a minimum standard of quality and hygiene, which reveals an insecurity when consuming these products, because the lack of hygiene in the handling, use of inferior quality raw material and inadequate conservation in the commercial establishments cause contamination, causing the risk of the emergence of diseases to consumers.

Food quality control is of paramount importance, as the search for healthier diets in recent years and the intake of processed fruits and their derivatives have increased considerably. Parameters such as reducing total sugars, soluble solids, titratable acidity, vitamin C and pH are extremely important for the standardization of the product and analysis of changes that occurred during processing and storage (Dantas et al., 2010). Moreover, fruits are extremely important in a worldwide scenario in relation to human nutrition, due to the fact that they present important vitamin, fiber and mineral levels (Souza, Carneiro and Gonsalves, 2011). Because of this, standards and regulations are being constantly developed and improved, in order to ensure that consumed food items are healthy, safe and present the quality specified on their packaging (Chitarra and Chitarra, 2005). In Brazil, the quality of marketed fruit pulps is regulated by Resolution RDC No. 12, of 01/02/2001 - Anvisa (Brazil, 2001) and Normative Instruction No. 1, of 01/07/2000 - Ministry of Agriculture and Supply (Brazil, 2000). In this context, the aim of the present study was to evaluate chemical and microbiological quality parameters of industrialized and artisanal bacuri and cupuaçu pulps, native Brazilian Amazon fruits.

Results and discussion

Chemical pulp characterization

For bacuri pulp, pH values ranged from 3.25 to 3.70, presenting a significant difference between the pulps and brands (Table 1). Artisanal pulps presented the highest values, but with no differences between the two analyzed brands. For bacuri pulp, pH values were close to those reported previously ranging from 3.25 to 3.29 (Fontenele et al., 2010). Other authors obtained pH values close to those reported herein, of 3.2 and 3.40 (Porte et al., 2010; Canuto et al., 2010). pH is an important parameter used in the assessment of the conservation state of a food product, since it provides an indication of deterioration degree (Macedo, 2001). The presence of organic acids important components in fruit flavor formation, can also contribute to pH variations (Santos et al., 2014).

Concerning bacuri pulp TTA, no significant difference between IP/A and IP/B were observed, and all pulps/brands presented values lower than those reported by Aquino et al. (2014), whose average acidity was of 1.34%. A significant difference was observed between artisanal pulps, and the highest value was found for AP/C (Table 1). This is a key fruit quality factor for industrial processing (Silva et al., 2010). The presence of organic acids, important components in the formation of fruit flavor, can also contribute to pH variations and determine acidity content (Santos et al., 2014).

Regarding TSS, statistical differences were observed between the four types/brands of bacuri pulps, highlighting pulp AP/C, which presented the highest value, indicating greater sweetness. However, the observed values were lower than those reported by Silva et al. (2010), ranging from 13.27 to 14.83 ° Brix. Thus, product dilution due to water addition during processing is inferred, often performed in order to increase product yield. The same author reported values ranging from 5.09 to 9.93, close to those observed herein, in which artisanal pulps differed from industrial pulps. The TSS/TTA ratio indicates the degree of sweetness of a fruit or its product, evidencing the predominant flavor, either sweet or acid, or a balance between flavors (Lima et al., 2015).

In relation to the pH of the cupuaçu pulps (Table 2), the pulps/brands presented significant values between artisanal pulps and industrialized pulps. Brand B (industrialized pulp) and C (artisanal pulp) presented the highest values but did not differ amongst themselves. In turn, Gonçalves et al. (2013) found a pH value of 3.68, relatively higher than in the present study, while Martim, Neto and Oliveira (2013) obtained a similar result, reinforcing that samples marketed in the local market were within consumption patterns.

The ratios (TTA and TSS) were differed between pulps and brands (Table 2), while the others we as follows: IP/B > AP/C> IP/A = AP/D. The higher acidity in relation to the other evaluated pulps can be explained by the maturation time of the fruits used in the processing of the AP/C pulp. It suggests that low TTA values are due to the use of fruits at an inappropriate maturation stage or to sample dilution, since ripening decreases acidity levels (Batista et al., 2013).

The TSS content that express the presence of sugars with the predominance of sucrose can be influenced by climatic factors, soil type, cultivar and fruit ripening stage, among others (Chitarra and Chitarra, 2005). The ratio values described by Freire et al. (2009) ranged from 5.13 to 6.10 º Brix, lower than the values found herein for brands B and C, which were significantly different in relation to A and D. According to Chitarra and Chitarra (2005), this relationship indicates the influence of acidity on palate sweetness. In addition, this relationship also indicates a subjective notion of the taste of a particular product (Silva et al., 2018).

Table 1. Chemical characteristics of industrial and artisanal bacuri (*Platonia insignis* Mart) pulp, a native Brazilian Amazon fruit.

Pulp/brand	pH	TTA (%)	TSS (ºBrix)	Ratio
IP/A	3.25 c	0.71 b	5.0 d	7.08 c
IP/B	3.29 c	0.72 b	5.6 c	7.80 c
AP/C	3.41 b	0.91 a	9.0 a	9.93 b
AP/D	3.70 a	0.56 c	8.2 b	14.53 a
CV(%)	0.75	4.89	3.94	5.22

Means followed by the same letters in the column do not differ by Tukey's test at a 5% probability level. IP = industrial pulp; AP = artisanal pulp; TTA = Total Titratable Acidity; TSS = Total Soluble Solids.

Table 2. Chemical characteristics of industrial and artisanal cupuaçu (*Theobroma grandiflorum* Schum) pulp, a native Brazilian Amazon fruit.

Pulp/brand	pH	TTA (%)	TSS (ºBrix)	Ratio
IP/A	3.44 c	1.35 b	7.5 b	5.55 c
IP/B	3.55 a	0.89 c	7.0 b	7.86 a
AP/C	3.56 a	1.55 a	9.8 a	6.32 b
AP/D	3.49 b	1.29 b	7.4 b	5.73 c
CV(%)	0.36	3.82	4.33	3.68

Means followed by the same letters in the column do not differ by Tukey's test at a 5% probability level. IP = industrial pulp; AP = artisanal pulp; TTA = Total Titratable Acidity; TSS = Total Soluble Solids.

Table 3. Microbiological analyses of industrially and artisanally processed bacuri (*Platonia insignis* Mart) and cupuaçu (*Theobroma grandiflorum* Schum) pulps, native Brazilian Amazon fruits.

Pulp/brand	Bacuri			Cupuaçu		
	Mold and yeast ($CFU.g^{-1}$)	Staphylococcus aureus ($CFU.g^{-1}$)	Salmonela($MPN.g^{-1}$)	Mold and yeast ($CFU.g^{-1}$)	Staphylococcus aureus ($CFU.g^{-1}$)	Salmonella($MPN.g^{-1}$)
IP/A	$3.19x10^6$ b	$5.09x10^7$ ab	absent	$7.32x10^6$ b	$5x10^7$a	Absent
IP/B	$5.08x10^6$ b	$2.5x10^7$ b	present	$7.06x10^7$ a	$1x10^8$ a	Present
AP/C	$2.83x10^5$ b	$1.12x10^4$ b	absent	$2.803x10^7$ ab	$7.5x10^7$a	Absent
AP/D	$9.36x10^7$ a	$1.17 x 10^8$ a	absent	$4.54x10^7$ab	$1.13x10^8$ a	Absent
CV%	65.10	67.36	---	90.84	34.94	---

Means followed by the same letters in the column do not differ by Tukey's test at a 5% probability level. IP = industrial pulp; AP = artisanal pulp.

Microbiological conditions

For bacuri pulp, the mean mold and yeast values found in AP/D compared to the other brand (A, B and C), while significantly different, ranging from $2.83x10^5$ to $9.35x10^7$ $CFU.g^{-1}$ (Table 3). These values are above the available standards of $5x10^3$ $CFU.g^{-1}$ for *in natura* pulp, frozen or not, and $2x10^3$ $CFU.g^{-1}$ for chemically preserved and/or heat-treated pulp (Brazil, 2001).

High mold and yeast values have been reported in previous studies carried out with bacuri pulps, which may represent a strong indication of raw material contamination or inadequate storage conditions for both artisanal and industrialized pulps (Nascimento et al., 2006; Santos and Nascimento, 2014).

The presence of *Staphylococcus aureus* in bacuri pulp ranged from $1.12x10^4$ to $1.17 x 10^8$ $CFU.g^{-1}$, with the latter being the highest value found in AP/D. The applied analysis of variance test indicated no differences between IP/A, IP/B and AP/C samples. The results described herein differed from those reported by Nascimento et al. (2006), who did not detect presence of this bacterium in 100% of analyzed fruit pulps. *S. aureus* is considered the main agent responsible for foodborne disease outbreaks (Brandão et al., 2013). Its infection is mainly related to the personal hygiene deficiencies, such as hand, nose and skin, that are the main transmission sources. Furthermore, improper handling during processing also contributes to intoxication.

For *Salmonella* spp., bacuri B pulp (industrialized) levels were in disagreement with the quality standard stated in RDC No. 12, of January 2, 2001 (Brazil, 2001). Concerning cupuaçu pulp samples, mold and yeast values were in disagreement with the maximum standard stated by legislation for all pulps, ranging from $7.32x10^6$ to $7.06x10^7$ CFU. g^{-1}, the lowest and highest value, respectively, for industrial pulps A and B. Santos and Nascimento (2014) carried out microbiological characterizations of four regional fruit pulps commercialized at fairs in São Luís/MA, and reported mold and yeast counts ranging from 4 $x10^4$ to $1.28x10^7$ $CFU.g^{-1}$, considered high. Significant counts of these microorganisms may indicate pulp deterioration and represent health risks, considering that some mold species produce mycotoxins (Greco et al., 2012).

No statistically significant differences between pulps were observed for *Staphylococcus aureus* in cupuaçu pulp, which ranged from $5x10^7$$CFU.g^{-1}$ (industrialized) to $1.13x10^8$$CFU.g^{-1}$ (artisanal). According to Penteado et al. (2014), this bacterium may be present in soil, in the environment and even on human skin, making it impossible to identify exactly the origin of contamination. Asepsis of equipment used in frozen fruit pulp processing is essential to avoid microorganism contamination, which can remain viable for many months, even at -25 ° C.

Brand B (industrialized) was positive for *Salmonella* in cupuaçu pulp, while the same was observed for bacuri pulp. This indicates that hygienic conditions during raw material selection, cleaning operations, processing and storage conditions are not in accordance to good manufacturing practices (GMP), posing risks to consumer health.

In general, no significant difference was observed in sample contamination, comparing pulps at fairs and supermarkets. In contrast, Silva et al. (2010) and Santos et al. (2013), reported that food items from fairs are exposed to various situations that lead to contamination, such as improper handling, increased exposure of the product for sale purposes and packaging and storage under inappropriate conditions.

Materials and Methods

Samples

The bacuri and cupuaçu pulp samples were purchased from supermarkets and fairs at the city of São Luís, in the state of Maranhão, and transported to the laboratory in ice box and placed in a vertical freezer (CVU30 model, Consul) at -18°C for further chemical and microbiological assessments. Four fruit pulp brands were selected: industrialized pulps (IP), brands A and B and artisanal pulps (AP), brands C and D. Each IP was identified concerning origin, lot number, manufacture date and expiration date. Brands A and B, obtained in a supermarket, were the same for both pulps. Five replicates of each brand and fruit pulp were collected for the chemical and microbiological analyses, totaling 40 experimental units for each fruit pulp. The samples were obtained in 100 g^{-1} packages.

Chemical analyses

The chemical analyses comprised initial pH, total titratable acidity (TTA), total soluble solids (TSS) and their ratio (TSS/TTA). The pH, TTA (%, citric acid equivalent) and TSS (^{o}Brix) (AOAC, 2016) were analyzed. All determinations were performed in triplicate.

Initial pH determinations

A total of 10 g of concentrated pulp were weighed on an analytical balance (QUIMIS, model Q500B210C) and transferred to a 100 mL beaker containing 60 mL of distilled water, maintained under magnetic stirring. The readings were performed using a digital potentiometer (Tecnopon, model mPA-210) calibrated with pH 4.0 and 7.0 buffer solutions.

Total titratable acidity determinations

To determine the titratable total acidity, 10 g of each sample was weighed on an analytical balance (QUIMIS, model Q500B210C), transferred to a 100 mL beaker containing 60 mL of distilled water, maintained under magnetic stirring. Titration was carried out a 0.1 N sodium hydroxide solution (NaOH) using a standard burette until the pH of the sample reached the turning point (pH: 8.1) (AOAC, 2016). A Tecnopon model mPA-210 digital potentiometer was used to monitor the solution pH. The results were expressed as a percentage of citric acid (AOAC, 2016).

Total soluble solids determinations

Total soluble solids (TSS) were determined by means of direct readings using a manual refractometer (ATAGO, Model HHR-2N), placing 1 to 2 drops of the pulp samples on the prism and carrying out two readings in triplicates for each sample. The results were expressed in ºBrix.

TSS and TTA Ratio

The ratio was obtained by dividing TSS and TTA values.

Microbiological analyses

The microbiological analyses evaluated the presence of mold and yeast, *Staphylococcus aureus* and *Salmonella* spp., adapted from Ital (1995), Dantas et al. (2012) and Brazil (2003), respectively. The pulp packages were opened using scissors disinfected with cotton soaked in 70% alcohol. All determinations were performed in duplicate.
The results were evaluated as to their suitability according to the RDC Nº12 Resolution, 02/01/2001 – Anvisa (Brazil, 2001), which establishes the absence of *Salmonella* in 25 g of pulp, and Normative Instruction nº1, 07/01/2000 - Ministry of Agriculture and Supply (Brazil, 2000), which establishes a maximum limit of 5 x 10^{3} Colony Forming Units (UFC.g^{-1}) for mold and yeast in fresh fruit pulps and 2 x 10^{3} CFU.g^{-1} in thermally treated pulps.
After thawing the samples at room temperature, 25 g of each sample were weighed and aseptically transferred into vials containing 225 mL of sterile peptone water (10^{-1}dilution). From this dilution, serial dilutions were prepared, namely a 10^{-2} dilution (9 mL of peptone water + 1 mL of a 10^{-1} sample) and a dilution 10^{-3} (9 mL of peptone water + 1 mL of a 10^{-2} sample).

Mold and yeast determinations

The standard counting method (SCM) was used for mold and yeast counting, termed depth seeding (pourplate technique). Briefly, 1 mL of each dilution (10^{-1}, 10^{-2} and 10^{-3}) were aseptically transferred to identify sterile petri dishes, in duplicate. About 15 mL of Dextrose Potato Agar (DPA, Micro MED) previously melted and maintained at 45 °C were added to each petri dish. After homogenization, the petri dishes were incubated inverted between 22 and 25 °C in a BOD oven (FANEM, Model 347 CDG) for 72 hours. The results were expressed as the number of colony forming units per gram of material according to each dilution (UFC.g^{1}) (Ital, 1995).

Staphylococcus aureus **determinations**

To analyze the presence of *S. aureus*, 1 mL of each dilution (10^{-1}, 10^{-2} and 10^{-3}) followed by the addition of mannitol culture medium (KASVI) were homogenized. After waiting a few minutes for complete medium solidification, petri dishes were placed in a BOD oven (FANEM, Model 347 CDG) for 48h at 37 ° C. After 48 h counts and means calculations were performed. The results were expressed as the number of colony forming units per gram of material according to each dilution (UFC.g^{-1}) (Dantas et al., 2012).

Salmonella spp. determinations

Only the presence or absence of *Salmonella* spp. was determined. In the pre-enrichment stage, 25 mL of each sample were homogenized in 225 mL of peptone water and then incubated in an oven (QUIMIS, Model Q316M4) at 36 ° C for 24h. Then, 1mL of each pre-enriched sample was transferred into tubes containing 10mL of Selenite broth and incubated again under the same conditions. Subsequently, isolation in *Salmonella*-Shigella Agar (SS) and Xylose Lysine Deoxycholate Agar (XLD) (KASVI) was carried out using the seeding technique, with incubation carried out in an oven at 36 ° C for 24 h for typical colony verification. Typical colonies were transferred to Nutrient Agar, followed by biochemical tests. The results were expressed as the Most Likely Number (MPN) per gram of material according to each dilution ($NMP.g^{-1}$) (Brazil, 2003).

Statistical analyses

The assays were conducted in a completely randomized design, consisting of five replicates for each treatment. Statistical data evaluations concerning the microbiological and chemical analyses were performed using the ASSISTAT beta program, version 7.7, 2014 (Silva, 2014), by applying an analysis of variance (ANOVA) and Tukey's test for comparison of the means at a 5% level of significance.
The results were also individually compared to the standards established by Brazilian standards for each type of fruit pulp (Brazil, 2000, 2001).

Conclusions

Chemical and microbiological parameters of industrialized and artisanal bacuri and cupuaçu pulps of the four brands were evaluated. Overall, the chemical characterization demonstrated that bacuri pulps had lower level of TTA and TSS than cupuaçu pulp. However, ration between TSS and TAA showed higher level in artisanal bacuri brand D. The microbiological analyses indicated non-uniformity for bacuri and cupuaçu fruit pulps of all brands, with emphasis on brand B (industrialized), which tested positive for Samonella. Thus, possible failures in the choice of raw material, handling, processing and/or storage are noted, may contribute to the consumer health risks.

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