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Microbiological Analysis of Tucumã Pulp (*Astrocaryum aculeatum* Meyer) Commercialized at Fairs in the City of Manaus

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A B S T R A C T

The tucumanzeiro is a palm tree of the Arecaceae family, and *Astrocaryum aculeatum* is the best known and most appreciated species. The fruit called tucumã has a significant cultural and economic value, and despite being widely consumed, the pulp of this fruit has a relatively short shelf life. At fairs, it is possible to see the pulp being stored inappropriately, contributing to the proliferation of microorganisms. Given the importance of this fruit for local trade, this study aimed to investigate the microbiological profile of tucumã pulp sold in the main fairs in Manaus. The pulps were obtained from 18 points of sale divided between the six zones of the city. The samples were placed in a thermal box and submitted to microbiological analysis. The microorganisms evaluated were coliforms (AOAC 991.14), *Escherichia coli* (AOAC 991.14), *Salmonella* spp. (ISO 6579), molds and yeasts (APHA 21:2015) in triplicate. The results showed that 13 samples showed coliform growth with amounts ranging from 6.4x10 to 1.2x10^4 CFU/g. Regarding the amount of yeast, only three samples showed results above what is considered ideal for fruit pulps. Samples that showed growth for molds were within the permissible microbiological limits. None of the samples showed growth for *E. coli* and *Salmonella* spp.

Introduction

The Amazon is well known for its diversity of both fauna and flora. It presents a wide variety of fruits with local economic potential and, among these, tucumã stands out (Braga *et al.*, 2010). The tucumanzeiro (*Astrocaryum aculeatum* G. Mey) is a palm with great economic potential due to its various uses (Xisto, 2020). One of the main ways of using the tree is to obtain the fruits. In the municipality of Manaus, tucumã - the fruit of

the tucumanzeiro - has greater preference and consumption, arousing interest in the cultivation of the species (Flor, 2013).

There are two ways in which the fruit is generally sold: (i) in nature, sold in a dozen or a hundred; and (ii) locally processed by the traders themselves, where the pulp is sold by weight. As it is a regional product of forest origin, it is rarely sold in supermarkets, but it is abundant in open markets, markets, and strategic points of sale on the streets of Manaus, where it can be found during much of the year (Costa *et al.,* 2005; Didonet and Ferraz, 2014). Tucumã pulp is consumed in nature, accompanied by manioc flour, as a sandwich and in the form of juices and ice cream (Moussa and Kahn, 1997).

Among the desirable attributes in tucumã pulp is the microbial quality within the standards required by Normative Instruction (NI) nº 60, of December 23, 2019, and by NI nº 1 of 2000 (Brazil, 2019; Brazil, 2000; Flor, 2013).

Despite being widely consumed, the pulp of this fruit has a relatively short shelf life. According to Schroth *et al.,* (2004), when obtaining the tucumã pulp, it must be consumed in one day, at room temperature, or in one week, under refrigeration. However, what can be seen in the fruit pulp trade is quite different from what is recommended by scientific studies.

For Azevedo *et al.,* (2017), it is possible to verify the tucumã pulp being stored in inadequate conditions, a fact that contributes to the proliferation of microorganisms and the development of food-borne diseases (DTA). Among the microorganisms that cause DTA are coliforms, *Salmonella* spp., molds, and yeasts. Faced with this problem, this study aimed to investigate the microbiological profile of tucumã pulps sold in the main fairs in Manaus.

Materials and Methods

Sample Collection

The samples of tucumã (Figure 1) previously fractionated were acquired at fairs and stalls in six different areas of Manaus (North, East, South, West, Center-South, and Center-West) which were identified as follows:

North – Point 4 (P4), Point 15 (P15), Point 16 (P16).

East – Point 5 (P5), Point 6 (P6), Point 14 (P14).

South – Point 3 (P3), Point 9 (P9), Point 10 (P10), Point 11 (P11), Point 12 (P12), Point 13 (P13).

West – Point 8 (P8).

Center-South – Point 17 (P17), Point 18 (P18).

Midwest – Point 1 (P1), Point 2 (P2), Point 7 (P7).

From each zone, points of sale of tucumã pulp were randomly chosen for analysis. The samples were transported in thermal boxes for immediate processing at the Microbiology Laboratory of Estácio Amazonas College.

Microbiological Evaluation

Coliforms and *Escherichia coli*

The method used was the Petrifilm™ plate for counting *E. coli*/Coliforms (AOAC Methods 991.14 and 998.08) (Silva *et al.,* 2017). 25 g of sample were weighed and added to a flask containing 225 ml of Phosphate Buffer. After homogenization, the plate was placed on a flat surface, the top film was lifted and the volume of 1 ml of the solution was deposited. After waiting 2-5 minutes, the plates were incubated

in a bacteriological incubator at 35 ± 1 ºC for 24 hours. After incubation, only typical colonies with the following characteristics were counted: total coliforms, all red, blue, or bluish-red colonies with gas bubbles, and *E. coli* only blue or bluish-red colonies with gas bubbles. As there was no development of typical *E. coli* colonies within 24 hours, the plates were reincubated to repeat the count at 48 hours. The result was expressed in a colony-forming unit (CFU) per gram of sample (CFU/g).

Salmonella spp.

The method used was the Petrifilm™ *Salmonella* Express System Plate (ISO 6579 Method) (Silva *et al.*, 2017). 10 g of sample was weighed, added in a flask with 90 ml of Supplement Enrichment Broth for *Salmonella* spp. and the homogenized solution. The flask with this solution was incubated in a bacteriological incubator at 41.5°C ± 1°C for 24 hours.

Petrifilm™ plates were prepared by aseptically adding 2 ml of sterile distilled water to each plate and stored at room temperature (25°C) for 1 hour in the dark. At the end of the hydration period of the plates, 10 µl of the sample were removed from the bottle containing Enrichment Broth and carefully striated on the surface of the plate. Plates were incubated at 41.5°C ± 1°C for 24 hours. After this period, the typical colonies with the following characteristics were presumptively quantified: red colonies with yellow zones and associated gas bubbles, red colonies with yellow zones, and red colonies with gas without surrounding yellow zones.

For biochemical confirmation, the Petrifilm™ *Salmonella* Express System disc was used. On the plate with grown colonies, the top film was lifted and the confirmation disc was carefully inserted. The upper film was placed again, taking care to remove the excess bubbles resulting from the procedure. Plates were incubated again at 41.5°C ± 1°C for 4 hours and typical colonies were quantified. Only dark blue/black colonies with blue precipitate and dark blue/black colonies with a dark red center and blue precipitate were considered. The result was expressed in the CFU/g of a sample.

Molds and Yeasts

The method used was counting molds and yeasts in Dicloran Rose Bengal Chloramphenicol Agar – DRBC (APHA Method 21:2015) (Silva *et al.*, 2017). 25 g of sample were weighed and added to a flask containing 225 ml of 0.1% peptone water. The solution was homogenized, and then serial dilutions were made up to 10^{-3}. 0.1 ml of the 10^{-3} dilution was transferred to Petri dishes with about 20 ml of DRBC agar where the inoculum was spread with Drigalski loop. After 15 minutes the plates were incubated without inverting at 25 ± 1°C for 7 days in the dark. After this period, the colonies grown were counted by separating molds and yeasts. The result was expressed in the CFU/g of a sample.

Test results for the search for *Salmonella* spp. and *E. coli* were analyzed based on NI nº 60 of 2019 (Brazil, 2019) that establishes the lists of microbiological standards for foods. The results of coliforms, molds, and yeasts were analyzed as established in NI nº 1 of 2000 (Brazil, 2000), which determines identity and quality standards for fruit pulps.

Results and Discussion

Microbiological analyzes revealed the presence of coliforms, yeasts, and filamentous fungi (Table 1) in the evaluated samples. No samples were contaminated by *Salmonella* spp. and *E. coli*. Coliform bacteria are defined

as facultative anaerobic, gram-negative, non-spore-forming rods that ferment lactose vigorously in acid and gas at 35°C in 24 or 48 h (Halkman and Halkman, 2014). Coliform bacteria generally belong to four genera of Enterobacteriaceae: *Citrobacter freundii*, *Enterobacter cloacae*, *Enterobacter aerogenes*, *E. coli* and *Klebsiella pneumoniae*.

Although NI nº 60 does not set limits for coliforms, most samples had significant amounts of bacteria from this group. The NI nº1 of 2000 (Brazil, 2000) determines identity and quality standards for some fruit pulps. Although tucumã is not on the list, the Amazonian fruit of the same botanical family as the açaí (*Euterpe oleracea* Mart.) that is described in the IN can serve as a basis for evaluating the microbiological profile of the tucumã. Among the analyzed tucumã pulps, it was found that there was a significant growth of coliforms in samples P1, P2, P4, P7, P8, P9, P11, P12, P14, P15, P16, P17, P18. The high number of these microorganisms indicates that the circumstances are suitable for the presence of enteric pathogens and may mean insufficient sanitary conditions in the processing and/or packaging of the fruits. This type of problem can be easily solved with the adoption of good manufacturing practices combined with the quality of storage of this type of input. Azevedo *et al.,* (2017) verified that the use of vacuum packaging, associated with freezing, allows the preservation of the main nutritional characteristics of tucumã pulp in nature, for 30 days of storage.

An alternative for using the pulp more efficiently was also presented by Yuyama *et al.,* (2008) who suggested the application of dewatering and spraying methods. Through periodic tests, they concluded that dehydrated and pulverized tucumã pulp, regardless of the type of packaging and storage temperature, could be stored and consumed for up to 150 days, while maintaining its nutritional and microbiological potential.

Other Amazonian fruit pulps have also been studied for the presence of microbiological contaminants associated with the lack of application of good sanitary practices. Santos *et al.,* (2016), when evaluating samples of açaí pulp, an Amazonian fruit that is widely consumed in the form of juices, they also found bacteria from the coliform group, including *E. coli*, in about 35% of the samples.

Costa *et al.,* (2020) analyzing açaí samples in five open markets in Manaus, found that all were unfit for consumption due to the presence of pathogenic bacteria. A similar result was observed by Jones and Lemes (2014) who found 83.3% of samples contaminated even in açaí pulps that underwent pasteurization process. Santos and Nascimento (2014) also found coliforms above the allowed limit in acerola, bacuri, cupuaçu and guava pulps.

Molds and yeasts are considered a group of quality indicator microorganisms, being slightly related to inadequate manufacturing practices. In this context, high counts of this group of microorganisms can lead to a reduction in the shelf life of the product, because of food deterioration, and consequently, significant economic losses to the production chain (Moraes and Machado, 2021).

Yeast and mold counts are more relevant indicators of a shelf life than bacteria. They are commonly listed in foods as quality indicators. Yeasts are a common cause of food spoilage, especially in acidic foods such as fruits and fruit juices (Halhman and Halkman, 2014).

Table.1 Microorganisms quantified in tucumã pulps from different parts of the city of Manaus.

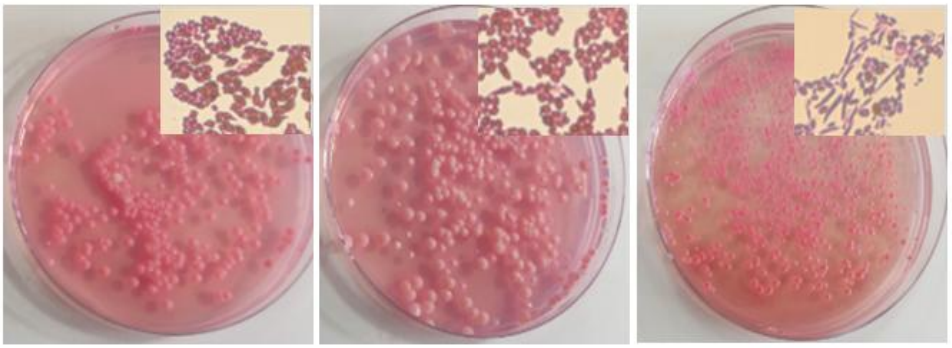
Sample	*E. coli*	*Salmonella*	*Coliforms (CFU/g)	*Yeasts (CFU/g)	*Molds (CFU/g)
P1	Absent	Absent in 25 g	$2.7 \times 10^{3**}$	2.6×10	0
P2	Absent	Absent in 25 g	$3.5 \times 10^{3**}$	5.2×10	1.3×10^{-2}
P3	Absent	Absent in 25 g	0	$7.6 \times 10^{3**}$	1.0×10^{2}
P4	Absent	Absent in 25 g	$4.0 \times 10^{3**}$	6.0×10^{2}	2.6×10^{-2}
P5	Absent	Absent in 25 g	0	5.6×10^{2}	1.2×10
P6	Absent	Absent in 25 g	0	$1.2 \times 10^{4**}$	0
P7	Absent	Absent in 25 g	$2.9 \times 10^{3**}$	0	0
P8	Absent	Absent in 25 g	$1.2 \times 10^{2**}$	2.6×10	0
P9	Absent	Absent in 25 g	$4.0 \times 10^{**}$	0	0
P10	Absent	Absent in 25 g	0	0	0
P11	Absent	Absent in 25 g	$4.0 \times 10^{3**}$	3.6×10^{2}	0
P12	Absent	Absent in 25 g	$1.2 \times 10^{4**}$	2.5×10^{2}	2.6×10^{-2}
P13	Absent	Absent in 25 g	0	0	0
P14	Absent	Absent in 25 g	$6.4 \times 10^{**}$	0	0
P15	Absent	Absent in 25 g	$8.0 \times 10^{**}$	0	0
P16	Absent	Absent in 25 g	$1.2 \times 10^{4**}$	$1.2 \times 10^{4**}$	0
P17	Absent	Absent in 25 g	$6.4 \times 10^{2**}$	9.0×10	0
P18	Absent	Absent in 25 g	$1.2 \times 10^{4**}$	2.5×10^{2}	0

*There is no standard in NI nº 60 of 2019
**Samples that presented values above what is considered safe for some fruit pulps (Brazil, 2000).

Fig.1 Tucumã samples obtained for the experiment

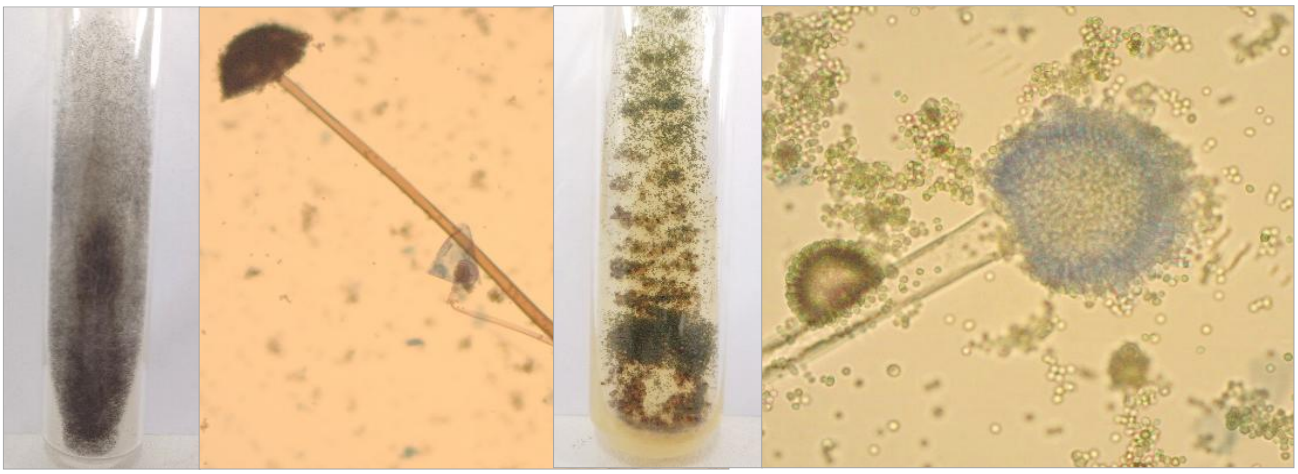
A – Packages containing tucumã pulp obtained at fairs. B – Tucumã pulp. C – Erlenmeyer flasks with 25 g of sample in 225 ml of Phosphate Buffer.

Fig.2 Dichloran Rose Bengal Chloramphenicol (DRBC) agar plates after 7 days of incubation



Yeast isolated from samples. A – Sample P3. B – Sample P6. C – Sample P16. Highlights, yeast microscopy at 100X magnification.

Fig.3 Some fungi isolated from tucuma pulp



Fungi isolated from the samples. A – Sample P4. B – Sample P12. Highlights, microscopy of filamentous fungi at 40X magnification

In the present work, only 33.3% of the analyzed tucumã samples showed negative results for yeast growth (Table 1). The IN nº 60 also does not set limits for the presence of molds and yeasts in fruit pulps however Brazil (2000) establishes for some fruit pulps that values above 5.0 x 10^3 CFU/g (or CFU/ml) may not be considered safe. Evaluating from this perspective, only samples P3, P6, and P16 (Figure 2) could be considered unfit for consumption.

Although fungi do not cause infection through food, some strains can produce mycotoxins, which can cause serious chronic illness if consumed. As quality indicators, they can be used to assess ingredient acceptability, organoleptic characteristics, stability, and shelf life of a product (Halhman and Halkman, 2014).

Furthermore, the presence of certain species of filamentous fungi such as *Penicillium*, *Aspergillus*, and *Fusarium* can result in serious damage due to their ability to produce mycotoxins (Moraes and Machado, 2021). In this work, only samples P2, P3, P4, P5, and P12 showed the growth of filamentous fungi

(Figure 3). Despite these results, according to NI nº1 from 2000 (Brazil, 2000) all samples are within the microbiological limits established for molds.

The results demonstrate that, despite the samples complying with the microbiological standards of NI nº 60 of 2019 regarding the absence of *E. coli* and *Salmonella*, it is necessary to implement good manufacturing practices. This is because a good part of the samples was not within the microbiological standards defined by NI nº1 of 2000 regarding the presence of coliforms and yeasts in fruit pulps. For this reason, works such as this one highlights the importance of constant microbiological surveillance to prevent possible outbreaks of food-borne diseases (DTA).

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