Microbiological evaluation of acerolas from a private cultivated orchard in the city of Macapá, Amapá, Brazil

Avaliação microbiológica de acerolas provenientes de um pomar de cultivo particular na Cidade de Macapá, Amapá, Brasil

Jaqueline Freitas Souza¹, Evanilza Aristides Santana², Anne do Socorro Santos da Silva³, Antonio Carlos Freitas Souza⁴

¹Pharmacist, Master’s student in Animal Science, State University of Maranhão-UEMA, São Luís-MA Brazil. https://orcid.org/0000-0002-3046-3212 E-mail: jackilinefn@hotmail.com * Corresponding author
²Pharmacist graduated from the Mapaense Institute of Higher Education-IMMES, Macapá-AP Brazil. E-mail: evasantana@live.com
³Nutritionist, Family Health Specialist, Researcher at the Institute of Scientific and Technological Research of the State of Amapá-IEPA, Macapá-AP Brazil. E-mail: annedoscorro@hotmail.com
⁴Biologist, Master in Health Sciences, PhD student in Animal Science, Researcher at the Institute of Scientific and Technological Research of the State of Amapá-IEPA, Macapá-AP Brazil. https://orcid.org/0000-0002-6921-9030 E-mail: jr_bio2005@yahoo.com.br

Worldwide, there is a significant increase in the consumption of tropical fruits, due to its characteristics and advantages of cultivation is the high number of harvests per year, normally four, and can reach seven harvests in the case of irrigated crops (JUNQUEIRA et al., 2004). Its production normally ranges from 20 to 100 kg/plant/year, and its fruits usually weigh between 2 and 15 g and show 1 to 4 cm in diameter (DELVA; SCHNEIDER, 2013a; NUNES et al., 2011), and in their development phase they show green color and with the arrival of maturation they obtain orange pigments until finally reaching an intense red color when fully ripe, a process that lasts an average of 5 days (BARBOZA; TAVARES; MELO, 1996).

INTRODUCTION

Worldwide, there is a significant increase in the consumption of tropical fruits, due to its characteristics and advantages of cultivation is the high number of harvests per year, normally four, and can reach seven harvests in the case of irrigated crops (JUNQUEIRA et al., 2004). Its production normally ranges from 20 to 100 kg/plant/year, and its fruits usually weigh between 2 and 15 g and show 1 to 4 cm in diameter (DELVA; SCHNEIDER, 2013a; NUNES et al., 2011), and in their development phase they show green color and with the arrival of maturation they obtain orange pigments until finally reaching an intense red color when fully ripe, a process that lasts an average of 5 days (BARBOZA; TAVARES; MELO, 1996).

Brazilian industries demand about 34.40 thousand tons of this fruit per year, which is equivalent to 7.16% of the total of fruits harvested in the country (ALDRIGUE et al., 2002). This...
intense production occurs through the great interest due to its nutritional aspects, which made acerola a highly requested fruit in the world market for the preparation of juices and consumption "in natura" (SOUZA et al., 2006).

The great success of the industrialization of acerola is also due to the amount of edible pulp that the fruit produces, about 70 to 80% (AGUIAR et al., 2010), given this great productivity it is necessary to obtain a fruit with quality control ideal, allowed for human consumption because it is known that there may be errors in several processes before reaching the consumer’s table, resulting in risks (SILVA-JÚNIOR et al., 2015; SILVA-JÚNIOR et al., 2017a; SILVA-JÚNIOR et al., 2017b; COSTA; NASCIMENTO; SILVA-JÚNIOR, 2018; JESUS et al., 2018; SILVA-JÚNIOR et al., 2018; SOUZA; NASCIMENTO et al., 2019; SOUZA, 2019; SOUZA et al., 2020a; SOUZA et al., 2020b; SOUZA; SOUZA; MENDES, 2020).

Therefore, the objective of this study was to carry out the microbiological evaluation of aceroleira fruits from a private cultivated orchard in the city of Macapá Amapá.

MATERIAL AND METHODS

The acerola fruits were collected in February 2019 in a private cultivated orchard in the municipality of Macapá-AP, BR 156, Km 14, branch of Km 9. They were collected directly from the trees, physiologically mature and manually (Figure 1). The process started with the preparation of the pulp through pulping, freezing and pasteurization.

Figure 1. Aceroleira fruit from private cultivation in Macapá.

Microbiological analysis

For the microbiological determinations, aseptic collection of 20g of acerola was performed and homogenized in 180 ml of 0.1% sterile peptone water (HIMEDIA, RM001), with the aid of the homogenizer (Stomacher). Subsequently, the serial dilution of 1 ml of the samples was carried out in 9 ml of the sterile peptone water 0.1% followed by sowing in plates or tubes for microbiological counts, which were performed in duplicate and according to Brazil (2003) and Silva et al, (2010).

For presumptive analysis of coliforms, three appropriate dilutions of the sample were selected and inoculated into a series of three tubes of lauryl sulfate tryptose broth (LST) (ACUMEDIA, 7142) by dilution, adding 1 ml of the dilution per tube in 10 ml of LST. The tubes were incubated at 37 ± 1ºC / 24 ± 12h and observed if there was growth through turbidity and gas production.

In a positive case, for total coliforms, elevations of the positive tubes were transferred to bright green broth (VB) (KASVI, K25-610010) and incubated at 35ºC for 48 hours, and for thermotolerant coliforms, a loaded section of each culture was transferred to tubes of Escherichia coli broth (EC) (ACUMEDIA, 7206) incubated at 42ºC for 24 ± 2h in a water bath (they remained submerged in water up to a height above the surface of the culture medium) and it was observed growth with gas production.

For the presumptive counting of total and thermotolerant coliforms, the numbers of tubes of Broth EC and Broth VB were recorded and compared with the Most Likely Number table (NMP for decimal dilutions)/g.

To determine the absence/presence of Salmonella sp, 25 g of each sample was weighed and transferred to a sterile homogenization bag, where 225 ml of buffered peptide water was added. Then the samples were incubated at 37 ± 1ºC for 18 ± 2 hours. After incubation, 0.1 ml and 1 ml aliquots were transferred to Rappaport Vassiliadis Soja broths (incubated at 37 ± 1ºC / 24 ± 3h) and Muller Kauffmann Novobiocina Tetrathionate broth (incubated at 41.5 ± 1ºC / 24 ± 3h) respectively. From the tubes were transferred elevated by streak of exhaustion on the surface of Xylose Lysine Deoxycholate Agar (XLD agar) incubated at 37 ± 1ºC / 24 ± 3h.

The confirmation was carried out with the transfer to Triple Sugar Iron agar (TSI) with a deep prick and striated movements on the surface, being incubated at 35 ± 0.5ºC/18-24 hours. After this period, it was passed through a biochemical series (glucose, sucrose and lactose fermentation test; indole test; malonate; citrate; urea; decarboxylation of lysine in broth). The samples that presented biochemical characteristics compatible with the genus Salmonella sp. and those that did not show autoagglutination, were submitted to polyvalent serology.

RESULTS AND DISCUSSION

Microbiological analysis of acerola, Malpighia emarginata DC.

Total and thermotolerant coliforms with values ranging from 2.1x10² to> 1.1x10³ NMP/g were found in the samples of fresh acerola. For thermotolerant coliforms, the values are in disagreement with the resolution RDC nº 12 (BRASIL, 2001),...
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Table 1. Microbiological analysis of fresh acerola and acerola after pasteurization.

| Sample | CTotal (NMP/g) | Cterm (NMP/g) | Salmonella sp. |
|--------|----------------|---------------|----------------|
| A1     | > 1,1x10³      | > 1,1x10³     | Absent         |
| A2     | > 1,1x10³      | > 1,1x10³     | Absent         |
| A3     | 2,1x10²        | 2,1x10²       | Absent         |

| Sample | CTotal (NMP/g) | Cterm (NMP/g) | Salmonella sp. |
|--------|----------------|---------------|----------------|
| A1     | <3             | <3            | Absent         |
| A2     | <3             | <3            | Absent         |
| A3     | <3             | <3            | Absent         |
| Standard* | -            | 10²      | Absent         |
| Standard** | -            | 1         | Absent         |

*Brazil (2001); **Brazil (2018)

which establishes a maximum value of 10² NMP/g, whereas for total coliforms, the legislation does not present a parameter; however, this microorganism is an indication of hygienic-sanitary quality, even compared to Normative Instruction No. 49/2018 (BRAZIL, 2018) which establishes the identity pattern of fruit juice and pulp, still remain in disagreement. For Salmonella sp. all samples were negative, in accordance with the recommendations of the legislation that determines the absence of 25 g of sample.

The pasteurization process was carried out in order to decontaminate the fruit, this process caused a 100% reduction in contamination by microorganisms, leaving the fruit in accordance with both current legislation (BRAZIL, 2011; BRAZIL, 2018), as shown in table 1.

Santini (2017), analyzing frozen fruit pulps acquired in supermarkets in the city of Campinas, SP did not find significant counts of thermotolerant coliforms and Salmonella in the acerola pulps. In the study by Muniz, Reis and Vieira (2017), evaluating samples of frozen fruit pulps where the acquisition was carried out in supermarkets in Southwest Bahia, it was reported that none of the samples of acerola pulps showed growth of total coliforms.

While in the work of Santos and Barros (2012), assessing the health profile of fruit pulps randomly collected at different points of sale in the municipality of Paragominas-PA, found in the analyzes for thermotolerant coliforms pulps of acerola outside the established standard.

For Castro et al. (2015) during the counting of microorganisms, the absence of groups of coliforms and Salmonella sp. in fruit pulps, is directly linked the satisfactory quality of the product, which probably occurs through the appropriate application of storage and/or processing techniques.

According to Siqueira (1995), the total coliform index is used to assess hygienic conditions and the thermotolerant coliform index is adopted as an indicator of faecal contamination. Since high counts mean failures during manufacturing, such as the employees’ hygienic-sanitary conditions, poorly washed fruits, poor cleaning and sanitation, inefficient heat treatments and multiplication during the storage process (SOUZA; CARNEIRO; GONSALVES, 2011).

CONCLUSION

In the initial microbiological analyzes, high counts of microorganisms were demonstrated, making pasteurization necessary for the decontamination of the fruits, meeting the standards established by the current legislation. The findings evidenced the need for a technique to eliminate microorganisms before consumption, as it may bring about a risk to the health of those who choose to consume the fruit in
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