



## MICROBIOLOGICAL AND PHYSICOCHEMICAL QUALITY OF FRUIT PULPS MARKETED IN SALINAS-MG, BRAZIL

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### ABSTRACT

The fruit pulp production can minimize losses, waste and add value to fruits, allowing availability to consumers in the off-season. The objective of this work was to evaluate the microbiological and physicochemical quality of frozen fruit pulps of passion fruit (*Passiflora edulis*), guava (*Psidium guajava*), and caja (*Spondias mombin*) commercialized in the region of Salinas, Minas Gerais, Brazil. Analyses of mesophilic and psychrotrophic aerobic microorganisms, mold and yeast count, coliforms at 35 °C and *Salmonella spp.* were performed. In regard to the physicochemical attributes, total soluble solids, pH and Total Titratable Acidity were evaluated. The results of the microbiological analyses were in accordance with the recommendations of Brazilian legislation. Non-conformities for Total Soluble Solids (TSS) were verified in passion fruit pulps of brands A and B, pH in guava pulp of brand B and, for Total Titratable Acidity (TTA), in passion fruit and caja pulps of both brands evaluated.

**Keywords:** Characterization; Frozen product; Consumption; Legislation.



## 1. INTRODUCTION

Brazil is the largest producer of fresh fruits, however, because they are highly perishable products, most of these fruits deteriorate in few days, and their commercialization is difficult, especially over long distances (Morais, 2010). In this sense, obtaining products such as fruit pulp can minimize losses, waste and add value to fruits, allowing consumer availability during the off-season.

According to the Legislation of the Ministry of Agriculture, Livestock and Supply (Brasil, 2000), fruit pulp is defined as unfermented, unconcentrated and undiluted product, obtained by crushing pulpy fruits, through an appropriate technological process, with a minimum content of total solids from the edible part of the fruit, specific to each fruit pulp. The pulps should be obtained from fresh, healthy and ripe fruits while maintaining the physical, chemical and sensory characteristics of the fruit. This product shall not contain fragments of the inedible parts or substances which are foreign to their normal composition.

The fruit pulp has a great importance as raw material, and can be produced in harvest seasons, stored and processed in the most favorable periods (Bueno et al., 2002) or according to the demand of the consumer market, as sweet in mass (Dias et al., 2018), jams (Silva et al., 2020a), edible ice cream (Öztürk et al., 2017), candies (Miranda et al., 2020), alcoholic beverages (Karabagias et al., 2020) and non-alcoholic (Silva et al., 2020b; Furtado et al., 2020), among others.

There are several alternative processes used in the preparation and conservation of pulp, such as: pasteurization (Branco et al., 2016), chemical additive conservation (Neves et al., 2007) and freezing (Machado et al., 2019). However, this practice may involve problems related to the break of the cold chain during the



distribution of the product, favoring microbial growth and compromising pulp quality (Santos, 2004).

The growth perspective of this market is directly linked to the awareness of the urban population about this healthier consumption activity and, consequently, to changes in habits caused by various causes, highlighting the adjustment of urbanization and the facilities of modern life (Oliveira & Donato, 2019).

In this regard, the objective of this study was to evaluate the microbiological and physicochemical quality of fruit pulps commercialized in Salinas, Minas Gerais, Brazil, in order to verify whether there is adequacy regarding the quality standards required by Brazilian legislation.

## **2. METHODOLOGY**

### **2.1 Acquisition of samples**

The pulps were collected in the Municipal Market of Salinas-MG, from a rural cooperative in the community of Jacurutu, and the flavors of guava (*Psidium guajava*), caja (*Spondias mombin*) and passion (*Passiflora edulis*) fruit were selected for presenting a higher prevalence of commercialization. They were kept under freezing until microbiological and physicochemical analyses and separated according to the two brands found (A and B).

### **2.2 Microbiological analyses**

Microbiological analyses were performed in a Microbiology Laboratory (latitude: 15°27`S, longitude: 44°22`W), using the methodologies recommended by the



American Public Health Association (Apha, 2015). The count analyses of mesophilic and psychrotrophic aerobic microorganisms, mold and yeast count, coliforms at 35 °C, and the presence of *Salmonella spp.* were verified.

For the quantification of total mesophilic aerobic microorganisms and total psychrotrophic aerobic microorganisms, the total counting and plating method was performed using Plate Counting Agar (PCA). For the analysis of total mesophiles, the plates were incubated at  $37 \pm 1$  °C for 48h, while for the analysis of psychrotrophic microorganisms were incubated at  $7 \pm 1$  °C for 10 days. The results were expressed in Colony Forming Units per gram (CFU/g).

For the counting of molds and yeasts, the surface plating method was used in Potato Dextrose Agar (PDA) with 10% tartaric acid solution. Thus, aliquots of 100 µL were transferred over the surface of PDA and the plates were incubated at  $25 \pm 1$  °C for 5 days. The results were expressed in Colony Forming Units per gram (CFU/g).

The quantity of coliforms were determined by multiple tubes and quantified by Most Probable Number (MPN) techniques. The results were expressed in MPN/g. *Salmonella spp.* analysis was performed using a pre-enrichment stage with buffered peptone water, followed by transference into selective and differentiation media according to APHA (2015). The results were expressed as absence or presence in 25g of sample. All analyses were performed in triplicate.

## 2.3 Physicochemical analyses

The physicochemical analyses were performed in triplicate and according to the analytical standards of the Adolfo Lutz Institute (2008).



- pH: The pH of the fruit pulps were determined using a pHmeter, and the results were expressed in pH unit.
- Total Titratable Acidity (TTA): It was determined by the alkalimetric method, using as phenolphthalein indicator at 1% and titling the solution with NaOH 0.1M. The results were expressed as a percentage of citric acid.
- Total Soluble Solids (TSS): Soluble solids content were determined in the pulps using refractometer. The results were expressed in °Brix.
- TSS/TTA ratio: It was obtained by the algebraic operation of division of values found for SST and ATT.

## 2.4 Statistical analysis

The results of the physicochemical analyses were analyzed by means of variance analysis (ANOVA) and the means compared by the Tukey test for  $p \leq 0.05$ , using the Excel software version 2010.

## 3. RESULTS AND DISCUSSION

### 3.1 Microbiological parameters

The results obtained by counting of molds and yeasts, coliforms at 35 °C and *Salmonella spp.* are shown in Table 1. The mold and yeast counts ranged values between  $1.1 \times 10^2$  to  $2.4 \times 10^3$  CFU/g. This fact can be partially attributed to the high carbohydrate content and acid character of the pulps. The current legislation for fruit pulps defines as a maximum limit  $5 \times 10^3$  CFU/g for these microorganisms for frozen or non-frozen pulp (Brasil, 2000).



**Table 1.** Counting of mold and yeast, coliforms at 35 °C, and *Salmonella spp.* In fruit pulps marketed in Salinas, Minas Gerais, Brazil.

Brands	Pulps	Molds and yeasts (CFU/g)	Coliforms at 35 °C (MPN/g)	<i>Salmonella</i> <i>spp.</i> (absence/25 g)
A	Passion fruit	$1,0 \times 10^3$	< 3	Absence
A	Guava	$4,0 \times 10^2$	< 3	Absence
A	Caja	$1,6 \times 10^3$	< 3	Absence
B	Passion fruit	$1,3 \times 10^3$	< 3	Absence
B	Guava	$1,1 \times 10^2$	< 3	Absence
B	Caja	$2,5 \times 10^3$	< 3	Absence

Source: Authors

Coliforms at 35 °C were not detected by the most probable number method in all samples analyzed. Normative Instruction No. 01 of January 7, 2000, only establishes limits of 1/g of thermotolerant coliforms, while Normative Instruction No. 60 of December 23, 2019 sets limits for *Escherichia coli*. Considering the results found in this study, all samples presented good microbiological quality, since the low concentration of total coliforms indicates that good manufacturing practices (GMPs) were fulfilled, as well as good quality raw materials were used. The same behavior that occurred for coliforms can be extrapolated to the *Salmonella spp.* analysis, since all samples were negative in 25 g of the product, is classified as suitable for consumption according to Normative Instruction 01/2000 and in 60/2019 standards.

Table 2 highlights the results of the analysis of mesophilic and psychrotrophic aerobic microorganisms from fruit pulp samples. It was observed that there was a development of mesophilic aerobic microorganisms, on the other hand, there was no growth of psychrotrophic aerobic microorganisms in any of the samples. Although



current legislation does not set values for mesophilic and psychrotrophic microorganisms for fruit pulps, the counting of these microorganisms are important indicators of food quality (Silva et al., 2010).

The total mesophilic counts found in this study are not considered high from the microbiological point of view, considering that the limit for batch acceptance for most foods is three samples (c) containing between  $1.0 \times 10^5$  (m) to  $1.0 \times 10^6$  (M) (Brasil, 2019).

**Table 2.** Counting of mesophiles and psychrotrophic in fruit pulps commercialized in Salinas, Minas Gerais, Brazil.

Brands	Pulps	Mesophilic (CFU/g)	Psychrotrophic (CFU/g)
A	Passion fruit	$2,5 \times 10^3$	$< 1,0$
A	Guava	$1,8 \times 10^2$	$< 1,0$
A	Caja	$1,6 \times 10^2$	$< 1,0$
B	Passion fruit	$2,5 \times 10^3$	$< 1,0$
B	Guava	$2,3 \times 10^2$	$< 1,0$
B	Caja	$9,6 \times 10^2$	$< 1,0$

Source: Authors

Thus, all samples could be considered as of good microbiological quality, since the presence of high counts comes from poor hygienic and sanitary conditions in the handling and/or use of contaminated raw material. It is noteworthy that most pathogenic microorganisms found in food are mesophilic and therefore the importance of keeping their count low (Silva et al., 2010).

According to these results, it can also be inferred that pulps kept at temperatures in the range of 30 to 35 °C suffer deterioration by mesophilic aerobic microorganisms. On the other hand, pulps stored at freezing temperature are less subject to bacterial multiplication since freezing is an efficient method of conservation and used for many years (Chaves & Zaritzky, 2018). In addition, freezing is not



indicated for samples that will be evaluated for their psychrotrophic microbiota, since this unitary operation can cause injury or death of several of these microorganisms. As the samples evaluated were previously frozen, it is possible that part of the psychrotrophic microbiota was lost in this process, which is positive from the point of view of microbiological conservation (Silva et al., 2010).

### 3.2 Physicochemical parameters

Table 3 presents the results of Total Soluble Solids (TSS), pH, Titratable Total Acidity (TTA) and the relationship between TSS/TTA. It can be verified that in regard of pH, most of the pulps analyzed meet the Identity and Quality Standards (IQS), except for the pH of the guava pulp of brand B that exceeded the maximum value of 4.2, differing statistically from the other pulps analyzed. Sousa et al. (2020) evaluating the physicochemical characteristics of fruit pulps commercialized in Santarém (Pará, Brazil) observed that the pH parameter was in accordance with the legislation for the acerola (*Malpighia emarginata*), cupuaçu (*Theobroma grandiflorum*) and passion fruit (*Passiflora edulis*) flavors. According to Lira Júnior et al. (2005), the pH is established as an attribute of product quality by legislation because it favors pulp conservation, avoiding the growth of microorganisms.

In relation to the SST, the passion fruit pulps of both brands A and B and the guava pulp of brand B did not meet the minimum value established, in which the minimum is 11 °Brix for passion fruit and 7 °Brix for guava. Santos et al. (2016) also found soluble solids values lower than the minimum established by the current legislation (8.20 and 6.50 °Brix) in the passion fruit pulps evaluated. Several factors, such as climate, rainfall during cultivation and addition of water during the





manufacturing process, may have an effect on the soluble solids content in the pulps, which would justify the lack of uniformity between the values presented for the different brands (Freire et al., 2009). The low soluble solids content can also be caused by inadequate processing and low quality of the raw material (BRASIL, 2000).

**Table 3.** Results of Total Soluble Solids (TSS), pH; Total Titratable Acidity (TTA) and the relation between TSS and TTA (TSS/TTA) of fruit pulps marketed in Salinas, Minas Gerais, Brazil.

Brand	Pulp	TSS (°Brix)	pH	TTA (% de ácido cítrico)
A	Passion fruit	7,5 ± 0,00 <sup>a</sup>	3,09 ± 0,006 <sup>a</sup>	2,43 ± 0,057 <sup>a</sup>
A	Guava	12,06 ± 0,451 <sup>b</sup>	3,93 ± 0,006 <sup>a</sup>	0,59 ± 0,001 <sup>b</sup>
A	Caja	10,26 ± 0,153 <sup>b</sup>	3,19 ± 0,006 <sup>a</sup>	0,077 ± 0,00 <sup>b</sup>
B	Passion fruit	9,6 ± 0,265 <sup>a</sup>	3,37 ± 0,006 <sup>a</sup>	1,88 ± 0,118 <sup>c</sup>
B	Guava	5,63 ± 0,208 <sup>a</sup>	4,32 ± 0,00 <sup>b</sup>	0,051 ± 0,011 <sup>b</sup>
B	Caja	9,06 ± 0,058 <sup>a</sup>	3,13 ± 0,015 <sup>a</sup>	0,076 ± 0,004 <sup>b</sup>

<sup>(1)</sup> Means ± standard deviation (n=6), followed by the same letter in the session column of the samples, do not differ from each other by the Tukey test (p<0.05).

The content of organic acids in fruits varies according to the species and its content decreases with ripening in most tropical fruits due to their use in the Krebs cycle or their transformation into sugars during the respiratory process (Chitarra & Chitarra, 2005). In regard of Total Titratable Acidity, Brazilian legislation determines minimum values of 2.5, 0.4 and 0.9 for passion fruit, guava and caja pulps, respectively. Only guava pulps were in accordance with the established.



#### 4. CONCLUSION

The results of the microbiological analyses were in accordance with the recommendations of Brazilian legislation. Non-conformities for TSS were verified in passion fruit pulps of brands A and B, pH in guava pulp of brand B and, for the TTA parameter, in passion fruit and caja pulps of both evaluated brands.

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