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Physical-chemical and microbiological profile of frozen fruit pulps commercialized in Limoeiro do Norte (Ce)

Perfil físico-químico e microbiológico de polpas de frutas congelada comercializadas em Limoeiro do Norte (Ce)

Perfil físico-químico y microbiológico de pulpas de frutas congeladas comercializadas en Limoeiro do Norte (Ce)

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Abstract

According to legislation for identity and quality, fruit pulp is the unfermented, non-concentrated product, the consumption of polished fruits, by an appropriate technological process with a minimum content in suspension. Objective to evaluate the quality of frozen

pineapple pulps sold in Limoeiro do Norte - Ce. To do so, Physico-chemical and microbiological analyzes were carried out on four commercial pineapple flavor brands named (A, B, C and D). The characteristics analyzed were color, pH, soluble solids, titratable acidity, total sugars and vitamin C. The microbiological tests consisted of determining the probable number of molds and yeasts, total and thermotolerant coliforms and Salmonella. The data were subjected to analysis of variance and media compared by the Tukey test at 5% significance level. The results obtained indicate that the physicochemical point of view of the values found for pH, SS, acidity and sugars is the total of all analyzed brands, which are evaluated in accordance with the values recommended by the legislation, except in relation to the vitamin C content, as the values found for brand B and C are above the established limits. In the evaluation of the microbiological profile, all samples are applied within the microbiological standards RDC No. 12, of January 2, 2001, for values of coliforms and salmonella, demonstrating that they are using consumption statistics only regarding mold and yeast counts, while only the D brand was found to be within the defined standards.

Keywords: Fruit; Quality; Frozen food; Pineapple.

Resumo

Segundo a legislação para identidade e qualidade, polpa de fruta é o produto não fermentado, não concentrado, obtido de fruta polposa, por processo tecnológico adequado, com teor mínimo em sólidos em suspensão. Objetivou-se avaliar a qualidade de polpas de abacaxi congeladas comercializadas em Limoeiro do Norte - Ce. Foram realizadas análise físico-químicas e microbiológicas em quatro marcas comercias denominadas (A, B, C e D) do sabor abacaxi. As análises realizadas foram cor, pH, sólidos totais, sólidos solúveis, acidez total expressa em ácido cítrico, açúcares totais e vitamina C. As microbiológicas consistiram em determinar o número provável de bolores e leveduras, coliformes totais e termotolerantes e Salmonella. Os dados foram submetidos a análise de variância e médias comparadas pelo teste de Tukey ao nível de 5% de significância. Os resultados obtidos indicaram que do ponto de vista físico-químico os valores encontrados para pH, SS, acidez e açúcares totais de todas as marcas analisadas apresentaram-se em conformidade com os valores determinados pela legislação, porém em relação ao teor de vitamina C, os valores encontrados para a marca B e C estão acima do estabelecido. Na avaliação do perfil microbiológico, todas as amostras encontraram-se dentro dos padrões microbiológicos RDC n° 12, de 02 de janeiro de 2001 para os valores de coliformes e Salmonella, demonstrando estarem adequadas para o consumo,

porém para quanto as contagens de bolores e leveduras, somente a marca D mostrou estar dentro dos padrões estabelecidos.

Palavras-chave: Fruta; Qualidade; Alimento congelado; Abacaxi.

Resumen

De acuerdo con la legislación de identidad y calidad, la pulpa de fruta es el producto no fermentado, no concentrado obtenido a partir de fruta despulpada, mediante un proceso tecnológico adecuado, con un contenido mínimo de sólidos en suspensión. El objetivo fue evaluar la calidad de las pulpas de piña congeladas vendidas en Limoeiro do Norte - Ce. Se realizaron análisis físico-químicos y microbiológicos de cuatro marcas comerciales denominadas (A, B, C y D) del aroma de piña. Los análisis realizados fueron color, pH, sólidos totales, sólidos solubles, acidez total expresada en ácido cítrico, azúcares totales y vitamina C. Las pruebas microbiológicas consistieron en determinar el número probable de mohos y levaduras, coliformes totales y termotolerantes y Salmonella. Los datos fueron sometidos a análisis de varianza y comparación de medias mediante la prueba de Tukey al nivel de significancia del 5%. Los resultados obtenidos indicaron que, desde el punto de vista fisicoquímico, los valores encontrados para pH, SS, acidez y azúcares totales de todas las marcas analizadas estaban de acuerdo con los valores determinados por la legislación, pero en relación al contenido de vitamina C, los valores encontrados para la marca B y C están por encima del valor establecido. En la evaluación del perfil microbiológico, todas las muestras estuvieron dentro de los estándares microbiológicos RDC No. 12, del 2 de enero de 2001 para los valores de coliformes y Salmonella, demostrando que son aptas para el consumo, sin embargo por cuánto el moho cuenta. levaduras, solo la marca D se encuentra dentro de los estándares establecidos.

Palabras clave: Fruta; Calidad; Congelados; Piña.

1. Introduction

Due to the great variety of fruits and their very diverse flavors, the sale of fruit pulps has grown significantly in recent years in the Northeast Region of Brazil. Fruits in their natural state are more likely to suffer microbial deterioration, and the speed will depend on the intrinsic and extrinsic factors linked to the food. Most of this microbial charge is present on the external side of the fruit, with its interior being practically sterile, unless a break in the skin occurs (da Rocha et al., 2010).

Pineapple (Ananas comosus (L). Merril.) is one of the main fruits produced and commercialized in tropical regions of the world, with Southeast Asia and Latin America being the main producers. Large quantities of fresh fruit, juices, jams and dehydrated pineapples are exported to North America and Europe. Pineapple and its derivatives are popular and highly consumed because they have a pleasant flavor. They also have a relevant concentration of polyphenols, vitamins and other compounds which play an important role in health (Difonzo et al., 2019). Pineapple is a fleshy fruit, which can present various shapes such as conical, cylindrical and rounded through the coalescence of individual fruits spirally fused into a central axis (Noronha et al., 2016). Its composition is rich in sugar when ripened in the plant, and also rich in minerals and vitamins (A, B1, B2 and C). These compounds contribute to bone formation in adolescents and also help prevent arteriosclerosis, arthritis and throat infections. Each 100g of pineapple pulp contains approximately 50 kilocalories, and 89% water, 0.3% protein, 0.5% lipids, 5.8% glycides, 3.2% cellulose and 0.3 of salts, also having a good amount of potassium, iron, calcium, magnesium, and manganese (Erkel et al., 2015).

Brazil is the world' s largest producer of fresh fruit, but because they are perishable, they deteriorate rapidly and their fresh sale is difficult, especially when they need to be transported over long distances (Honorato et al., 2015). It is also estimated that post-harvest losses vary from 15 to 50%, so the production of frozen fruit pulps has become a favorable way to fully use the fruits, which also avoids problems related to seasonality. The diversity of pulp flavors due to the great variety of fruits produced in the country has been causing its commercialization to grow year after year. This success is due to the ease and simplicity of the production process, as well as the practicality of the preparation that these products offer to the consumer. Another growth factor is its nutritional value and concern that the public has had in consuming healthy foods (Gadelha et al., 2009; Honorato et al., 2015). However, it is of fundamental importance that these products all have the ideal nutritional and sensory characteristics, as well as meet quality and hygiene standards (Santos et al., 2017).

The quality of the pulp concerns both the nutritional properties as well as the physical-chemical and sensory characteristics which need to be close to those of the fresh fruit, so that it meets consumer needs, as well as the current legislation. Thus, factors such as pH, titratable acidity, total sugars, soluble solids, total solids, and vitamin C, must obey the rules which are established for each type of pulp, as well as the microbiological parameters that must be within the standards (Santos et al., 2017).

According to the legislation for identity and quality, fruit pulp is an unfermented, non-concentrated product, obtained from pulped fruit by an appropriate technological process with

a minimum content of suspended solids. All of these factors are important for standardizing the product, and thus to analyze possible changes which may occur during processing and / or storage (Brasil; Sigarini; Pardinho, 2016). Thus, all physical-chemical, sensory and microbiological characteristics of the food must be safeguarded, and cannot be altered by equipment, utensils, containers, packaging or marketing (Honorato et al., 2015).

In view of the above, the present study aimed to physicochemically and microbiologically characterize the frozen pineapple pulp, marketed in the city of Limoeiro do Norte (CE), and to analyz and verify if they are within the established standards for the identity and quality of the product.

2. Materials and Methods

This study is a quantitative, experimental research, performed through data collection by sampling, thus yielding precise and safe numerical results (Pereira et al., 2018).

To carry out this study, different commercial brands of frozen pineapple pulps were purchased from various establishments located in the city of Limoeiro do Norte - Ce. Samples were acquired in triplicates of three lots each, from July to September 2019.

The samples were transported in their original packaging to the laboratory of the Master's degree in food technology at the Federal Institute of Education, Science and Technology of Ceará campus Limoeiro do Norte and were kept frozen in a freezer for later analysis.

Each sample was identified as to origin, batch number, manufacture date and expiration date. The experimental portion consisted of 1 package of each lot and brand. In total 36 experimental samples were analyzed in the laboratory. Prior to the analyzes, the samples were thawed in their original packaging under refrigeration for a period of 10 hours, after which they were put to establish room temperature and subsequently homogenized.

The coloring was determined by a digital colorimeter with L*, a* and b* readings expressed in brightness values; a* reflects red (+) or green (-), and b* yellow (+) or blue (-).

The pH, total solids (TS) and soluble solids (SS) analyzes were performed on the pulp without dilution. The acidity (citric acid%) was determined by titration with a standardized solution of 0.1 N NaOH using the phenolphthalein indicator. Soluble solids (SS) were measured by refractometry and expressed in (ºBRIX) for TS analysis and other parameters following the methodology described by Aoac (2005).

Total sugars were quantified by the Antrona method, which consists of carbohydrates

present in the sample being hydrolyzed by treatment with an acid, thereby forming furfural and hydroxymethylfurfural (HMF). These furfurals are then condensed by antrona, forming a blue-green complex. Vitamin C was determined by the method proposed by Strohecker and Henning. To do so, 5 g of the sample was weighed and diluted to 100 ml with oxalic acid. Next, 5 ml of the extract was removed, 50 ml of distilled water was added and titration with Tillman's solution was performed.

Microbiological analyzes were determined according to standards established by Resolution RDC 12, of January 2, 2001 to measure total and thermotolerant coliforms, mold and yeast count and Salmonella.

The coliform quantification was by the most likely number method (NMP.g-1). In the presumptive test, 1 ml aliquots of each sample dilution were aseptically inoculated in series of three tubes containing 9 ml of lactated broth and an inverted Duhran tube. Next, the tubes were incubated in an oven at 35 °C for 48 hours. Confirmatory tests were subsequently carried out from the tubes with a positive reading for the presence of turbidity and gas formation to determine total coliforms in Bile Verde Brilhante (VB) broth at 35 °C for 48 hours, and the thermotolerant coliforms in Escherichia Coli (EC) broth.

The mold and yeast counts was performed using the plating method in three dilutions of 10-1, 10-2 and 10-3 with series of two plates per sample dilution. The culture medium used was Acid Batata Dextrose. Aliquots of 0.1 mL were sown on the surface of the plate using the drigalski loop to spread evenly, and the plates were incubated in the greenhouse for 3 to 5 days at 28 °C. The results were expressed in number of Colony Forming Units per gram of the sample (CFU g-1).

The Salmonella determination consisted of three steps. The first stage was the pre-enrichment involving transferring 25 g of the sample to a flask containing lactated broth and incubated at 35º C for 24h. The second stage was selective enrichment by transferring a 0.1 mL aliquot from the previous stage to Broth Rappa-Vassiliadis (RV) and incubated at 35 ºC for 24h. The third step was the differential plating, from which the tubes that formed gas were transferred to plates with Brilliant Green Agar (BG) and Enteric Hektoen Agar (EH) incubated in an oven at 35 ºC for 24 hours.

The results were analyzed using analysis of variance (ANOVA). The means were compared by the Tukey test with a 5% significance level ($p \leq 0.05$) using the STATISTICA software version 7.0 and also compared to the standards established by the Brazilian norms for fruit pulps.

3. Results and Discussion

In order to maintain the quality of the commercialized fruit pulps, it is essential to ensure the preservation of nutrients, and their microbiological, physical-chemical and sensory characteristics as close as possible to fresh fruit in order to meet consumer requirements and current legislation (Dantas et al., 2012).

Identity and quality standards for pineapple pulp according to M.A.P.A. (Ministry of Agriculture, Livestock and Supply) Brasil (2018), are shown in Table 1.

The results obtained for the physical-chemical analysis of frozen pineapple pulps are shown in Table 1, in which they were compared to the legislation in force Brasil (2018), with the values for pH, acidity, soluble solids, total sugars and vitamin C shown.

Table 1. Mean ± physical-chemical parameters of frozen fruit pulps.

Parameters	Brands				Legislation
	A	B	C	D	
pH	4.20a ± 0.12	4.21a ± 0.02	3.89b ± 0.08	4.03ab ± 0.06	-
Titratable Acidity (g/100g)	0.65a ± 0.14	0.67a ± 0.03	0.82a ± 0.06	0.72a ± 0.16	0.3 min
Soluble Solids (ºBrix a 20º C)	13.18a ± 1.00	11.63a ± 0.23	12.47a ± 1.19	11.49a ± 1.40	11 min
Total sugars (g/100g)	8.38a ± 1.44	7.37a ± 0.53	8.47a ± 1.11	7.17a ± 0.47	15 max
Ascorbic acid (mg/100g)	10.24b ± 5.60	27.61a ± 0.01	24.41a ± 4.20	12.52b ± 5.24	21.5 max.

Same lowercase letters on the same line indicate that there was no significant difference by the Tukey test ($p \geq 0.05$). Source: Authors (2019).

According to the physical-chemical analysis found in Table 1, it can be seen that there was no significant difference between the brands A, B and D in the pH parameter with average values of 3.89 to 4.21. The hydrogen potential (pH) is a quality attribute in frozen fruit pulps as it allows the maintenance of its conservation (do Nascimento et al., 2018). According to Santos (2017), most fruits and vegetables are within the group of acidic foods (pH 4.0 - 4.5) or very acidic foods (pH <4.0), thus enabling inhibition of the growth of pathogenic microorganisms.

Regarding the titratable acidity expressed in (citric acid%) they are between 0.65 and 0.82 (g/100g) for the studied samples and there was no significant difference. In addition, all the fruit pulp samples are within the standards of the legislation, which defines minimum values of (0.3 g/100g). According to dos Santos et al. (2019), citric acid values in fruits between 0.08% to 1.95% provide a smooth and pleasant flavor, directly implying good

acceptance by the consumer.

Likewise, the values obtained for soluble solids of 11.49 to 13.18 (ºBrix) in all brands did not show any significant difference and were adequate to the legislation which establishes 11 in ºBrix as a minimum quantity for this parameter and a temperature of 20 ºC. According to Gadelha et al. (2009), the soluble solids content can vary due to the influence of some factors such as the fruit's growing conditions, climatic factors, the amount of rain during the harvest, the variety, the soil, etc.; in addition, it must be considered that some producers add water to aid in the processing, leading to a decrease in the levels of soluble solids in the final product.

The soluble solids content increases in the fruit maturation process because there is a breakdown of compounds and this is considered an important parameter to indicate the fruit maturity stage (Chitarra; Chitarra, 2005). In the study by Morgado et al. (2019), who analyzed the useful life of frozen pulp of araticum in vacuum packed and unpackaged treatments, they observed that the soluble solids content tended to increase during pulp storage.

In the study by Honorato et al. (2015) who evaluated the physical-chemical parameters of fruit pulps in different flavors produced and marketed in the city of Petrolina-PE, they found average values for total solids between 13.34 and 14.65 (g/100g) in pineapple pulp, which is close to that obtained in the present study.

Regarding the values found for total sugars, it can be seen that there was no significant difference between the samples of the 4 brands, also showing that they are within the standard required by law, which establishes (15 g/100g) as the maximum value. These levels are close to those found by Fonseca (2018), who also analyzed pineapple pulps of different brands, and obtained values of 9.85 and 9.67. A study by Gadelha et al. (2009) also found similar results, approximately 6.8181. According to the authors, the importance of determining total sugars is to indicate whether or not sucrose was added to the product. These values can also be affected by the maturity stage the fruits used for manufacturing the pulps were in.

The results found for vitamin C demonstrated that the A and D samples do not differ significantly from each other and are within the standard established by legislation, which is 21.5 mg/100g. The samples of brands B and C also do not present any significant difference and are within the standard established by legislation considering the value of their standard deviation. In the study by Honorato et al. (2015) analyzing two brands of pineapple pulp, they found levels close to those obtained by brands A and D, which found 6.76 mg/100g for one brand and 15.99 mg/100g for the other. However, values much higher than those determined in this study were found by Silva, Oliveira e Jales (2010) in their analysis, about 65.28

mg/100g. According to Brasil, Sigarini e Pardinho (2016), this variation in vitamin C levels is due to storage conditions of both time and temperature, as well as the amount of this vitamin present in the fruit that was processed.

The color evaluation was performed through its coordinates with values of L*, a* and b* for each brand described in Table 2.

Table 2. Average color values (L*, a* and b*) of frozen fruit pulps.

Brand	L*	a*	b*
A	61.73ab ± 1.50	0.54a ± 1.57	26.19a ± 2.42
B	68.91a ± 0.73	-1.06ab ± 0.12	28.07a ± 1.54
C	57.82b ±3.21	-1.47ab ± 0.75	28.51a ± 3.84
D	57.25b ± 7.41	-2.03b ± 0.31	16.86b ± 3.16

Same lowercase letters on the same line indicate that there was no significant difference by the Tukey test ($p \geq 0.05$). Source: Authors (2019).

The results show that samples A and B do not differ significantly in relation to the value of L*, as well as samples A, C and D. Regarding the values of a*, the data showed that samples A, B, C do not show significant difference, as well as B, C and D. The values of b* for the A, B and C samples also do not differ significantly. The legislation does not establish minimum and maximum values for color, so only the visual aspects are considered, which are presented accordingly. Color is an attribute of paramount importance for product quality, being one of the main factors of consumer choice. According to Castro et al. (2015), the color determination by the L*, a* and b* parameters is valid because they can represent the differences between the analyzed foods, stating that this is due to the maturation stage of the raw material; a fact which they observed in their analysis with different brands and flavors of fruit pulps.

In order to determine the quality of a food among the most important parameters, those which define its microbiological characteristics stand out, allowing them to be evaluated in terms of processing conditions, storage, distribution for consumption, useful life and possible risks to the health of the population (Dantas et al., 2012).

Table 3 presents the values found in the microbiological analyzes for the total and thermotolerant coliform counts, molds and yeasts, and Salmonella. RDC No. 12, of January 2, 2001, from the Ministry of Health Brasil (2001), recommends limits for the counting of thermotolerant coliforms at 45 °C of $5x10^2$ NMP/g for fruits and derived products, as well as requiring an absence of Salmonella spp./25g.

Table 3. Microbiological analysis for total and thermotolerant coliforms, molds and yeasts.

Brand	Total coliforms ($MPN.g^{-1}$)	Thermotolerant coliforms ($MPN.g^{-1}$)	Molds and yeasts ($CFU.g^{-1}$)	Salmonella ($g\ 25g^{-1}$)
A1	<3.0	Absent	4.8×10^4	Absent
A2	3.6	Absent	8.0×10^4	Absent
A3	<3.0	Absent	6.75×10^4	Absent
B1	7.4	Present	11.2×10^4	Absent
B2	7.4	Absent	21.4×10^4	Absent
B3	3.0	Absent	17.2×10^4	Absent
C1	43	Absent	14.55×10^4	Absent
C2	3	Absent	14.5×10^3	Absent
C3	9.2	Absent	19.2×10^3	Absent
D1	<3.0	Absent	1.1×10^3	Absent
D2	<3.0	Absent	0.75×10^3	Absent
D3	<3.0	Absent	0.7×10^3	Absent

CFU.g – 1 = Colony forming units per g; MPN.g – 1 = Most probable number per g.
Source: Authors (2019).

The results obtained in coliform counts showed positive values for 7 pulp, which were analyzed comprising different batches of brands A, B, and C. Only brand D did not obtain any positive value for total coliforms among the analyzed batches. On the other hand, a batch in brand B confirmed the presence of a thermotolerant coliform. All analyzed brands showed coliform values below the maximum limit required by law. In the study by Diniz et al. (2017), analyzing the microbiological profile of three pulp brands in the acerola, cajá and guava flavors, the authors observed that the samples did not show total coliform or E. coli growth.

Freezing stands out among the conservation methods used in fruit pulps, which can act by inhibiting the development of microorganisms and enzyme activity, thereby extending the shelf life and microbiological safety of these products (Morgado et al., 2019).

As can be seen, the analysis verified the absence of Salmonella sp in 25g of sample, as established by the current legislation, thus obtaining satisfactory results, which detected absence in 100% of the analyzed samples. The same was observed by Santos et al. (2017), who evaluated the microbiological quality of frozen fruit pulps of different flavors and brands marketed in the municipality of Vitória da Conquista - BA, in which there was no presence of this microorganism. Altered values demonstrate non-compliance with hygiene in any of the production stages.

The results obtained for counting molds and yeasts showed that only the D brand samples are within the values determined by legislation, with 1.1 x 103, 0.75 x 103, 0.7 x 103 in their counts, while other samples are above the maximum established value. A similar answer was found by Santos et al. (2017), in analyzing pineapple pulp, in which they

observed that their counts were outside of what is allowed by the legislation. They also claim that high values in the counts, in addition to interfering with the deteriorating aspect, also result in rejection of the product by the consumer, as it promotes risks to public health due to the possible production of mycotoxins by some species of fungi, which can lead to growth and produce toxins in foods subjected to technological processes, since they have resistance to heat, freezing, some antibiotics and irradiation.

4. Final Considerations

According to the results, the pulps complied with the current legislation regarding physical-chemical parameters. The pulps evaluated are within the microbiological standards established by A RDC No. 12, of January 2, 2001, for the values of coliforms and Salmonella, demonstrating that they are suitable for consumption; however, only the brand D showed to be in compliance with the standards established by the legislation for mold and yeast count, and this result may be due to failures in the processing and hygiene of the fruits used in manufacturing the pulps.

To ensure physicochemical and microbiological quality in compliance with the standards required by legislation is fundamental in the production of pulps. Thus, for further studies, microscopic analysis shall be necessary for an accurate identification of the presence or absence of foreign materials in the food. This shall ensure greater safety in hygienic and sanitary conditions in this product's manufacture.

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