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Physicochemical and microbiological characterization and antioxidant capacity of açaí pulps marketed in the states of Minas Gerais and Pará, Brazil

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ABSTRACT: The frozen açaí (Euterpe oleracea Mart.) pulp market has had acceptable development and presents great market potential due to its high nutritional level and fruit's seasonality. However, due to the lack of standards for all types of fruit, products without uniformity are in the market. This study aimed to evaluate frozen açaí pulp belonging to different trademarks related to chemical, physicochemical, and microbiological characteristics. Regarding the total titratable acidity (TTA) and pH, two trademarks from Pará and two from Minas Gerais were rejected compared with the current legislation. It was observed that K (1,253.6mg 100 g¹), Ca (312mg 100g¹), Mg (178mg 100g¹), and P (145mg 100g¹) were the main minerals reported in the analyzed samples. Fe showed an average content from 9.65mg 100g¹ to 22.66mg 100g¹. As for microbiological characteristics, the counts for E. coli were in compliance with legislation. Counts of mesophilic aerobic, filamentous fungi, and yeasts showed variations in the results. The count of coagulase-positive S. aureus met the standards [10² Colony Forming Units (CFU) g¹]; and presence of Salmonella sp. in a trademark was detected among the six evaluated trademarks. The analyzed pulp of açaí showed total phenolics values ranging from 240.14mg AGE 100g¹ to 372.43mg AGE 100g¹, and anthocyanin total levels ranging from 12.05 to 24.98mg 100g¹. The obtained physicochemical and microbiological results indicated the need for immediate implementation of Good Manufacturing Practices; conversely, the frozen pulp of açaí showed considerable antioxidant potential.

Key words: Euterpe oleracea Mart, frozen açaí pulp, sanitary conditions, antioxidant compound.

Caracterização físico-química, microbiológica e capacidade antioxidante de polpas de açaí comercializadas nos Estados de Minas Gerais e Pará, Brasil

RESUMO: O mercado de polpa de açaí (Euterpe oleracea Mart.) congelada tem tido crescimento aceitável e apresenta grande potencial mercadológico em função da sazonalidade e do elevado nível nutricional do fruto. Porém, devido à inexistência de padrões para todos os tipos de frutas, encontram-se no mercado produtos sem uniformidade. O objetivo do presente estudo foi avaliar polpas de açaí congeladas de diferentes marcas comerciais, quanto às características químicas, fisico-químicas e microbiológicas. Para ATT e pH, duas marcas do Pará e duas de Minas Gerais foram reprovadas, quando comparadas com a legislação vigente. Observou-se que K (1253,6mg 100g¹), Ca (312mg 100g¹), Mg (178mg 100g¹), e P (145mg 100g¹) foram os principais minerais encontrados nas amostras avaliadas. O Fe apresentou conteúdo médio entre 9,65mg 100g¹ e 22,66mg 100g¹. Quanto às características microbiológicas, as contagens para E.coli atenderam à legislação. As contagens de mesófilos aeróbios, fungos filamentosos e leveduras apresentaram variações nos resultados. A contagem de S. aureus coagulase positiva apresentou-se dentro dos padrões (10²UFC g¹) e foi detectada a presença de Salmonella sp. em uma marca, dentre as seis avalidads As polpas de açaí analisadas apresentaram valores de fenólicos totais variando de 240,14mg AGE 100g¹ a 372,43mg AGE 100g¹ e os níveis de antocianinas totais variando de 12,05 a 24,98mg 100g¹. Os resultados físico-químicos e microbiológicos obtidos indicaram a necessidade de implementação imediata das Boas Práticas de Fabricação; em contrapartida, as polpas congeladas de açaí apresentaram considerável potencial antioxidante.

Palavras-chave: Euterpe oleracea Mart, polpa congelada, qualidade higiênico-sanitária, composto antioxidante.

INTRODUCTION

In addition to its traditional way of consumption, the açaí in recent years is used in very versatile ways, e.g., pasteurized, with guaraná syrup (*Paullinia cupana* Kunth.) in powder, with

condensed milk, and also as jam form, and as liquor form. Development of these products is leading the market - highlighting açaí under frozen pulp form. Many companies in Brazil and worldwide are using açaí included in marketed products, such as pills, juices, energetic drinks, and nutritional supplements,

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especially in the United States of America, Europe, and Japan (HEINRICH et al., 2011). The great importance of this fruit is due to its high nutritional and energetic value, and the presence of phenolic compounds in its composition, particularly anthocyanins, responsible for high antioxidant capacity.

The açaí pulp is extracted from the eatable part of the açaí palm fruit, using water and depulper; it must be preserved with physical processes (pasteurization) and addition of citric acid; the use of chemical preservatives or colorings is prohibited, excepting the colouring obtained from the açaí fruit itself (BRAZIL, 2000). Physicochemical and microbiological characteristics must comply with Instruction No. 1 of January 7, 2000 from the Ministério da Agricultura, Pecuária e Abastecimento (MAPA) (Ministry of Agriculture, Livestock, and Supply), which establishes the identity and quality standards for fruit pulps and the Board Resolution (RDC) No. 12 of January 2, 2001 (BRAZIL, 2001).

These treatments that improve the quality of açaí pulp, mandatory in Brazil, increase the production cost, hindering its application by small producers. Considering the good quality of food, essential to public health, this study aimed to characterize and outline the quality profile by chemical, physicochemical, and microbiological analyzes of six trademarks of frozen açaí pulps traded in the states of Minas Gerais and Pará.

MATERIALS AND METHODS

Six trademarks of açaí pulp were collected during September 2014, of which, three trademarks were traded in Minas Gerais and three trademarks in Pará. Five samples of each trademark were acquired at different commercial establishments, totaling thirty samples; lots and expiration dates were different for samples of the same trademark. The three trademarks of Minas Gerais were named A, B, and C; and the three trademarks of Pará were named D, E, and F. By the label information, only the D trademark was pasteurized; B, C, and D trademarks had added sodium benzoate (INS211) and sodium metabisulfite (INS223) preservatives. Analyses were carried out in triplicate forms.

The physicochemical characteristics related to the total titratable acidity (TTA), pH, and total soluble solids (TSS) were evaluated following analytical procedures proposed by the Instituto Adolfo Lutz (IAL) (Adolfo Lutz Institute) (ALI) (2008). Moisture percentage and crude fiber (CF) analyzes were determined according to Association of

Official Analytical Chemists (AOAC) methodology (2000). Reducing sugar (RS) and non-reducing sugar (NRS) contents were expressed in g 100g⁻¹ of açaí pulp, in accordance to the Somogy-Nelson's methodology (1945). Analysis of mineral was carried out in accordance to DEFELIPO & RIBEIRO (1997); results were expressed in mg 100g-1 of dry basis açaí pulp. Total nitrogen determination (N) for protein calculation (6.25 conversion factor) followed the ALI's methodology (2008). Total phenolics (TF) quantification was performed by spectrophotometric method of Folin-Ciocalteu, described SINGLETON & ROSSI (1965), and the results were expressed in mg of equivalent gallic acid (EGA) g-1). The total anthocyanins (TA) were quantified in accordance with LEES & FRANCIS (1972) and FULEKI & FRANCIS (1968) and expressed in mg of anthocyanins 100mg-1 of açaí pulp. Antioxidant capacity (AC) determination was performed using the assay Trolox equivalent antioxidant capacity (TEAC), in accordance with the RE et al.'s methodology (1999). Results were expressed in µmol L-1 Trolox g-1 per mL of sample.

For microbiological analyzes, fast methods with plates of Petrifilm® AC and EC were used to enumerate, respectively, mesophilic aerobic and *E. coli*, in accordance to WEHR & FRANK (2004). Staph Express method (3M Microbiology, St. Paul, MN, USA) was used for analyzes of coagulase-positive *S. aureus*, in accordance to AOAC (2001). In the research of *Salmonella* sp., the analysis was carried out in accordance to the methodology described by International Standards Organization (ISO 6579). Results were expressed by the number of colony forming units per gram (CFU g⁻¹).

To find the difference among the treatments means, the Tukey test at 5% level of probability was applied, using the Statistical Analysis System (SAS) program, version 9.3, licensed for the Universidade Federal de Viçosa, Viçosa City, Minas Gerais State, Brazil. Pearson's coefficient (r) was used to correlate the variables of total phenolic compounds and total anthocyanins with antioxidant capacity of the trademarks of açaí pulps. Microbiological data were interpreted descriptively.

RESULTS AND DISCUSSION

Among the evaluated trademarks of frozen pulps, it was observed that D and E trademarks showed, respectively, the lowest and highest moisture content (86.14 and 91.48%); however, the A trademark did not differ from C trademark; and B trademark did

not differ from F trademark, statistically (Table 1). The significant moisture fluctuation suggested lack of uniformity in the production process of the marketed açaí pulp. According to CHAVES et al. (2004), products with high moisture are more susceptible to microorganisms attack, so, the use of physical conservation methods such as pasteurization and lowering the pH to near 4.0 value are important for the preservation of açaí pulp. Overall, there was difference in protein concentration among the analyzed açaí pulp trademarks, with mean values between 7.5g 100 g⁻¹ (E trademark) and 10.75g 100g⁻¹ (A trademark), all in compliance with current legislation (BRAZIL, 2000) that establishes a minimum value of 6g 100g-1 dry weight. The RS shows content ranging from 0.17 (C trademark) and 1.09g 100g-1 (D trademark), and NRS in the range of 0.06g 100g-1 (C trademark) to 0.47g 100g⁻¹ (F trademark).

The processed açaí pulp is classified considering the total solids content and the addition or not of water. Under current law, the açaí A (special), B (medium), and C (popular) types should be extracted with the addition of water and filtration, and should present, respectively, total solids contents above 14%; 11 to 14%; and 8 to 11%. Comparing the values found in this study with the Brazilian legislation (BRAZIL, 2000) (Table 2), samples of A, B, C, and E trademarks were classified for total solids as thin or popular açaí, and D and F trademarks, as medium or regular açaí. Ours results of B and E trademarks were in disagreement with the label information because it reported that the açaí pulp was of medium type. The variation among

the pulps of different trademarks for the solids content may have been caused by variation in the amount of water used for extracting the pulp, which, besides promoting the dilution of their constituents, can change the physicochemical composition, counteracting the legislation. The pH values ranged between 3.83 (E trademark) and 4.9 (D trademark). Acidity expressed in citric acid was between 0.17g 100g⁻¹ (A trademark) and 0.49g 100g⁻¹ (E trademark) values. Comparing the TTA and the prevailing values in the legislation, the C trademark (from 0.28 to 0.40g 100g⁻¹) and the E trademark (from 0.45 to 0.56g 100g⁻¹) were not within the proposed parameters.

Regarding the pH, the C and E trademarks showed lower levels than those established by law. One of the acceptable reasons for pulps with higher acidity values was due to the use of preservative, for example, benzoic acid as salt added to the açaí pulp (INS211) remains in balance by hydrolysis and shows the effect of the fruit maturation degree. In the label on the evaluated açaí pulp, there was the specification that the INS211 preservatives - sodium benzoate (bactericide and fungicide) and INS223 - sodium metabisulphite (sterilizing and antioxidant) were added to the B, C, and D trademarks (in disagreement with current Brazilian legislation); the addition of citric acid was not informed.

Evaluating the results for crude fiber, contents showed values ranging from 1.16% (A trademark) to 5.75% (B trademark). FREGONESI et al. (2010) found values of 1.68% to 2.09%, slightly lower than the values reported in this research. According

	Trademarks of Minas Gerais State			Trademarks of Pará State			
	A	В	С	D	Е	F	
M^1	89.95±1.43 ^{ab}	88.93±1.51 ^b	89.64±0.86 ^{ab}	86.14±0.36°	91.48±1.30 ^a	88.76±0.52 ^b	
\mathbf{P}^2	10.75±0.06 ^b	7.75±0.08 ^a	9.31±0.06°	10.38±0.01 ^b	7.5±0.06 ^a	10.50 ± 0.02^{b}	
pН	4.60 ± 0.09^{b}	4.22±0.06°	4.00 ± 0.18^{d}	4.90±0.02 ^a	3.83 ± 0.14^{d}	4.84±0.05 ^a	
TTA^2	0.17 ± 0.01^{d}	0.33 ± 0.01^{b}	0.35 ± 0.03^{b}	0.24±0.01°	0.49 ± 0.03^{a}	0.23±0.01°	
TSS^3	1.94±0.20 ^d	2.78 ± 0.38^{c}	1.29±0.12e	3.74 ± 0.18^{a}	1.87 ± 0.12^{d}	3.20 ± 0.10^{b}	
CF^1	1.16±0.62 ^b	5.75 ± 0.60^{a}	4.95±1.35 ^a	2.09 ± 0.80^{b}	1.64±0.19 ^b	4.72±2.03 ^a	
RS^2	0.47 ± 0.05^{b}	1.02 ± 0.07^{a}	0.17 ± 0.03^{c}	1.09 ± 0.12^{a}	0.49 ± 0.04^{b}	1.05±0.19 ^a	
NRS^2	0.21 ± 0.04^{c}	0.39 ± 0.05^{ab}	0.06 ± 0.01^{d}	0.37 ± 0.01^{ab}	0.31 ± 0.16^{bc}	0.47 ± 0.03^{a}	
TF^4	301.36±44.57 ^{ab}	349.42±32.14 ^a	372.43±16.27 ^a	290.86±15.88 ^{ab}	240.14±77.90 ^b	343.74±43.47 ^a	
TA^5	22.86±2.4a	24.98 ± 2.06^{a}	24.84±4.58 ^a	22.6±1.74 ^a	12.05±9.13 ^b	21.22±2.36 ^a	
AC6	5 00±1 62b	7 26±0 06ab	0.06±9.62ab	11 92±2 26ª	7 02+1 20ab	4.45±1.00b	

Table 1 - Chemical and physicochemical characteristics of frozen açaí pulps from different trademarks.

 $^{^1}$ Moisture and Crude Fiber expressed in %, 2 Proteins, Total titratable acidity, reducing sugar and non-reducing sugar expressed as g 100 g $^{-1}$; 3 Total soluble solids, expressed in 6 Brix; 4 Total phenolics expressed in mg AGE 100 g $^{-1}$; 5 Total anthocyanins, expressed in mg 100 g $^{-1}$; 6 Antioxidant capacity, expressed in μ mol L $^{-1}$ Trolox g $^{-1}$. Data expressed as mean of triplicate \pm standard deviation. Means followed by the same letter in the rows do not differ at 5% probability by Tukey test.

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Table 2 - Minimum and maximum values of pH, titratable acidity (TTA) and total solids from different trademarks of frozen açaí pulp and the current legislation.

Origin	Trademark	pН	TTA1 (% m/m)	Total solids (%)	
	A	4.38 – 4.77	0.15 - 0.19	10.05	
Minas Gerais	В	4.11 - 4.32	0.30 - 0.35	11.07	
	C	3.45 - 4.26	0.28 - 0.40	10.36	
	D	4.84 - 4.94	0.23 - 0.26	13.86	
Pará	Е	3.45 - 3.95	0.45 - 0.56	8.52	
	F	4.76 - 4.93	0.22 - 0.26	11.24	
			Maximum 0.27*	8 – 11*	
Legislation (BRAZIL, 2000)		4.00 - 6.20	Maximum 0.40**	$11 - 14^{**}$	
, ,			Maximum 0.45***	Above 14***	

Special Açaí*; ***Medium Açaí; ****Popular Açaí. Data expressed in minimum and maximum of pH parameters, 1Titratable acidity and total solids compared with current legislation.

to OLIVEIRA et al. (2007), 90% of the fibers are insoluble, and these fibers, in turn, help in preventing intestinal constipation and colorectal cancer, acting primarily in the enteric transit by accelerating the movement of the fecal matter in the intestine.

The lower and upper values reported for total phenolic among the trademarks were 240.14mg EGA 100g⁻¹ (E trademark) and 372.43mg EGA 100g⁻¹ (C trademark). For total anthocyanins, there was no significant difference among the trademarks, except for the E trademark. Average values ranged from 12.05mg 100g⁻¹ (E trademark) to 24.98mg 100 g⁻¹ (B trademark); the reported results for the antioxidant capacity ranged from 4.45 (F trademark) to 11.83 µmol L⁻¹ Trolox g⁻¹ (D trademark) (Table 1). HOGAN et al. (2010) reported total phenolic values of 312±5.6mg AGE 100g⁻¹, similar to those of the present study; Nevertheless, the results for the antioxidant capacity were below those reported by RUFINO et al. (2010) (15.1±4.1µmol L-1 Trolox g-1). Pearson correlation among the variables of total phenolic and anthocyanin compounds with antioxidant capacity of the açaí pulp trademarks was 0.869 and 0.863, respectively. According to KUSKOSKI et al. (2006), the higher the anthocyanin contents the greater the observed antioxidant capacity.

In evaluated açaí pulp, it was reported that minerals present in greater abundance were K (ranging from 797.5 to 1,253.6mg 100g⁻¹), Ca (ranging from 211.5 to 312.5mg 100g⁻¹), P (ranging from 112 to 145mg 100g⁻¹), and Mg (ranging from 90 to 178mg 100g⁻¹). The consumption of this pulp rich on these minerals can help to ensure the growth and the proper functioning of the human body in general. However, the açaí pulp cannot be considered a good source of Mn, Fe, Zn, Cu,

and B, found in lesser amounts in this study. In general, there was difference in mineral content among different evaluated trademarks (Table 3).

In all pulps trademarks of frozen açaí, it was noted a wide variation of presence of the mesophilic bacteria, with values between 6.0x10¹ (trademark A) and 5.8x10⁵CFU g⁻¹ (F trademark), (Table 4). In all trademarks, the presence of *E. coli* was reported with values less than 10²CFU g⁻¹, considered below the upper tolerance limit. For filamentous fungi and yeast, the trademarks collected in Minas Gerais (A, B, and C) ranged from <10² to 4.4x10⁵CFU g⁻¹, and those ones collected in Pará (D, E, and F) ranged from <10² 8.6 to 10⁵CFU g⁻¹. Results for positive-coagulase *S. aureus* ranged from <10 to 3.2 to 10²CFU g⁻¹. *Salmonella* sp. was found on the D trademark from Pará State.

Compared to the current Brazilian legislation (BRAZIL, 2000), D trademark that has undergone heat treatment showed the maximum value of 1.4x10⁴CFU g⁻¹ for fungi and yeasts, indicating that pasteurization and addition of preservatives were inefficient. The A, B, and F trademarks showed maximum results of 4.4x10⁵; 1.4x10⁴, and 8.6x10⁵CFU g⁻¹, respectively, values above the maximum limit for pulp *in natura* (5x10³CFU g⁻¹), according to the legislation. Among the values reported for *E. coli*, all trademarks were below the permitted in the Real Decreto no. 3484/2000 of Spain, which establishes for foods based on raw vegetal, prepared and packaged crude, the count limit below 10²CFU g⁻¹ (ESPANHA, 2000).

Maximum values reported for mesophilic aerobic bacteria classified the A (4.2x10⁵CFU g⁻¹) and F (5.8x10⁵CFU g⁻¹) trademarks as products under unsatisfactory hygienic conditions. On the packaging label, it was registered that B, C, and D trademarks

Table 3 - Average mineral content of the frozen açaí pulps from different trademarks.

M^1	Tra	demarks of Minas Ge	rais	Trademarks of Pará			
M^1	A	В	С	D	Е	F	
K^2	1,253.60±5.46 ^{ab}	1,141.00±9.47 ^{bc}	1,051.50±14.43bc	$1,134.00\pm14.93^{ab}$	797.50±2.50°	1,238.50±1.97 ^a	
Ca ³	261.00±0.79 ^b	242.00 ± 0.20^{bc}	214.00±0.53°	312.50±0.17 ^a	232.00±1.27 ^{bc}	211.50±0.50°	
P^4	136.0 ± 0.30^{ab}	124.5 ± 0.03^{bc}	125.0 ± 0.27^{bc}	145.0±0.33 ^a	112.0±0.73°	133.0 ± 0.60^{ab}	
Mg^5	143.60 ± 0.46^{b}	152.00±0.13 ^b	114.50±0.37°	133.00±0.20 ^{bc}	90.00 ± 0.47^{d}	178.00 ± 0.80^a	
Mn^6	23.62 ± 1.33^{de}	30.86 ± 0.53^d	44.72 ± 1.83^{b}	35.12±0.67°	21.86±1.29e	66.76 ± 1.55^{a}	
Fe ⁷	11.56 ± 0.64^{bc}	9.65 ± 0.07^{bc}	11.40 ± 0.38^{bc}	10.93±1.73°	22.66 ± 1.25^{a}	11.71 ± 0.06^{b}	
Zn^8	4.40 ± 0.47^{a}	2.44 ± 0.01^{bc}	2.39 ± 0.09^{bc}	2.61 ± 0.02^{bc}	2.05±0.11°	2.96 ± 0.09^{b}	
Cu ⁹	1.90 ± 0.07^{a}	1.54 ± 0.04^{bc}	1.65 ± 0.03^{b}	1.55 ± 0.01^{b}	1.34 ± 0.06^{c}	1.64 ± 0.09^{b}	
B^{10}	1.28 ± 0.05^a	1.11 ± 0.05^{abc}	1.22±0.03 ^{abc}	1.28 ± 0.09^{ab}	1.02 ± 0.02^{bc}	1.04 ± 0.03^{c}	

¹Mineral expressed in mg 100g⁻¹, dry basis. ²Potassium; ³Calcium; ⁴Phosphorus; ⁵ Magnesium; ⁶Manganese; ⁷Iron; ⁸Zinc; ⁹Copper; ¹⁰Boron. Data expressed as mean of triplicate ± standard deviation. Means followed by the same letter in the rows do not differ at 5% probability by Tukey test.

had the addition of INS211 and INS223 preservatives; therefore, in violation of current legislation, which prohibits the use of preservatives. Pasteurization associated with other heat treatments is still the most widely used for conservation of fruit pulps. The anthocyanins degradation is the most common effect caused by the use of heat treatments. The açaí pulp of D trademark that underwent heat treatment and had added preservatives, according to the information

label, did not differ in total phenolic, and total anthocyanins contents, when compared to pulps that were not thermally treated, but the treatment was not effective for reduction of microorganisms.

CONCLUSION

The analyzes results of the evaluated pulp trademarks for the physicochemical and

Table 4 - Microbiological counts (CFU g⁻¹) of aerobic mesophilic, *Escherichia coli*, filamentous fungi and yeasts, coagulase-positive *Staphylococcus aureus* and presence/absence of *Salmonella* sp. in frozen açaí pulps.

	Trademarks of Minas Gerais						
Analyzes (CFU g ⁻¹)	AA		B		C		
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	
Aerobic mesophilic	6.0×10^{1}	4.2×10^5	6.2×10^3	2.0×10^4	2.9×10^4	2.0×10^4	
Escherichia coli	<102	<10 ²	<10 ²	<10 ²	<10 ²	<10 ²	
Fungi and yeasts	<10 ²	4.4×10^5	<10 ²	1.4×10^4	<10 ²	1.7×10^3	
S. aureus	5.0×10^{1}	2.0×10^{1}	1.8×10^{2}	3.2×10^2	<10	2.7×10^2	
Salmonella sp. in 25g	Absence	Absence	Absence	Absence	Absence	Absence	
	Trademarks of Pará						
Analyzes (CFU g ⁻¹)	D		E		F		
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	
Aerobic mesophilic	5.5×10^2	3.0×10^3	2.0×10^2	9.0×10^3	6.0×10^{1}	5.8×10^5	
Escherichia coli	<10 ²	<10 ²	<10 ²	<10 ²	<10 ²	<10 ²	
Fungi and yeasts	6.6×10^2	1.4×10^4	3.1×10^2	4.0×10^2	<10 ²	8.6×10^5	
S. aureus	5.0 x 10 ¹	5.4×10^4	4.0×10^{1}	1.1×10^2	<10	5.0×10^{1}	
Salmonella sp. in 25g	Absence	Presence	Absence	Absence	Absence	Absence	

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microbiological characteristics showed compliance with current legislation, suggesting deficiency in monitoring and process standardization, showing that the product is not being produced so as to fulfill the legislation requirements. It is emphasized the importance of continuous improvement in the pulp quality control that involves these aspects, since the product is widely consumed in Brazil and abroad. Regarding the microbiological analyzes, only the C and E trademarks fully met the requirements of Brazilian sanitary legislation for fruit pulp, showing its misuse. Total phenolics compounds and total anthocyanins were also the subject of this study and showed good antioxidant capacity in evaluated pulps.

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