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Thermal and non-thermal processing effect on açai juice composition

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ABSTRACT

This study evaluated the effects of High-Temperature Short Time (HTST), Ultra High Temperature (UHT), and the non-thermal processes High Power Ultrasound (US), UV-pulsed-light and Low Pressure Plasma (LPP) on the composition, stability, and bioactive compounds bioaccessibility of açai juice. 1H NMR based approach, coupled to chemometrics, was applied to evaluate the changes in the juice composition. All the non-thermal processes increased the sugars content (glucose and fructose), and the amino acid betaine, except the combined processing of ultrasound followed by low-pressure plasma (US.LPP). HTST and UHT increased the fatty acids and phenolic compounds content in the açai juice. The bioaccessibility of phenolic compounds decreased due to the processing. After thermal sterilization (UHT), the anthocyanin bioaccessibility was 2-fold higher. The combined non-thermal treatment reduced the biocompounds bioaccessibility to 40% of the non-processed juice. However, the combined US.LPP improved the bioaccessibility of vitamin C by 8%. UHT increased the anthocyanin's bioaccessibility but sharply decreased vitamin C bioaccessibility. Higher impact of thermal processing on vitamin C, anthocyanins, total phenolics, PPO, POD, DPPH, ABTS, and FRAP was verified after 45 and 60 days of storage compared to the non-thermally processed samples.

1. Introduction

Açai (*Euterpe oleracea*) is a large palm tree found in marshes and upland regions in northern Brazil. The increase of açai consumption is due to numerous health benefits related to its high content of bioactive compounds (polyphenols and anthocyanins), as well as fatty acids and fibers, among others (de Lima Yamaguchi, Pereira, Lamarao, Lima, & da Veiga-Junior, 2015). The food industry is continuously searching for new processing technologies (Aadil et al., 2015; Alves Filho et al., 2018).

Since thermal processing may promote non-desirable changes in the organic composition of foods, innovative non-thermal technologies are under investigation. Among the non-thermal technologies, plasma, ozone, ultrasound, high-pressure processing (HPP) and pulsed light are some of the food treatments with less undesirable influence after processing on the main composition of the food matrix related to sensor, flavor, and nutritional characteristics after processing (Alves Filho et al., 2018). The processing should preserve bioactive compounds' integrity (Barba et al., 2017; Carbonell-Capella, Buniowska, Barba, Esteve, & Frígola, 2014). Recent studies reported a positive impact of non-thermal processing on food quality. Optimized non-thermal protocols enhanced the content of the bioactive compounds in fruit juices (Alves Filho et al., 2018; de Castro et al., 2020; Porto et al., 2020; Paixão, Fonteles, Oliveira, Fernandes, & Rodrigues, 2019; Rodríguez, Gomes, Rodrigues, & Fernandes, 2017). Hurdle technology, combining more than one non-thermal processing or thermal and non-thermal processing, is regarded as the future of non-thermal technology application in the food industry (Zhang, Wang, Zeng, Han, & Brennan, 2019). The use of combined non-thermal processing might offer advantages, such as higher enzyme and microorganism inactivation, and the reduction of the harmful effects on the food matrix (Oliveira et al., 2018).

Although some studies reported the thermal, non-thermal or combined processes applied to açai pulp (de Jesus, Cristianini, dos Santos, & Maróstica Júnior, 2020; de Jesus, Leite, & Cristianini, 2018) they lack information about on the bioaccessibility of açai bioactive compounds. In this regard, it is paramount to evaluate the impact of processing conditions on the release, transformation, and absorption of bioactive

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compounds. The concentration of bioactive compounds that are released from the food matrix into the gastrointestinal tract and becomes available for absorption is much more important than that in the corresponding beverage. Processing and storage conditions play an essential role in the release, transformation, and absorption of health-related compounds during gastrointestinal digestion. Furthermore, food processing can increase the concentrations of bioactive compounds, improving their bioaccessibility. The present study evaluated the effect of low-pressure plasma (LPP), UV-pulsed light and ultrasound processing and, their combination on açai juice, contributing to understanding the effect of such technologies on açai juice quality and composition. High-temperature short time (HTST) pasteurization and ultra-high temperature (UHT) sterilization were the thermal treatment reference. The objective was to compare the effect of the thermal and non-thermal treatments on açai quality. Nuclear Magnetic Resonance (NMR) was used as a non-target analysis to evaluate the changes in product composition. NMR is a non-destructive spectroscopy technology that offers detailed results of many compounds simultaneously without previous separation or purification, being appropriate to evaluate food composition (Alves Filho et al., 2018; Spraul et al., 2009). Chemometrics tools evaluated the changes in the açai juice (organic composition and bioaccessibility of bioactive compounds).

2. Materials and methods

2.1. Açai juice

The raw material was non-pasteurized frozen pulps (type A with 14% of solids) free of preservatives, which were purchased from a local producer (Petrus Fruit, Castanhal, Pará-Brazil) and kept frozen (−18 °C) until processing. For processing, the juices were produced by dilution in potable water (1:2) and then submitted to different thermal and non-thermal processes, as described in Sections 2.2 and 2.3, respectively. After the pulp dilution, the açai juice presented 4.7% of suspended solids.

2.2. Thermal processing

High-Temperature Short Time (HTST) and Ultra High Temperature (UHT) were the thermal processes evaluated in the present study (Sucupira et al., 2017). Initially, the açai juice was submitted to UHT processing at 138 °C ± 0.3 °C for 6 s and cooled to 25 °C at a flow rate of 210 mL min^{-1}, using an Armfield fully automated tubular heat exchanger system (model FT74) has an Armfield FT63 chiller and an aseptic package unit. The juice was aseptically packaged in transparent 210 mL polyethylene terephthalate (PET) bottles closed with polypropylene screw caps. The same equipment was used for HTST processing at 90 °C ± 1.2 with a retention time of 6 s. After the HTST processing, the juice was bottled by hot-fill processing using transparent 210 mL bottles. All bottles were previously sterilized with a peracetic acid solution (0.5 v/v %) and rinsed with sterile water before filling. The samples were kept frozen at −80 °C until the analyses. For each thermal processing, 5 L of açai juice was treated in replicate.

2.3. Non-thermal processing

Açai juice was subjected to five different non-thermal processes such as low-pressure plasma (LPP), pulsed light (PL), ultrasound (US), and two combinations of LPP followed by US, and US followed by LPP, as shown in Table 1. The PL equipment has only one independent variable (the light intensity), and thus only the UV energy was changed. For the combined non-thermal processing, the most efficient LPP and US processing, regarding the product quality (bioactive compounds content and activity POD and PPO enzyme activity levels). As non-thermal processing operates without external heat, all thermal processing was carried out at room temperature (25 °C ± 2). Replicates were done for all non-thermal processes.

Table 1
Experimental parameters applied to each non-thermal processing.

Exp.	*Non-thermal*	*Parameters*
1	LPP	5 min; 10 mL min^{-1}
2		15 min; 10 mL min^{-1}
3		5 min; 30 mL min^{-1}
4		15 min; 30 mL min^{-1}
5		15 min; 20 mL min^{-1}
6		10 min; 30 mL min^{-1}
7	PL	2 V (0.0857 J/cm^2)
8		6 V (0.6000 J/cm^2)
9		10 V (0.9473 J/cm^2)
10	US	5 min; 75.34 W cm^{-2}
11		5 min; 226.02 W cm^{-2}
12		5 min; 372.93 W cm^{-2}
13		2 min; 372.93 W cm^{-2}
14		10 min; 372.93 W cm^{-2}

2.3.1. Low-pressure plasma processing

Açai juice was processed in 50 mL Falcon tubes containing 45 mL of juice each. The tubes were vacuum-sealed in a nylon-polyethylene bag and processed in a glow discharge plasma generator (model Venus PE-50, PlasmaEtch, USA), composed by one horizontal electrode (4.5″W × 6″D + 2.5″ Clearance); 80 W and 50 kHz power supply (continuously variable with Automatic Matching Network); a 5CFM-2-Stage Direct Drive Oil Pump (Oxygen Service – Krytox Charged); and an aluminum chamber (5.5″W × 7″D × 3.5″H). The pressure reached within the chamber remained was lower than 300 mbar (Rodríguez et al., 2017). The juice was processed by plasma irradiation using synthetic air (grade FID 4.5, purity 99.95%, White Martins, Brazil). The airflow rate and processing time were changed from 5 min to 30 mL.min^{-1}, as shown in Table 1.

2.3.2. UV-Pulsed light processing

The UV-pulsed light was carried out in an Intense Pulsed Light System XeMatic (SteriBeam Systems GmbH) composed by two xenon filled flash lamps (19 cm; inner diameter 7 mm and external diameter is 9 mm), Xe pressure: 700 Torr. An aliquot of 15 mL of the açai juice was inserted in polyethylene open Petri Dish (90 × 15 cm; liquid film maximum 0.5 cm) and submitted to different levels of UV-pulsed light, as follow: 2 V (0.0857 J.cm^{-2}); 6 V (0.6000 J.cm^{-2}); 10 V (0.9473 J.cm^{-2}).

2.3.3. Ultrasound processing

The ultrasound processing was carried out using a 19 kHz probe ultrasound (Unique model USD500). A 13 mm titanium probe was immersed 15 mm below the liquid surface. For each experiment, 150 mL of açai juice was placed in a glass jacketed beaker (250 mL). The juice was subjected to ultrasonic irradiation under different time and potency, as shown in Table 1. US processing generates internal heat. Depending on the food matrix and processing conditions, temperature gradients up to 40 °C are reached (Fonteles et al., 2012). Thus, to keep the room temperature, the processing was thermostated at 25 °C.

2.3.4. Combined non-thermal processing

The effect of two different combined processing was evaluated on açai juice. Firstly, a volume of 150 mL was placed in a glass jacketed beaker (250 mL) and processed by US for 10 min at a potency density of 372.93 W cm^{-2}. Then, the resultant volume was transferred for LPP and was processed at 30 L min^{-1} for 10 min. The inverse combined processing with plasma exposure before the ultrasound processing was also carried out. The UV-pulsed-light equipment processing capacity

did not allow combined processing using this technology. The combined non-thermal treatment was carried out at the best conditions regarding the product quality of each non-thermal treatment.

2.4. Sample preparation and NMR spectroscopy

Before the ^{1}H NMR experiments, all açai juice samples were centrifuged (3 min at 6000*g*). An aliquot of 400 μL of D_2O (99.9%) and 35 μL of a solution containing 14 mM of EDTA and 1% of sodium-3-trimethylsilyl propionate (TMSP-d4) were added to 165 μL of the centrifuged açai juice, reaching 600 μL of the final solution. The EDTA was added to the sample to minimize the ionic strength effect on frequency shifts in the NMR spectra. Then, the final solution was inserted in a 5 mm NMR tube.

The NMR experiments were performed on an Agilent 600-MHz spectrometer equipped with a 5 mm (H-F/^{15}N-^{31}P) inverse detection One Probe™ with actively shielded Z-gradient. The ^{1}H NMR analysis was carried out in quantitative mode: 5 min of waiting before to start the acquisition in order to stabilize the sample temperature with the probe fixed to 298 K; calibrated hard pulse to 90° (8.03 μs); acquisition time of 5.0 s and relaxation delay of 20.0 s achieved by the inversion-recovery pulse sequence considering seven times the longest T_1 of the observed protons (99.9% of complete spins relaxation accuracy) (Malz & Jancke, 2006; Pauli, Jaki, & Lankin, 2005); and fixed receiver gain value to 40 for all acquisitions in order to receive the signals at the same amplitude (Alves Filho et al., 2018). The ^{1}H NMR spectra were acquired with 48 scans using the PRESAT pulse sequence for non-deuterated water suppression at 4.76 ppm, 32 k of time-domain points in a spectral window of 16.0 ppm. The TMSP-d_4 was used as an internal standard (simplet at 0.0 ppm). The spectra were processed by applying exponential multiplication of the FID by a factor of 0.3 Hz, and Fourier transformation of 16 k points. Phase correction was manually performed, and the baseline correction was applied over the entire spectral range.

The identification of the constituents on açai juices was performed through 2D NMR experiment as ^{1}H–^{1}H *g*COSY, ^{1}H–^{13}C *g*HSQC, and ^{1}H–^{13}C *g*HMBC. The results were compared to the existing data in open-access databases (www.hmdb.ca) and literature reports (Schauss et al., 2006; Sterling, Crouch, Russell, & Calderón, 2013). The molecular structures, ^{1}H and ^{13}C chemical shifts, multiplicity, constant coupling, 2D NMR data acquisition, and processing are available in Table 2.

2.4.1. Chemometric analysis of the ^{1}H NMR dataset

The unsupervised multivariate analysis by Principal Component Analysis (PCA) was developed to investigate the influence of thermal and non-thermal processing on açai juice composition. For numerical matrix construction, each ^{1}H NMR spectrum was converted to American Standard Code for Information Interchange (ASCII) and imported by Origin™ (version 9.4, 2016). The resultant numerical matrix presented dimensionality of 435,780 data points from 54 samples acquired in triplicate ×8070 variables into each spectrum between δ 0.7 and 9.0. The matrix was further exported to Excel™ software (Microsoft Office, 2010) and then imported by PLS Toolbox™ program (version 8.6.2, Eigenvector Research Incorporated, Manson, WA, USA, 2018) to develop the chemometric analysis. Previously, the area affected by the non-deuterated water suppressing according to the saturation profiling (between δ 4.65 and 5.05) was excluded.

For unsupervised chemometric analysis by PCA, algorithms for baseline correction (automatic weighted least squared, order 2), normalization to the area, and variables alignment using COW (Correlation Optimized Warping) with segment of 50 data points and a slack of 5 data points were applied prior the matrix decomposition (Alves Filho et al., 2018; Soares et al., 2017). The mean-centered pretreatment over the samples provided better differences among the açai juices, not allowing that noises and unidentified small signals negatively affect the samples distribution. For original matrix decomposition to scores, loadings, error (Q residuals), and influence (Hotelling's T^2) matrices, the Singular Value Decomposition (SVD) algorithm was applied. These data treatments enhanced the differences among the juices, and relevant information was obtained at the first two Principal Components (PC) under a confidence level of 95%.

2.5. Stability analysis of the açai juice

After processing, samples were stored at 4 °C for up to 60 days. During this period, analysis of the amounts of antioxidant compounds (total phenolics, anthocyanins, and vitamin C), antioxidant activity by DPPH, ABTS, and FRAP, and enzymatic activity by peroxidase (POD) and polyphenol oxidase (PPO) were performed after 0, 30, 45, and 60 days. Stability along the cold storage was performed for the thermally processed and the combined non-thermal processing samples. The combined non-thermal treatment was carried out at the best conditions regarding the product quality of each non-thermal treatment.

The concentration of the total phenolic compounds was determined according to the Folin-Ciocalteu method (Folin & Ciocalteu, 1927; Osae et al., 2019) with adaptations. In a 96-well microtiter plate, 10 μL of the sample (water as used as blank) was mixed with 200 μL of the Folin–Ciocalteu reagent, previously diluted in water (1:10). After 3 min of reaction and the development of the blue color, 10 μL of 20% (w/v) sodium carbonate solution was added to stop the reaction. The reading was taken at 765 nm using an Elisa reader spectrophotometer (Biotek Epoch, Winooski, VT, USA) using the software Gen 5 1.10 to handle the data (Almeida, 2015). The anthocyanin quantification was performed according to the pH differential method (Lee, Durst, & Wrolstad, 2005). The concentration of ascorbic acid (vitamin C) was spectrophotometrically determined (Selimović, Salkić, & Selimović, 2011).

The antioxidant activity of the açai juices by the methods ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid), DPPH (2,2'-diphenyl-1-picrylhydrazyl), and FRAP (ferric reducing antioxidant power) was spectrophotometrically measured (Osae, Zhou, Alolga, et al., 2019). The PPO and POD enzyme activity was also spectrophotometrically determined (Fonteles et al., 2012; Osae, Zhou, Tchabo, et al., 2019; Wissemann & Lee, 1980). All analyses were carried out in triplicate.

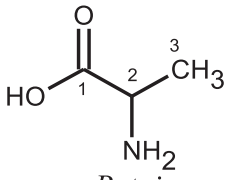
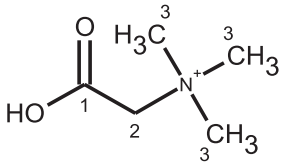
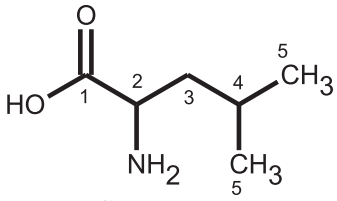
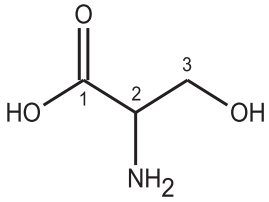
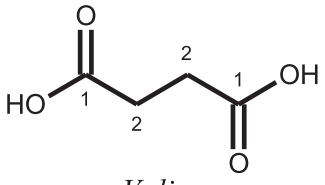
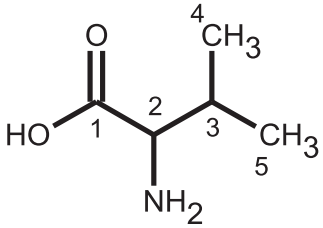
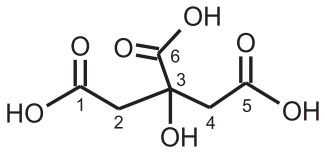
2.6. Bioactive compounds

An evaluation of the bioaccessibility of bioactive compounds in açai juices under simulated gastrointestinal conditions was developed based on a published method (Liserre, Re, & Franco, 2007), which was adapted to the food matrix (Buriti, Castro, & Saad, 2010). A volume of 10 mL of the açai juice (before and after processing) was transferred to three bottles performed in triplicate totaling 15 bottles. The pH was adjusted to 2 with HCl (1 M). Then, pepsin (3 g L^{-1}) and lipase (0.9 mg L^{-1}) enzymes were added, and all bottles were incubated at 37 °C under agitation at 150 rpm during 2 h, representing the gastric phase. After this period, the pH was adjusted to 5 using a buffer (150 mL de NaOH 1 M and 14 g of PO_4H_2Na. 2 H_2O to 1 L of distilled water). Bile (10 g L^{-1}) and pancreatin (1 g L^{-1}) were added, and the samples were incubated again under agitation during 2 h, which represented the enteric phase. At the end of digestion, aliquots were collected in triplicate for the quantification of the bioactive compounds.

2.7. Chemometric analysis of the bioactive compounds, antioxidant and enzymatic activities

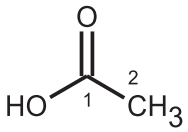
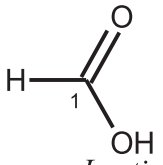
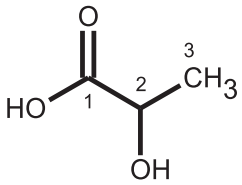
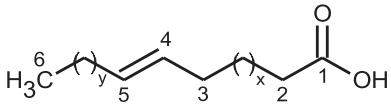
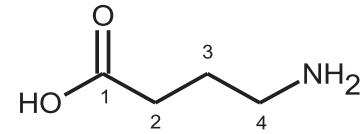
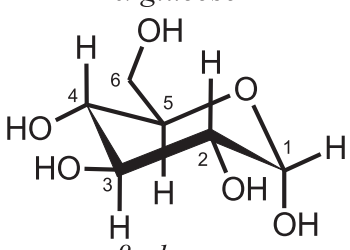
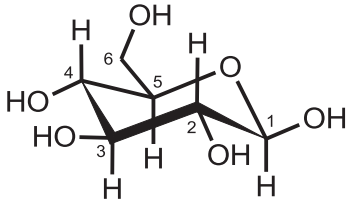
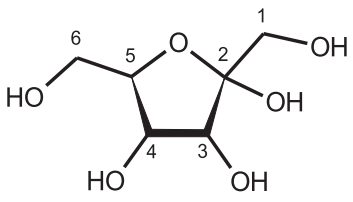
Due to the elevate number of experiments and different experimental responses, an unsupervised chemometric analysis by PCA was developed to investigate the influence of different thermal and non-thermal processes and the refrigerated storage time on açai juice quality. The product quality was evaluated as bioactive compounds, antioxidant, and enzymatic activity of spoiling enzymes (POD and PPO). Each experiment was performed in triplicate, and the data were

Table 2
Organic compounds identified in the açai juices before and after the thermal and non-thermal processing.

Structures	*δ ^{1}H (multip.* J in Hz)*	*δ ^{13}C*	*δ ^{1}H ref.*	*δ ^{13}C ref.*
AMINO ACIDS				
Alanine 	2–3.95 (o) 3–1.46 (*d* 7.2)	56.7 19.2	3.90 (*q* 7.3) 1.52 (*d* 7.3)	53.4 19.1
Betaine 				
Leucine 	2,3,4 - no 5,6–0.97 (*d* 6.0)	no 42.7 23.4 24.7	3.90 (no) 1.73 (*m*) 0.96 (*dt*)	n 42.8 23.9 27.0
Serine 	3–3.80 2–3.83	57.4 63.2	3.83 (*dd* 5.58; 3.80) 3.95 (*m*)	59.2 63.1
Succinic 	2–2.44 (*s*)	36.9	2.39 (*s*)	36.8
Valine 	2–3.62 (o) 3–2.28 (o) 4–0.98 (*d* 7.2) 5–1.05 (*d* 7.2)	o o 19.3 20.8	3.82 (*d* 4.4) 2.33 (*m*) 1.02 (*d* 7.1) 1.06 (*d* 7.1)	n 32.0 19.1 20.9
ORGANIC ACIDS				
Citric 	2–2.63 (*d* 15.0) 4–2.72 (*d* 15.0)	48.8 48.8	2.52 (*d* 15.8) 3.66 (*d* 15.8)	48.6 48.6

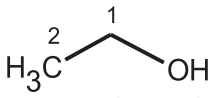
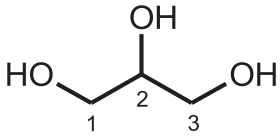
(*continued on next page*)

Table 2 (*continued*)

Structures	*δ ^{1}H (multip.* J in Hz)*	*δ ^{13}C*	*δ ^{1}H ref.*	*δ ^{13}C ref.*
Acetic 	1 – 2–1.96 (*s*)	181.2 25.1	2.08 (*s*)	184.1 26.0
Methanoic 	1–8.48 (*s*)	no	8.39 (*s*)	172.4
Lactic 	3–1.38 (*d* 7.2) 2–4.12 (*d* 7.2)	23.0 71.4	1.37 (*d* 7.2) 4.42 (*q* 7.2)	22.9 71.4
Fatty 	1 - 2–2.26 (*m*) 3–2.04 (*m*) 4,5–5.35 (*m*) 6–0.93 (*m*)	175.8 36.7 30.1 132.5 17.8	- 2.35 (*t* 7.3) 2.02 (*m*) 5.35 (*m*) 0.89 (*t* 6.8)	173.5 34.0 27.1 129.9 14.1
GABA (γ-aminobutyric acid) 	2–2.34 (*m*) 3–2.00 (*m*) 4–2.82 (*m*)	32.8 27.8 36.3	2.28 (*t* 7.60) 1.88 (*qui* 7.60) 2.99 (*t* 7.60)	37.1 26.3 42.2
SUGARS				
α-glucose 	1–5.20 (*d* 4.08) 2–3.47 (*m*) 3–3.77 (*m*) 4–3.56 (*m*) 5–3.72 (*m*) 6–3.85 (*m*)	95.6 72.3 75.6 74.0 63.9 75.5	5.25 (*d* 3.80) 3.89–3.36 (o) n n n n	95.4 72.2 76.0 72.8 64.2 74.5
β-glucose 	1–4.66 (*d* 7.80) 2–3.26 (*m*) 3–3.75 (*m*) 4–3.48 (*m*) 5–3.41 (*m*) 6–3.90 (*m*)	99.3 77.5 63.6 78.8 72.2 63.7	4.66 (*d* 8.10) 3.25 (*t* 8.40) n n n n	99.2 77.6 56.1 79.0 72.8 63.1
Fructose 	1–o 2 – no 3–4.10 (*dd* 3.55) 4–4.10 (*d* 3.55) 5–3.82 (o) 6–4.02 (o)	o 107.4 77.5 78.5 83.8 66.5	3.58 (*m*) no 4.11 (*m*) 4.11 (*m*) 3.82 (*m*) 4.01 (*m*)	65.6 104.2 78.2 77.4 83.6 66.1

(*continued on next page*)

Table 2 (*continued*)

Structures	*δ ^{1}H (multip.* J in Hz)*	*δ ^{13}C*	*δ ^{1}H ref.*	*δ ^{13}C ref.*
OTHER COMPOUNDS				
Ethanol 	1–3.65 (o) 2–1.16 (*t* 7.20)	54.5 21.2	3.64 (*q* 7.08) 1.17 (*t* 7.08)	60.3 19.6
Glycerol 	1.3–3.61 (o) 2–3.74 (*m*)	67.0 72.4	3.64, 3.56 3.77	65.4 75.0

s – simplet; *d* – duplet; *t* – triplet; *q* – quadruplet; *quin* – quintet; *dd* – double duplet; o – overlapping signal; n – no information; no – not observed.

evaluated after autoscaling.

2.8. Microbiological quality

The Brazilian regulation for fruit juices stored under refrigeration requires the absence of coliforms, thermotolerant coliforms, and *Salmonella* (Brazil, 2001). However, molds and yeasts were also determined because they are common spoiling contaminants in fruit juices. The microbial analysis was done according to the methods described by the American Public Health Association (APHA, 2001) in triplicate for each dilution. Microbiological analyzes were performed for thermal (HTST, UHT) or combined non-thermal processing (US + LPP or LPP + US), immediately after the processing and along the cold storage (60 days).

3. Results and discussion

3.1. NMR analysis and compounds variability

The composition of açai juice under different thermal and non-thermal processing was investigated by ^{1}H NMR coupled to chemometric analysis. Initially, it was performed the identification of the main organic compounds, which are illustrated in Fig. 1 into the chemical shifts between δ 0.7 and 9.0 from a control açai juice (before processing). The structures of the compounds, ^{1}H, and ^{13}C chemical shifts, multiplicity, correlations, and constant coupling are described in Table 2.

Fig. 2a illustrates the resultant scores with control samples in black color, after thermal processing in red, and after non-thermal processing in blue color, with respective loadings plotted in lines form in Fig. 2b. The opposite effect between thermal and non-thermal processing on açai juice composition according to PC1 and PC2 axes is evident in Fig. 2a. In general, all the non-thermal processing increased the sugars content (glucose and fructose), and the amino acid betaine, except the combined processing of ultrasound followed by low-pressure plasma (US.LPP) that clustered at positive scores of PC1 and PC2. Furthermore, both thermal processing (HTST and UHT) increased the fatty acids and phenolic compounds content in the samples. The PC3 axis helped to highlight the clustering according to the type of processing (thermal or non-thermal), as well as reveled a particular effect of some non-thermal processing on açai juice composition (highlighted in Fig. 3).

An additional PCA was developed on NMR spectra considering the respective dataset to detail the effect of the non-thermal processing on açai juice composition. Fig. 3a illustrates the scores after low-pressure

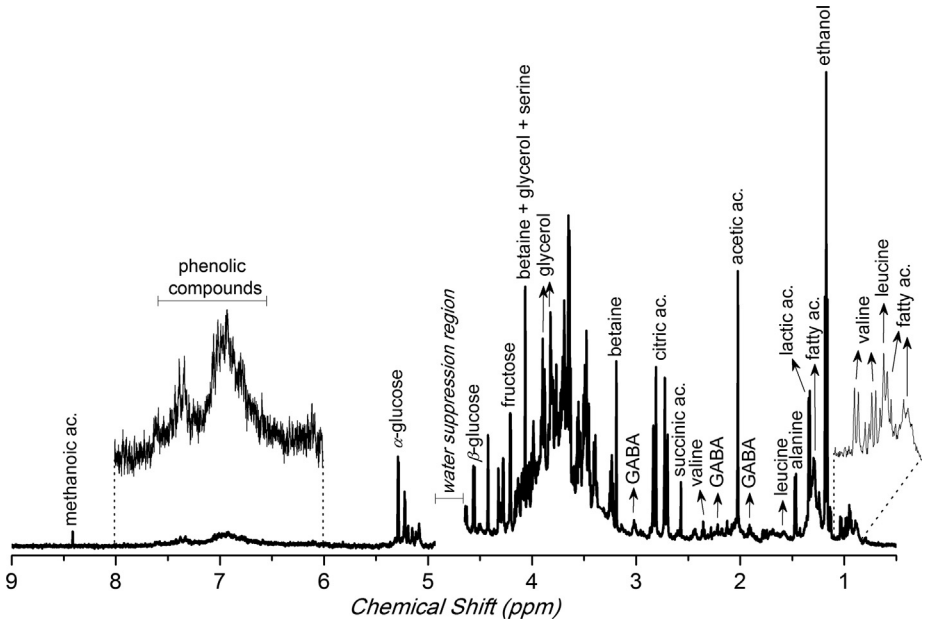


Fig. 1. ^{1}H NMR spectrum of the aqueous extract from non-processed açai juice (control sample) with the identified compounds.

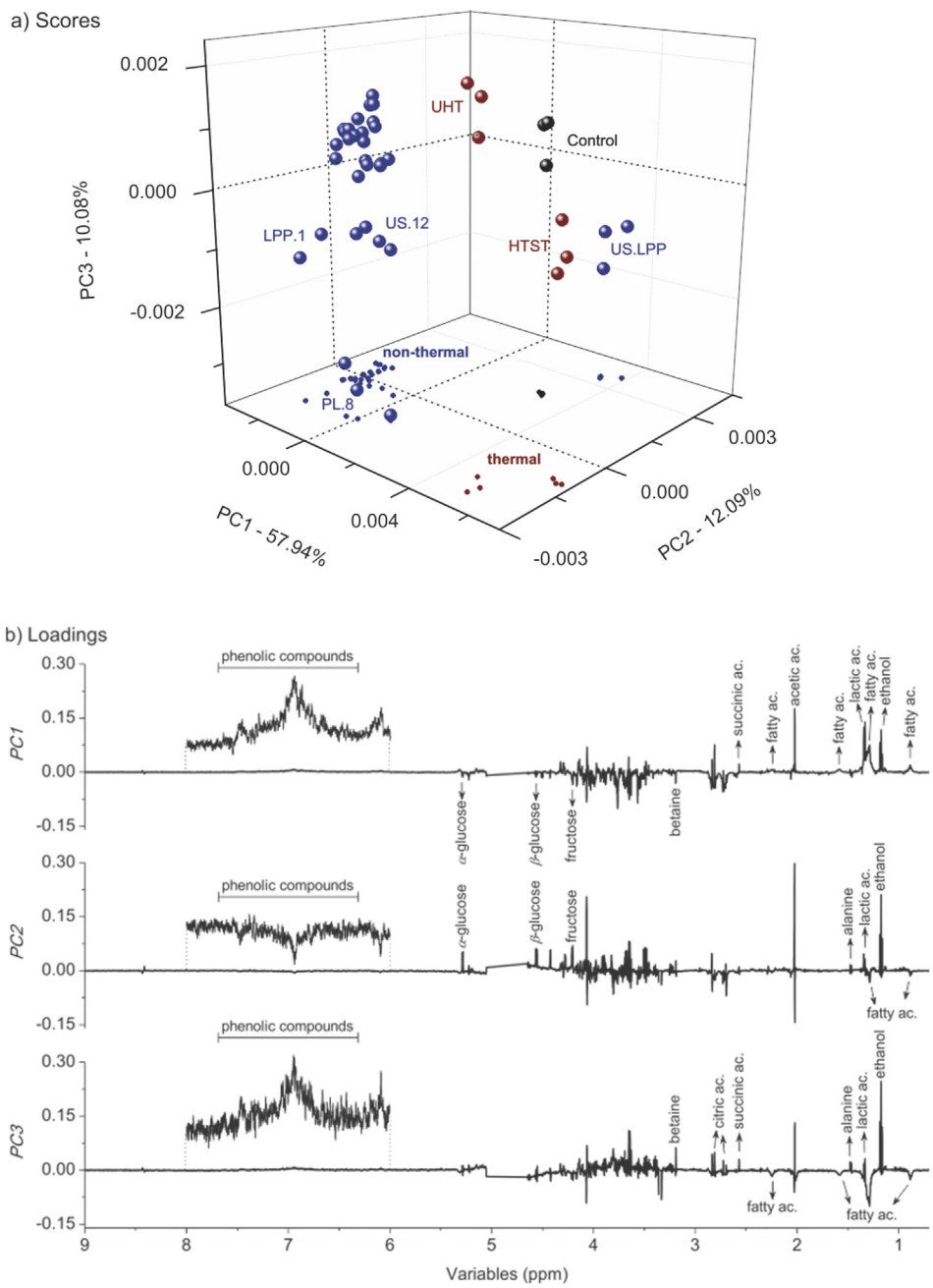


Fig. 2. 3D scores coordinate system with respective projections on PC1 × PC2 plane (a), and relevant loadings (b) from the PCA evaluation of açai juice under different processing. The control samples are illustrated in black color; after thermal processing by HTST and UHT in red; and after non-thermal processing by ultrasound (US), low-pressure plasma (LPP), pulsed light (PL), and the combination of US followed by PL in blue. The reference number along with the samples are related to the processing assay number: LPP.1 – 5 min at 10 mL.min^{-1}; PL.8 – 6 V (0.6 J/cm^2); US.12 – 5 min at 372.93 W.cm^{-2}. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

plasma processing in green color (LPP), pulsed light in blue color (PL), ultrasound in red (US), and the combined processing US followed by LPP in gray, with respective loadings plotted in lines form in Fig. 3b.

The non-thermal processing by low-pressure plasma by 5 min at 10 mL min^{-1} of flow rate (LPP.1), pulsed light under 6 V (PL.8), ultrasound for 5 min at 372.93 W cm^{-2} of potency (US.12), and ultrasound followed by pulsed light (US.LPP) presented significant effect on the açai juice composition. The non-thermal processing presented in Fig. 3, increased the amounts of ethanol, acetic, and lactic acid. Ultrasound and plasma also increased the fatty acids content, mainly after the combined processing. Plasma processing (LPP.1), pulsed light (PL.8), and ultrasound (US.12) decreased the amounts of glucose, betaine, and succinic acid proportionally to the others processing.

The non-thermal processes generate reactive species that interact in a complex way with the food matrix compounds. Depending on the technology, the processing parameters, and the food matrix, the effect is positive or negative. US processing is known as able to disrupt the cell tissue liberating compounds from the cell cytosol and those bound to the cell membrane (Silva, Almeida, Rodrigues, & Fernandes, 2015). The increase in cell permeability imparted by ultrasound allows higher mass transfer rates and, consequently, higher extractability of sugars from the cells (Ewe, Abdullah, Bhat, Karim, & Liong, 2012; Fernandes, Gallão, & Rodrigues, 2009; Fonteles et al., 2012). As açai juice has a high pulp content, the extraction phenomena promoted by US processing was due to the cell membrane disruption.

On the other hand, low-pressure plasma promoted sugar degradation on cashew apple juice (Rodríguez et al., 2017), because the ozone and nitrogen radicals and singlets formed can degrade sugars. Thus the effect of sugar increase was not observed in the combined treatment because LPP plasma was applied after the US processing. Regarding the increase of some compounds due to the thermal treatment, the phenomenon might be related to the solubility increase, especially for fatty acids because açai juice contains a high amount of suspended solids. Our group observed the same phenomena for Vitamin C in acerola juice

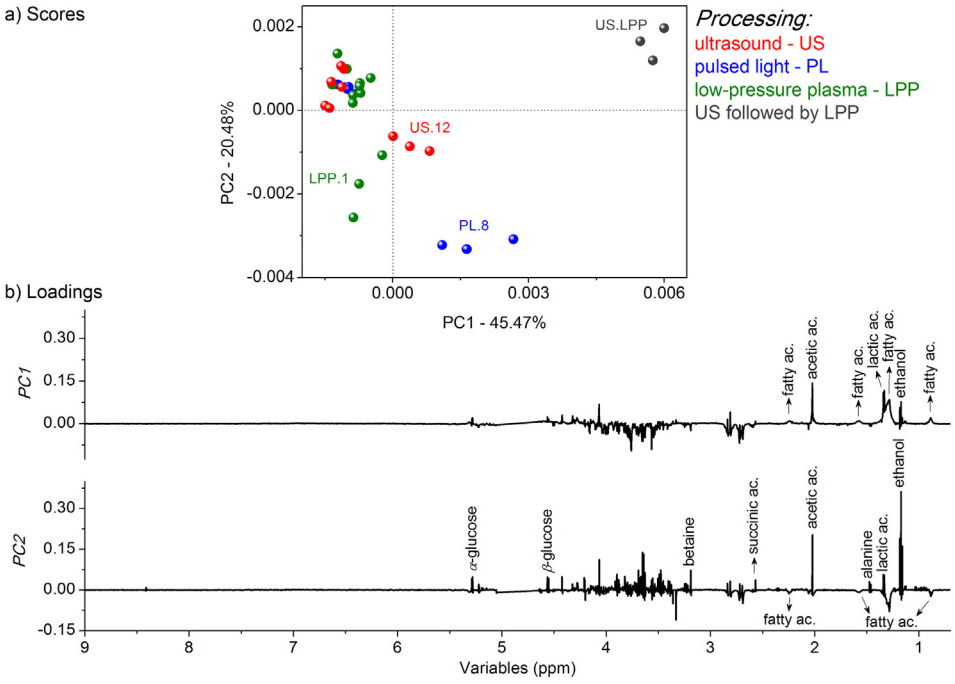


Fig. 3. 2D scores (a) and respective loadings (b) from the PCA evaluation of açai juice under non-thermal processing: ultrasound (US), low-pressure plasma (LPP), pulsed light (PL), and the combination of US followed by LPP in blue. The reference number along with the samples are related to the processing assay number: LPP.1 – 5 min at 10 mL.min^{-1}; PL.8 – 6 V (0.6 J.cm^{-2}); US.12 – 5 min at 372.93 W.cm^{-2}. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(Alves Filho et al., 2018).

3.2. Thermal and non-thermal processing on bioactive compounds, enzymes, and antioxidant activity during storage

To verify the influence of the thermal and non-thermal processing on bioactive compounds, as well as on the enzymatic and antioxidant activity during different periods of storage, PCA was applied to the resultant matrix. Fig. 4 illustrates the scores plotted in two dimensions (PC1 × PC2), retaining 59.62% of the total variance with error bars related to the standard deviation from triplicate of processing, and loadings are represented by vectors from the axes origin.

In general, PC1 was the main axis for sample distribution based on processing, which is mainly related to the storage period. Fig. 4 depicts the higher effect of thermal processing on vitamin C, anthocyanins, PPO, POD, DPPH, ABTS, and FRAP after 45 and 60 days of storage in comparison with the non-thermally processed juices, which followed the control samples.

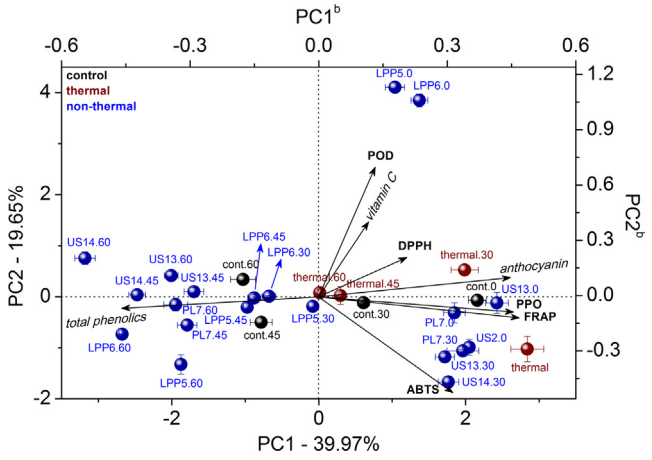


Fig. 4. PCA biplot of açai juice after thermal processing by HTST (red) and non-thermal processing (blue): [a]refers to scores axes; [b]refers to loadings axes with variables represented by vectors from the origin. Legend: values 0, 30, 45, and 60 into the samples name refer to the number of storage days; the values 13 and 14 into the ultrasound processing name (US) refer to 2 and 10 min of processing at 372.93 W cm^{-2}; PL refers to pulsed light processing under 2 V at 0.0857 J.cm^{-2}; the values 5 and 6 into the low-pressure plasma processing name (LPP) refer to 20 L min^{-1} during 15 min, and 30 L.min^{-1} during 10 min, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Due to higher amounts of vitamin C, anthocyanins, PPO, POD, DPPH, ABTS, and FRAP, both thermal and non-thermal processing stored from 0 to 30 days is located in positive scores. However, the bioactive compounds in non-thermal processed samples decreased after more extended storage periods (45 and 60 days) in the same way observed for the control sample, placing these samples at negative scores. On the other hand, non-processed samples stored during 45 and 60 days are located at negative scores of PC1 due to the higher amounts of total phenolics.

Hellström, Mattila, and Karjalainen (2013) reported that anthocyanin stability is related to the storage temperature, removal of oxygen, and inactivation of enzymes. The authors also pointed out that the stability of anthocyanins in berry products is often enhanced by naturally existing phenolic co-pigments, such as phenolic acids and flavanols. The hydrogen peroxide, a reactive species from the plasma processing, may remain active after treatment exposure, and some oxidative species can occur after 30 days of storage (Surowsky, Fischer, Schlueter, & Knorr, 2013). In thermal processing, the reactive species formed interacts with the polysaccharides cell wall. The cell wall depolymerization releases the bounded compounds increasing their concentration in the liquid media (Sarangapani, Ryan Keogh, Dunne, Bourke, & Cullen, 2017). The increase in bioactive compounds might be due to extraction effects promoted by membrane disruption induced by cavitation, or due to chemical release of bonded compounds. The increase or decrease of bioactive compounds content depends on the food matrix and the processing technology (Avalos-Llano, Martín-Belloso, & Soliva-Fortuny, 2018; Chemat et al., 2017; Musielak, Mierzwa, & Kroehnke, 2016; Surowsky et al., 2013).

3.2.1. Detailed effect of combined non-thermal processing compared to thermal processing

Based on effects of thermal processing during more extended periods of storage (45 and 60 days), as well as the particularities of combined non-thermal processing (plasma and ultrasound), and storage under refrigeration, bioactive compounds content, enzymatic and

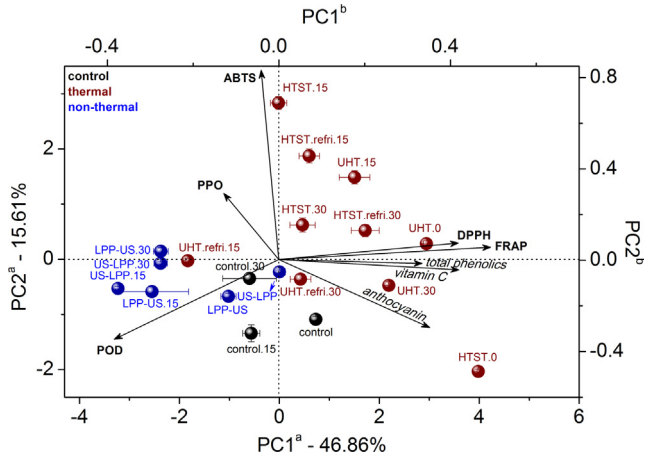


Fig. 5. PCA biplot from açai juices after thermal processing by HTST and UHT (red) and combined non-thermal processing by plasma-ultrasound (LPP-US) and ultrasound-plasma (US-LPP) illustrated in blue: [a] refers to scores axes; [b] refers to loadings axes with variables represented by vectors from the origin. Legend: values 0, 15 and 30 into the samples name refer to number of days under storage period; "refri" refers to storage under refrigeration. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

antioxidant activities of acai juices under the processing mentioned above were evaluated. Fig. 5 presents the scores plotted in two dimensions (PC1 × PC2) retaining 62.47% of the total variance with error bars related to the standard deviation of the processing triplicate, and loadings are represented by vectors from the axes origin.

The refrigerated storage did not present relevant effects on bioactive compounds, enzymatic and antioxidant activities of açai juice, as well as the combined non-thermal processing sequence (plasma before or after ultrasound). In general, positive scores of PC1 are located the samples after thermal processing due to the higher amounts of anthocyanins, vitamin C, and total phenolic, which decreased after all the non-thermal processing independent of the storage period. The percentage of PPO and POD increased after non-thermal processing, mainly after 15 and 30 days of storage. Enzyme activity is strongly related to its tridimensional structure (3D). Several factors such as hydrogen bonds, ionic bonds, and van der Waals bonds aside the hydrophobic and hydrophilic interaction among the amino acid residues of the enzyme are responsible for the enzyme 3D structure stabilization. The reactive species formation promoted by the non-thermal technologies, the UV radiation, and the cavitation affects those interactions breaking or forming new interactions leading the 3D enzyme structure to a more or less active conformation. The non-thermal technology effect on enzymes depends not only on the technology but also on the food matrix because the enzyme 3D structure depends on its medium composition as well. Plasma and ozone treatments decreased the POD activity on coconut water. However, ozone was able to complete POD inactivation while after plasma treatment, residual activities remained at different levels depending on the processing condition (Porto et al., 2020). High isostatic pressure (HIP) treated açai pulp resulted in different effects on POD and PPO. According to de Jesus et al. (2018), POD was baroresistant, while PPO was partially inactivated by the HIP processes at 65 °C by 5 min and 600 MPa and activated at the same pressure and lower temperature (25 °C). Furthermore, the thermally processed juices after 15 days of storage presented the highest antioxidant capacity determined by ABTS. It is known that thermal processing may increase compounds degradation (Alves Filho et al., 2018). However, the suspended pulps may reduce the heat transfer, which favors bioactive extraction over degradation. Therefore, the viscosity and the presence of suspended pulp are important parameters on the thermal processing of açai juice. Also, it is known that non-thermal processing by ultrasound and plasma may extract compounds, enzymes, and proteins from the matrix (Chemat et al., 2017; Musielak et al., 2016). In the present study, the enzymatic activity was higher after the combined non-thermal processing than that of non-combined processing. Enzyme denaturation can be an irreversible or a reversible phenomenon (Chakraborty, Kaushik, Rao, & Mishra, 2014). The same behavior was reported for açai PPO 24 h after the HIP (de Jesus et al., 2018).

3.2.2. *Bioaccessibility of anthocyanins, total phenolic, and vitamin C*

Since the bioaccessibility involves the nutrients available to be absorbed in the intestine after digestion (Carbonell-Capella et al., 2014). The analysis of anthocyanins, total phenolic, and vitamin C of açai juice samples were carried out after the complete simulated digestion. Fig. 6 presents the results of the effect of combined non-thermal processing and thermal processing by HTST and UHT on the bioaccessibility of total phenolic, anthocyanins, and vitamin C from açai juice. A single

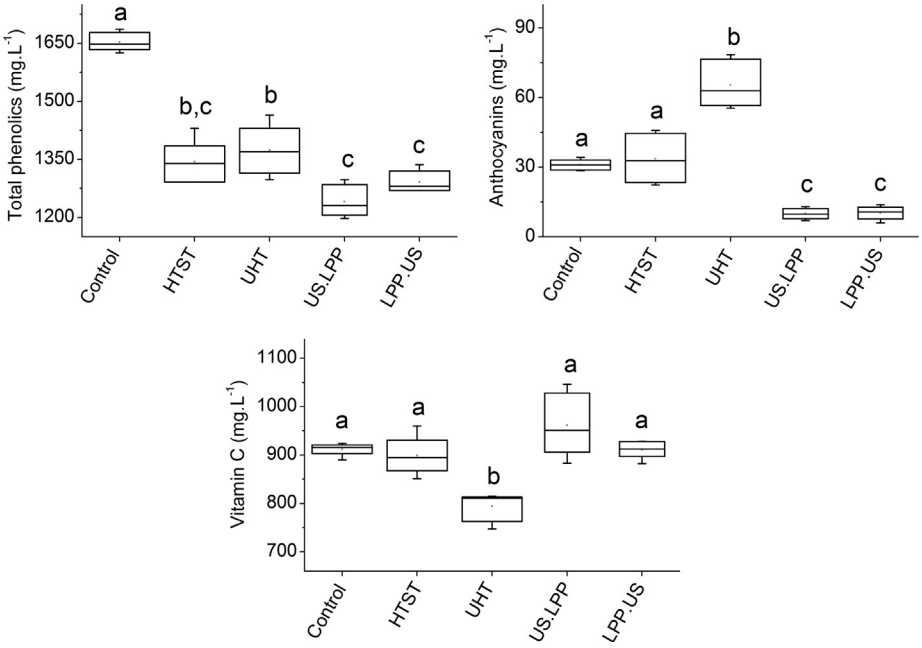


Fig. 6. Bioaccessibility of anthocyanins (a), total phenolic (b), and vitamin C (c) on açai juice according to combined non-thermal processing by low-pressure plasma followed by ultrasound (LPP-US) and US followed by LPP, and thermal processing by HTST and UHT. Different letters means significant differences according to the Tukey test.

Table 3
Microbial quality of processed açai juice (yeast and molds).

Time (days)	Control (log CFU/mL)	Past (log CFU/mL)	UHT (log CFU/mL)	US + PL (log CFU/mL)	PL + US (log CFU/mL)
0	5.04 ± 0.17^{aA}	3.20 ± 0.26^{aB}	2.89 ± 0.02^{aC}	4.45 ± 0.23^{aD}	4.12 ± 0.26^{aE}
15	6.85 ± 0.02^{bA}	3.84 ± 0.23^{aB}	3.23 ± 0.29^{bB}	5.84 ± 0.04^{bC}	4.90 ± 0.10^{bD}
30	7.24 ± 0.03^{cA}	6.12 ± 1.12^{cA}	4.84 ± 0.06^{cC}	7.83 ± 0.63^{cD}	7.06 ± 0.02^{cE}
4	8.50 ± 0.25^{dA}	6.75 ± 0.20^{cB}	5.50 ± 0.15^{dC}	8.23 ± 0.05^{dA}	7.60 ± 0.07^{dD}
60	8.75 ± 0.20^{dA}	7.50 ± 0.15^{dB}	6.43 ± 0.07^{eC}	$8.89 \pm 0,20^{eD}$	7.89 ± 0.12^{eE}

Average ± standard deviation of three replicates. Means with different lower caption in the same column and with different capital letter in the same line are significantly different according to the Tukey test ($p < 0.05$).

factor Analysis of Variance (One-way ANOVA) was performed to evaluate the differences among the concentrations.

The concentration of total phenolic reduced about 17–25% after complete digestion, independently of the type of processing (thermal or non-thermal). Despite the thermal processing that may modify the cellular wall, thus making phenolic compounds available (increasing their concentration), enzymes POD and PPO may oxidize the phenolic compounds compromising their bioaccessibility. Previous studies showed stable phenolic compounds available in apple, grape, and orange after thermal and high-pressure processing (Mannozzi et al., 2019). Most phenolic compounds are present in a glycosylated form in the vegetable tissue. The lower bioaccessibility of the phenolic compounds at the end of the gastric phase might be due to the acid hydrolysis of those compounds (Barba et al., 2017; Palafox-Carlos et al., 2011).

Most of the anthocyanins are degraded in monomers or dimmers forms before their absorption (Fang, 2015). Furthermore, some anthocyanins may be efficiently absorbed on the gastrointestinal lumen, pass through extensive metabolism to penetrate in systemic circulation as metabolites. The thermal and non-thermal processes may favor the anthocyanins absorption by molecular structure modification (Carbonell-Capella et al., 2014), as evidenced by the present study.

A similar result was found by Matheus de Sousa Carvalho et al. (2020). They also observed the improvement of the bioaccessibility of anthocyanins and carotenoids from açai and buriti juices after high energy ultrasound processing. This improvement was associated with the release of bioactive compounds retained in the plant cell structures that occurred by the cavitation disruption.

Anthocyanin bioaccessibility increased 2-foldincreased in 132% by thermal sterilization (UHT). On the other hand, the combined non-thermal treatment reduces the bioaccessibility to 40% of the control values. Martínez-Huélamo et al. (2015) attributed the increased release of phenolic compounds from the tomato matrix after thermal processing to the cell wall and cell membrane disruption, which increased the pool of bioaccessible lycopene. Colle, Lemmens, Van Buggenhout, Van Loey, and Hendrickx (2010) reported that only very intense heat processing conditions were able to improve the lycopene bioaccessibility in plain tomato pulp making it more approachable for digestive enzymes and bile salts.

The combined non-thermal processing of US followed by LPP improved by 8% ($p \geq 0.05$) the bioaccessibility of vitamin C. The vitamin C may be oxidized into the gastrointestinal tract and regenerate to the active structure based on others diet components (Fonteles et al., 2016). Therefore, the processing may influence positively or negatively the food composition during digestion, depending on the treatment parameters (like treatment time and intensity), as well as the metabolism, the material amount, and the components that may influence in absorption (food matrix).

3.2.3. Microbial stability

Salmonella and *coliforms* were absent both in raw and processed samples. The absence of *Salmonella* and *coliforms* corroborates with the study published by de Jesus et al. (2020) and might be attributed to the good manufacturer practice of the raw material. The yeast and molds count were 5 log CFU/mL in the raw material. Thermal processing (HSTS and UHT) decreased yeasts and molds counts to 1 log CFU/mL after the thermal treatment.

Despite the increase in açai consumption, its conservation is still a challenge. The fruit is highly perishable, presenting microbial populations up to 7 log CFU/g (de Jesus et al., 2020). Aside from the high microbial loads, the endemic prevalence of Chagas Disease requires the berries blenching at 80 °C or washing with free chlorine (200 ppm) for the decontamination process (Bezerra, Freitas-Silva, Damasceno, Mamede, & Cabral, 2017; Fujita, Nascimento, & de Andrade Júnior, 2019). The pasteurization of açai only decreases the microbial counts. Pasteurization is not enough to reach the microbiological stabilization. Thus, açai pulp is still commercialized frozen even after pasteurization. However, due to the fruit pulp viscosity, the thermal transfer is limited, and usually, long processing times are required imparting the severe product quality loss. Sousa et al. (Sousa, Yuyama, Aguiar, & Pantoja, 2006) recommended the pasteurization at 90 °C for 5 min, followed by frozen storage at −18 °C up to 120 days. The pure açai products are commercialized as frozen pulp for safe consumption. The açai pulp production regulation requires only the berry blenching to avoid Chagas disease. The microbial analysis required by the Brazilian legislation are *Salmonella* and coliforms. However, in the present study yeasts and molds were also determined, as shown in Table 3. The microbial analyses were done for further processing optimization by hurdle technology. Up to now the açai pulp and other açai products are commercialized frozen after pasteurization (85 °C/1 min), which only decreases the microbial loadings requiring the frozen storage and frozen commercialization of the product.

According to the results presented in Table 3, the combined non-thermal processing reduced less than 1 log CFU/mL. On the other hand, thermal treatment promoted a maximum of about 2 log CFU/mL of yeasts and molds reduction even for the sterilization processing (UHT). In the present study, only the thermal treatments were done in aseptic conditions using a pilot-scale fully automated equipment. The non-thermal processing was carried out in an open bench, which might affect the microbial counts immediately after the processing (Oliveira et al., 2018).

The microbial counts increased for all treatments along the refrigerated storage period, even for thermally treated samples. Açai is a problematic product for microbial stabilization, and because of that up to now, açai pulp is commercialized frozen. Some products containing açai are blended juice, and many of them contain preservatives and acidulants. Regarding the results presented in Table 3, the microbial quality (counts < 4 log CFU/mL) was kept only by thermal processing up to 15 days of cold storage, reinforcing the need for technology development for açai juice processing and stabilization.

The microbial inactivation by non-thermal treatment presented in Table 3 agrees to the reported by Oliveira et al. (2018). Those authors studied the açai juice processing by US and ozone with an açai juice prepared from the açai berry made in a domestic blender and reported a reduction of 1 log CFU/mL of yeasts and molds in açai juice after the combination of ozone and US treatment. Those authors did not report any data on thermal treatment nor on the product storage. de Jesus et al. (2020) reported yeasts and molds count less than 1 log CFU/mL

for pasteurized açai pulp (85 °C/1 min), and HPP processed açai pulp, keeping the counts level in the same range for 28 days of cold storage (5 °C) for both processes.

Regarding yeasts and mold inactivation, it seems that the HPP treatment for açai pulp is much better than the other studied non-thermal treatments, including the combination of US and ozone reported by Oliveira et al. (2018). However, the results presented by de Jesus et al. (2020) on pasteurization contradict the information available on açai processing. A previous study on açai thermal processing required longer times with severe product quality loss (Sousa et al., 2006). Besides, pasteurization at 85 °C/1 min (the industrial protocol) is not enough to avoid açai frozen storage - the current commercial practice. In their study, de Jesus et al. (2020) açai pulp was inoculated açai juice with lactic acid bacteria (LAB).

Other aspects of the working plan carried out by de Jesus et al. (2020) must be taken into accounts for a fair comparison such as the use of plastic bags (closed system) and batch pasteurization with a small volume. The raw material used by de Jesus et al. (2020) was not pure açai juice. The juice was inoculated with two microorganisms. One of then a lactic acid bacterium (LAB), which was not inactivated and showed stable counts along the storage period. Alive LAB produces bacteriocins and lactic acid (even not growing), which might have contributed to avoiding yeasts and molds growth. Previous studies on *Lactobacillus casei* (LAB) in fruit juices showed that LAB does avoid yeasts and molds growth (Pereira, Maciel, & Rodrigues, 2011; Fonteles et al., 2013; Pereira, Almeida, de Jesus, da Costa, & Rodrigues, 2013). This feature might have affected the results on microbial counts presented by de Jesus et al. (2020) because the results reported by simple batch pasteurization are not consistent with the UHT sterilization protocol and the state of the art for açai pulp preservation.

Industrial batch sterilization processing is carried out in stirred tanks, and the product is usually package by hot-fill technology. Industrial batch sterilization requires long exposure periods and imparts severe quality loss in fruit juices. Because of that, several studies suing HSTS and UHT have been carried out on fruit juice thermal stabilization. The present study used a fully automated heat exchanger system to avoid bioactive compounds degradation due to prolonged exposure to high temperatures, and the package processing was done in agreement with what is really done in an industry. The results reported by de Jesus et al. (2020) for microbial quality, especially for the pasteurized sample, seem to be affected by the addition of lactic bacteria. Also, the processing times and temperature of the studies are different, and a fair comparison would not be possible. The results obtained in the present study showed that nor thermal neither the evaluated non-thermal processing was enough to microbial stabilization of açai juice. Thus, this field requires more studies on hurdle technology.

4. Conclusions

The chemometric evaluation was suitable to monitor changes in açai juices under different processing, according to ^{1}H NMR dataset, stability, and bioactive compounds. The non-targeted monitoring of the effect of thermal and non-thermal treatments on the general composition of açai juice can be useful in searching for the processing improvement, and in the planning of preventive actions against related degradation products. The results showed in the present study showed that it is possible to increase the bioactive contents in açai juice by non-thermal processing and design future hurdle technologies for better conservation of açai juice. The increase in bioactive compounds promoted by non-thermal technologies allows the increase in thermal processing time in a hurdle technology optimized processing. Thus, the present study contributes to future investigations on hurdle technology to preserve açai juice. Despite the product quality preservation, microbial stabilization was not achieved. In fact, up to now there is no current thermal technology available that stabilizes pure açai products without preservatives.

The initial reduction on microbial counts obtained by non-thermal technology indicates that microbial cells may suffer sublethal injuries instead of complete inactivation. This information is important because it allows further studies combing different thermal and non-thermal technologies. The main limit of combining HSTS and UHT processing with non-thermal treatment is the scale. Most non-thermal technologies are not available in pilot scale because they are still under investigation. Up to now, there is no current thermal nor-non-thermal technology available that stabilizes pure açai products without preservatives.

CRediT authorship contribution statement

Maria de Fátima D. Linhares: Methodology, Investigation, Data curation, Writing - original draft. **Elenilson G. Alves Filho:** Methodology, Validation, Software, Investigation, Writing - original draft. **Lorena Mara A. Silva:** Methodology, Investigation, Validation. **Thatyane V. Fonteles:** Methodology, Investigation, Software. **Nédio Jair Wurlitzer:** Methodology, Investigation. **Edy S. de Brito:** Methodology, Investigation, Validation, Funding acquisition. **Fabiano A.N. Fernandes:** Methodology, Investigation, Validation, Funding acquisition. **Sueli Rodrigues:** Investigation, Methodology, Supervision, Project administration, Writing - review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2020.109506.

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