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Evaluation of thermal and non-thermal processing effect on non-prebiotic and prebiotic acerola juices using ^{1}H *q*NMR and GC–MS coupled to chemometrics

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ABSTRACT

The effects of thermal (pasteurization and sterilization) and non-thermal (ultrasound and plasma) processing on the composition of prebiotic and non-prebiotic acerola juices were evaluated using NMR and GC–MS coupled to chemometrics. The increase in the amount of Vitamin C was the main feature observed after thermal processing, followed by malic acid, choline, trigonelline, and acetaldehyde. On the other hand, thermal processing increased the amount of 2-furoic acid, a degradation product from ascorbic acid, as well as influenced the decrease in the amount of esters and alcohols. In general, the non-thermal processing did not present relevant effect on juices composition. The addition of prebiotics (inulin and *gluco*-oligosaccharides) decreased the effect of processing on juices composition, which suggested a protective effect by microencapsulation. Therefore, chemometric evaluation of the ^{1}H *q*NMR and GC–MS dataset was suitable to follow changes in acerola juice under different processing.

1. Introduction

Acerola (*Malpighia emarginata* DC.) is a valuable fruit for human diet and a potential source of Vitamin C (ascorbic acid). Acerola juice presents an appreciated flavor and a pleasant color. The insertion of prebiotic compounds as soluble dietary fiber has been reported as a way to improve the beneficial effects of fruit juices for human healthy (Araújo et al., 2015; da Silva, Rabelo, & Rodrigues, 2014). Prebiotics are non-digestible carbohydrates that reach the intestine where they selective stimulate the growth of the gut microbiota associated with health benefits. The insertion of prebiotic compounds helps significantly to improve the functional efficacy of probiotic fruit juices. Among the prebiotic compounds, inulin and other non-digestible *gluco*-oligosaccharides are considered important for health reducing risks of certain diseases (Tingirikari, Gomes, & Rodrigues, 2017).

Thermal processing is the standard industrial process to increase the shelf life of commercial fruit pulps and juices. Ultra High Temperature (UHT) is an efficient thermal processing for foodstuffs sterilization (Soares et al., 2017; Sucupira et al., 2017). However, nutritional changes in functional composition, vitamins, and other nutrients are usually reported for thermal processing. Therefore, new non-thermal processing technologies as cold plasma and ultrasound have been widely studied for inactivation of enzymes, microorganisms, besides minimizing the loss food quality nutritional value (Misra, Keener, Bourke, Mosnier, & Cullen, 2014).

The knowledge of the compounds affected by the food processing is an important part of the food products development. The composition of food might influence on flavor, aroma, color, stability, and microbial control of the final product. Among the analytical techniques, Nuclear Magnetic Resonance (NMR) spectroscopy allows the compounds assignment in food matrices as a whole (without previously chemical separation or purification). Untarget sample exploration, processing effect evaluation, characterization of the genotype of the fruit; evaluation of the environmental factors and different agronomical practices (irrigation condition, solar incidence, altitude and others) in the product quality are NMR useful applications (D'Imperio et al., 2007; da Silva et al., 2016; Spraul et al., 2009). Additionally, the Gas Chromatography coupled to Mass Spectrometry (GC–MS) is widely used to characterize the aroma composition of fruits juices (Alves Filho, Rodrigues, et al., 2017; Bicas et al., 2011). However, due to complex

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and extensive datasets obtained for food matrices, chemometric analyses are often necessary for a proper analytical evaluation, especially in untargeted analysis.

Pure and prebiotic acerola juice (juice containing inulin or *gluco*-oligosaccharides) samples were processed by High Temperature Short Time (HSTS); Ultra High Temperature Sterilization (UHT) and non-thermal processing (ultrasound and plasma). After processing, NMR spectroscopy and GC–MS (both combined with chemometrics) were used to detect possible chemical changes in the acerola juice samples composition.

2. Materials and methods

2.1. Raw material

The raw material used was non-pasteurized and free of preservatives acerola (*Malpighia emarginata* DC) frozen pulp purchased from a local producer (KIPOLPA™, Fortaleza-CE, Brazil). The product was kept frozen (−18 °C) until the processing. Initially, the pulp was diluted (1:2) with potable water. Three different juices were used for the thermal and non-thermal processing: pure juice (PJ); juice with the addition of inulin (JI); and juice containing *gluco*-oligosaccharides (JGO). The pure juice was obtained by the simple dilution of the pulp in water. The JI was obtained by adding 7% (w/v) of inulin (Beno-Oraft, Germany). The JGO were prepared by synthesizing the oligosaccharides directly into the juice as described further on. A total volume of 3 L of each juice sample was prepared. The samples were thermally or non-thermally processed just after the preparation.

2.1.1. Oligosaccharides synthesis in the acerola juices

The oligosaccharides synthesis in acerola juice was developed according to the protocol previously established by our group using the enzyme dextran-sucrase (Araújo et al., 2015; Coelho et al., 2015). The synthesis was carried out at the optimum enzyme conditions (30 °C and pH 5.2) in a reactor containing 3L of acerola juice. Amounts of 100 g of fructose, 100 g of glucose, and 200 g of sucrose were added to juice to act as a substrate for the enzyme synthesis. The pH was adjusted to 5.2 using NaOH. The enzyme dextran-sucrase (1 IU·mL^{-1}) was then added to the juice. The synthesis was carried out at 30 °C for 24 h (da Silva et al., 2014). After the enzymatic synthesis, the juice pH was adjusted to 3.1 (initial juice pH) using citric acid.

2.1.2. Evaluation of the oligosaccharides formed in the acerola juices

Samples of the juice containing *gluco*-oligosaccharides were taken after the synthesis. The dextran formed was precipitated using 3 volumes of ethanol 96% (v/v). The dextran was diluted in water and quantified as total carbohydrate according to the phenol–sulfuric method (DuBois, Gilles, Hamilton, Rebers, & t., & Smith, F., 1956). The alcoholic supernatant was used to evaluate the oligosaccharides formation by thin layer chromatography (TLC). Silica gel aluminum plates 60 (20 × 20 cm, Merck, product number 1.05553.0001) were used. Acetonitrile/ethyl acetate/2-propanol/water (85:20:50:90) was the solvent system used to separate the *gluco*-oligosaccharides (Rodrigues, Lona, & Franco, 2005; Tingirikari et al., 2017). The samples (5 μL) were applied at 1.5 cm of the plate border, and the plates were irrigated two times with the described solvent system. The plates were dried using a hair dryer after each development. The detection system was a solution containing 0.3% (w/v) of 1-naphtyletienediamine and 5% (v/v) of concentrated sulfuric acid in methanol. The TLC plate was quickly immersed using a Chromatogram Immersion Device 3 (Camag, Switzerland). The plates were then naturally dried inside a hood at room temperature. After that, the plate was heated for 10 min at 120 °C in a TLC plate heater 3 (Camag, Switzerland).

2.2. Thermal processing

A general procedure for pasteurization and sterilization of fruit juices was employed with modifications for acerola juice (Sucupira et al., 2017). The processing was performed in triplicate. Acerola juice was submitted to two types of thermal treatments: High Temperature Short Time (HTST) pasteurization processing at 90 ± 2 °C; and Ultra High Temperature (UHT) sterilization at 136 ± 1 °C. For UHT processing, the retention time at 136 °C was 4.1 s using an Armfield tubular heat exchanger (model FT74). The juice was cooled to 17 °C with the Armfield FT63 chiller and packaged in transparent 210 mL polyethylene terephthalate (PET) bottles and closed with polypropylene screw cap. The same equipment was used for the HTST processing, which was performed at 90 °C with a retention time of 2 min. The pasteurized juices were bottled by the hot-fill processing using clear 210 mL bottles. The bottles were kept upside-down for 2 min and then cooled in running water to 25 °C. All packages (PET bottles) were previously sterilized with a peracetic acid solution (0.5%) and rinsed with sterile water before filling. The samples were kept frozen at −80 °C until the analyses.

2.3. Non-thermal processing

Juice samples were submitted to low-pressure plasma and ultrasound (US) processing. The plasma treatments were carried out using a glow discharge plasma generation model Venus PE100 (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). Pressure reached within the chamber remained at 300 mbar. The process was carried out in indirect plasma irradiation using nitrogen gas at 30 mL·min^{-1} (grade FID 4.5, purity 99.95%, White Martins, Brazil) (Rodríguez, Gomes, Rodrigues, & Fernandes, 2017). The species produced using nitrogen plasma were nitrogen oxide (NO), nitrogen dioxide (NO_2), peroxynitrous acid (ONOOH), nitrite (NO^{2-}) and nitrate (NO^{3-}) ions in the aqueous phase. The juices were exposed to plasma treatment for 10 or 20 min.

The ultrasound processing was carried out using 18 kHz probe ultrasound (Unique model USD500). A 13 mm titanium probe was used, which was immersed 15 mm below the liquid surface. For each experiment, 100 mL of acerola juice was placed in a glass jacketed beaker (250 mL) and the US potency applied was 5000 W·L^{-1}. The juice was subjected to ultrasonic application during 5 or 15 min. The temperature was kept constant at 25 °C trough a circulating water bath.

2.4. Quantification of ascorbic acid using HPLC-UV

The concentrations of ascorbic acid were determined by an HPLC-UV procedure with modifications (Plaza et al., 2011). Each sample (0.3 g) was homogenized with 20 mL of the extraction solution (3% of *meta*-phosphoric acid with 8% acetic acid). The resultant mixture was adjusted up to 50 mL with distilled water, centrifuged, and filtered through 0.45 μm Nylon membrane filter to 2 mL vials. An LC20A Shimadzu Proeminance equipped with the SPD-20A photodiode array detector (PDA) was used for the analysis. An aliquot of 10 μL was injected at a reversed-phase Shim-pack CLC-ODS(M)™ C_{18} (150 mm × 4.6 mm × 5 μm) (Shimadzu, Kyoto, Japan) at 30 °C. The solvent system was an isocratic elution of 0.01% H_2SO_4 (pH 2.5–2.6) using a flow rate of 1 mL·min$^{-1,}$ and the chromatographic peaks were monitored at 245 nm. The identification of the ascorbic acid was carried out by comparing the retention time and UV–visible absorption spectrum with the reference standard of ascorbic acid 99% (Sigma). Quantification was carried out by a calibration curve ranging from 50 to 550 mg·L^{-1} of ascorbic acid.

2.5. Sample preparation, ^{1}H qNMR spectroscopy, and molecular identification

All samples were centrifuged (3 min at 605*g*) to remove solid particles. An aliquot of 165 μL was mixed with 400 μL of pure D_2O (99.9%), 35 μL of D_2O containing 14 mM of EDTA; and 1% of sodium-3-trimethylsilyl propionate (TMSP-d_4). The samples were then transferred to 5 mm NMR tubes.

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 spectra were acquired in quintuplicate using the PRESAT pulse sequence for water suppression (4.77 ppm). To ensure complete relaxation of all nuclei of the samples, the inversion recovery sequence was used after performing 90° pulse calibration (7.9 μs pulse length at 58 dB of power) and probe properly tuned and matched. This sequence consists of the application of a 180° pulse to reverses the magnetization vector, followed by a time interval (τ) where the magnetization evolves according to the longitudinal relaxation process (T_1), with a subsequent 90° pulse to obtain the NMR signals. The amplitude of the signals is directly proportional to the magnetization in the "z" axis. With this, the value of T_1 of all the nuclei into the samples was determined by varying the τ linearly from 0.5 s to 5.0 s, with increment times of 0.15 s. The recycling delay of 5 times T_1 between pulses was applied to ensure the full relaxation of all the hydrogens present in the samples. Therefore, it was used a relaxation delay of 22.0 s, acquisition time of 5.0 s, 32 scans, 48 k of time domain points with a spectral window of 16.0 ppm. The prefixed value for the receiver gain was achieve by comparison between spectra using the same signal to noise ratio, which was used for all the acquisitions. The temperature was controlled to 298 K and, the TMSP-d_4 was used as an internal standard (0.0 ppm). Free induction decays were multiplied by an exponential function equivalent to 0.3 Hz line-broadening before applying Fourier transform for 64 k points. Phase correction was manually performed and, the automatic baseline correction using polynomial degree 5 was applied over the entire spectral range.

The identification of the constituents within the matrices was performed through 2D-NMR evaluation using the correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), heteronuclear multiple bond correlation (HMBC), assessments using an open access database (*www.hmdb.ca*) (Wishart et al., 2012), and literature reports (Alves Filho et al., 2016; Alves Filho, Silva, Teofilo, Larsen, & de Brito, 2017; Nunes, Oliveira, & Alcantara, 2015; Silva, Alves Filho, Choze, Lião, & Alcantara, 2012). The molecular structures, ^{1}H and ^{13}C chemical shifts, multiplicity, correlations and constant coupling, 2D-NMR data acquisition and processing are available in the Supporting Information.

The compounds in pure acerola juice samples that presented high variations in chemometrics and did not exhibit overlapping resonances were quantified by the external reference method provided by VnmJ™ program (version 4.2, Agilent). This technique is based on the principle of reciprocity and, the NMR signals strengths are correlated with a reference sample. A stock solution of D_2O (99.9%) and sucrose (5.0 mg·L^{-1}) was used to calibrate the equipment and, then, the probe file was updated with all the parameters required to determine concentrations of the other compounds. The results were evaluated by the analysis of variance ANOVA single factor using Origin™ 9.4 program to statistically certify the differences in the concentrations at a a significance level of 0.05. Tukey and Levene test were applied to test the variance homogeneity. The combined uncertainties were based on the analytical errors and standard deviation from the quintuplicate of ^{1}H spectra acquisitions.

2.5.1. Chemometric analysis of the ^{1}H NMR data

The chemometric analysis was performed to investigate the variability of the composition in prebiotic and non-prebiotic acerola juices after thermal and non-thermal processing. Numerical matrices with 45 ^{1}H NMR spectra for each type of acerola juice (PJ, JI, and JGO) were created using the data acquired in quintuplicate. The NMR spectra were converted to American Standard Code for Information Interchange (ASCII) files for construction of the numerical matrices. To reduce the original data dimensionality and to observe the samples composition trends with a confidence level of 95%, the matrices were exported for chemometric analysis by Principal Component Analysis (PCA) and Partial Least Squares (PLS) using The Unscrambler X™ program 10.4 (CAMO software, Woodbridge, NJ, USA). The Singular Value Decomposition algorithm (SVD) was applied to decompose the matrices and PCAs were performed separately for each group of samples (PJ, JI, and JGO). For PCAs evaluation, the matrices data were reduced (averaged) along variables by a factor of 50. Afterward the application of a baseline correction algorithm, the spectral area were normalized and aligned using the Correlation Optimized Warping (COW) algorithm with a segment of 20 data points and a slack of 10 data points (Soares et al., 2017; Sousa, Magalhães, & Ferreira, 2013). PCAs were carried out after the mean-centered processing over the variables since this pretreatment enhanced the differences between the samples. The relevant information from the chemical data was obtained at the first two principal components (PCs) for all juices.

To improve the identification of chemical changes due to the different processing and to maximize the separation among the groups of samples, the supervised PLS method was employed using the ascorbic acid concentration previously determined by HPLC-UV as categorical variables (Y column). The NIPALS (Nonlinear Iterative Partial Least Squares) algorithm was applied to build the model, and the latent variables were selected in accordance to statistical parameters: slope, *SEC* (Square Error of Calibration); SEV (Square Error of Validation), and R^2 values. The full cross-validation method was applied to evaluate the performance of PCA and PLS models: each replicate of each juice sample was left out from the calibration data set and the sub-models were calibrated on the remaining data points.

2.6. GC–MS analysis

A general procedure for HS-SPME-GC/MS analysis of fruit juice was employed (Alves Filho, Rodrigues, et al., 2017). Before the chemical analysis, 1 g of the juice sample was mixed to 2 g of water, 1 g of NaCl, transferred to 10 mL vial, and equilibrated at 60 °C for 10 min with constant stirring (500 rpm). An automatic SPME holder (Supelco, Bellafonte, PA, USA) with a 30–50 μm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) Stable Flex fiber was used for all the experiments. The DVB/CAR/PDMS fiber was exposed to the sample headspace for 25 min.

Once the volatile compounds were extracted, the DVB/CAR/PDMS SPME fiber was introduced in the gas chromatograph (GC) splitless injector and maintained there at 240 °C for 3 min. The samples were analyzed in a GC–MS Agilent 5977A equipped with an HP-5MS (Agilent) fused silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) connected to a quadrupole detector operating in the EI mode at 70 eV with a scan mass range of 50–600 *m/z* and sampling rate of 2.7 scans·s^{-1}. Helium was used as carrier gas at 1 mL·min^{-1}. The injector and the interface temperatures were 240 °C and 280 °C, respectively. The temperature ramp was: 40 °C for 4 min, increased to 80 °C at 2.5 °C min^{-1}, to 110 °C at 5 °C min^{-1} and to 220 °C at 10 °C min^{-1}. The final temperature (220 °C) was held for 23 min. The identification of compounds was performed by comparing their retention indices with those of known compounds obtained by injecting a mixture of standards containing homologous series of C_7-C_{30} alkanes analyzed in the same column and the same chromatographic conditions (Van den Dool & Kratz, 1963). The peaks were identified by fragmentation patterns using the NIST Mass Spectral Search Program (Washington, DC, version 2.0 of 2008 – 287,324 compounds) and reported references (Bicas et al., 2011; Boulanger & Crouzet, 2001; Pino &

Marbot, 2001; Vendramini & Trugo, 2000).

2.6.1. Chemometric analysis of the GC–MS data

For each acerola juice (PJ, JI, and JGO), the region between 3.2 and 33.1 min was selected for chemometric analysis resulting in numerical matrices with the dimensionality of 76,722 data – 4038 variables × 19 samples (control and triplicate of six processing). The statistical analysis was the same as described in Section 2.4.1.

3. Results and discussion

The oligosaccharides formation in acerola juice detected by TLC showed *gluco*-oligosaccharides with a degree of polymerization up to 7. The present study evaluated three samples of acerola juice (pure juice, juice with the addition of inulin, and juice with *gluco*-oligosaccharides) using NMR, HPLC-UV, and GC–MS. The results were presented separately according to the analytical technique.

3.1. Quantification of ascorbic acid (Vitamin C) by HPLC-UV in acerola juice

Quantitative analysis of Vitamin C using HPLC-UV is usually applied to food matrices since it is a precise, robust, and low-cost analytical technique (Fontannaz, Kilinç, & Heudi, 2006). Fig. 1 presents the concentrations of Vitamin C in the three types of acerola juice (pure juice, juice with inulin, and juice with *gluco*-oligosaccharides) before and after both thermal (HTST and UHT) and non-thermal (plasma and ultrasound) processing. The purpose of this quantification was to create a categorical variable (Y column) to develop the regression analysis using Partial Least Square (PLS). The number of latent variables in X matrix (samples data) was evaluated to maximize the covariance between X and Y in the prediction model. Further, the quantitative results of Vitamin C using HPLC-UV were compared to those found by ^{1}H *q*NMR.

Based on ANOVA single factor, a clear increasing tendency on the concentration of Vitamin C was observed after thermal processing (HTST, and UHT), for acerola juice and acerola juice containing inulin. This result was consistent with the increase of Vitamin C in orange juice of 10% and 18% after 75 °C/30 s and 95 °C/30 s, respectively, due to its extraction from the orange solids parts (Gil-Izquierdo, Gil, & Ferreres, 2002). Despite the common sense that Vitamin C is degraded due to thermal treatment, the heat extraction can take place in foods with suspended solids like pulps. The Vitamin C degradation might be attributed mainly to oxygen and storage influence (Al Fata, Georgé, Dlalah, & Renard, 2017). However, in the present study, the thermal processing was carried out in a tubular heat exchange (closed system), and the amount of oxygen inside the equipment is limited avoiding atmospheric absorption as happen in open tanks. An increase in the Vitamin C concentration in juice samples submitted to ultrasound (US) processing was also observed for acerola juice and acerola juice containing inulin. The US processing is known to enhance the content of bioactive products due to the extraction effect (Costa et al., 2013; Fonteles et al., 2012; Fonteles, Leite, da Silva, Fernandes, & Rodrigues, 2017). In general, a decrease (15–25%) in the concentration of Vitamin C was observed in all acerola juices with *gluco*-oligosaccharides. The *gluco*-oligosaccharides were synthesized directly on the juice at 30 °C for 24 h. Such procedure could explain the decrease in the initial Vitamin C concentration. However, the presence of prebiotic carbohydrates (*gluco*-oligosaccharides and inulin) might be playing a protective effect on Vitamin C from further degradation. Inulin and oligosaccharides isomers are prebiotics commonly used for microencapsulation to protect certain compounds or microorganisms from harsh conditions, as high temperatures (Corona-Hernandez et al., 2013).

3.2. Variability of the organic composition using ^{1}H qNMR coupled to chemometrics

A comparison among ^{1}H NMR spectra from each acerola juice sample for an overall comprehension of the composition variability according to the different processing is shown in Fig. 2. The main compounds detected in all samples were ascorbic acid, *α*-glucose, *β*-glucose, fructose, choline, and malic acid. Others compounds as ethanol, alanine, trigonelline, 2-furoic and formic acid were detected in lower amounts. Trigonelline is an alkaloid produced by the metabolism of niacin (Vitamin B3), and 2-furoic acid is one of the degradation products of the Vitamin C (Ordóñez-Santos, Martínez-Girón, & Arias-

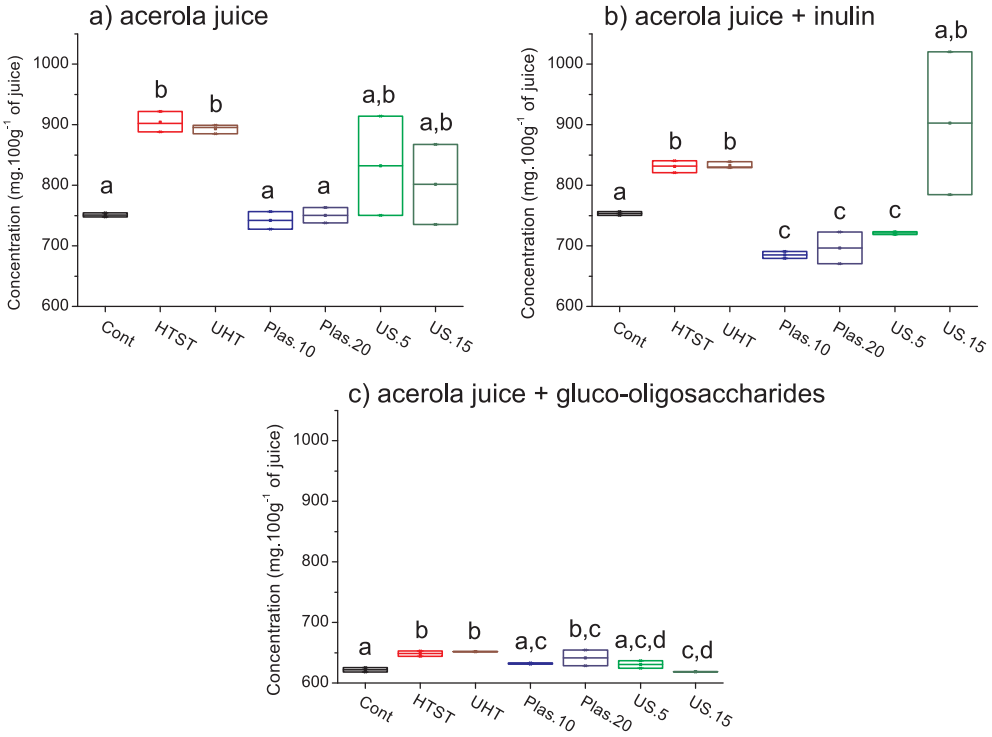


Fig. 1. Concentrations of Vitamin C (mg·100 g^{-1}) in acerola juice (a), acerola juice + inulin (b), and acerola juice + *gluco*-oligosaccharides (c) subject to different processing: Cont – control sample (non-processed); HTST – pasteurized sample; UHT – sterilized; Plas.10 – 10 min of plasma treatment; Plas.20 – 20 min of plasma treatment; US.5 – 5 min of ultrasound application; US.15 – 15 min of ultrasound application. The overwritten letters in concentrations values represent equality or difference between them.

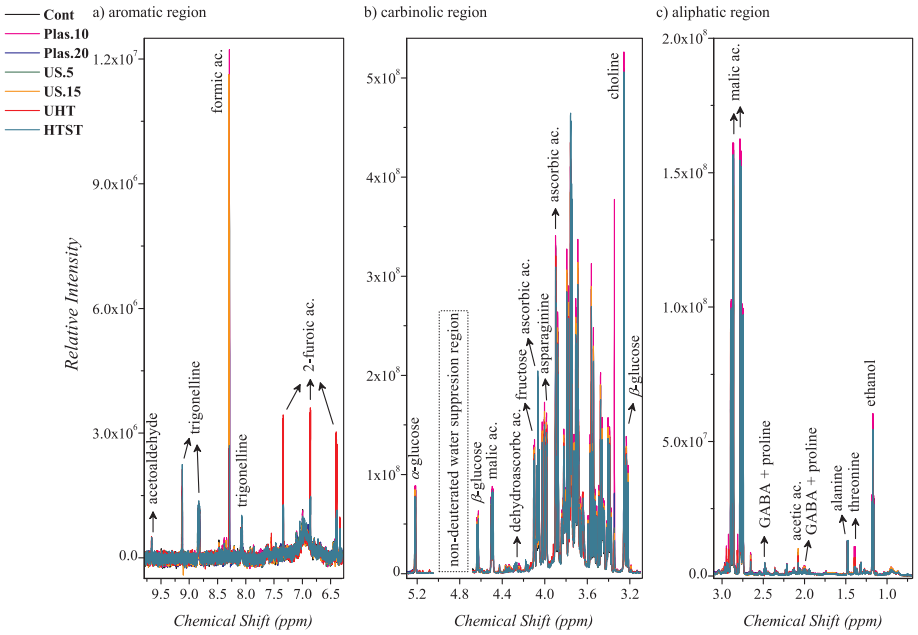


Fig. 2. a) ^{1}H NMR spectra with identified compounds in acerola juice subject to different processing: Cont – control sample; HTST – pasteurized; UHT – sterilized; Plas.10 – 10 min of plasma treatment; Plas.20 – 20 min of plasma treatment; US.5 – 5 min of ultrasound application; US.15 – 15 min of ultrasound application.

Jaramillo, 2017).

Due to the juices composition similarity and the high number of identified compounds (variables), chemometric evaluation using Principal Component Analysis (PCA) was applied to investigate the variability according to the different processing (Fig. 3). It is known that variations in pH among samples may induce the chemical shift scatter, mainly for organic acids signals. However, as a standard protocol for juice analysis developed by the group, EDTA was used during the samples preparation to minimize the ionic strength effect on frequency shifts in the NMR spectra (Alves Filho et al., 2016). Therefore, careful peak alignment procedure by COW (Correlation Optimized Warping) algorithm was preformed to precisely adjust the ^{1}H NMR signals alignment. Fig. 3a illustrates the scores graph plotted in two dimensions (PC1 × PC2) representing 75.32% of the total variance, and Fig. 3b presents the respective loadings plotted in lines. The PCA graphs of the acerola juice containing inulin and those containing *gluco-*

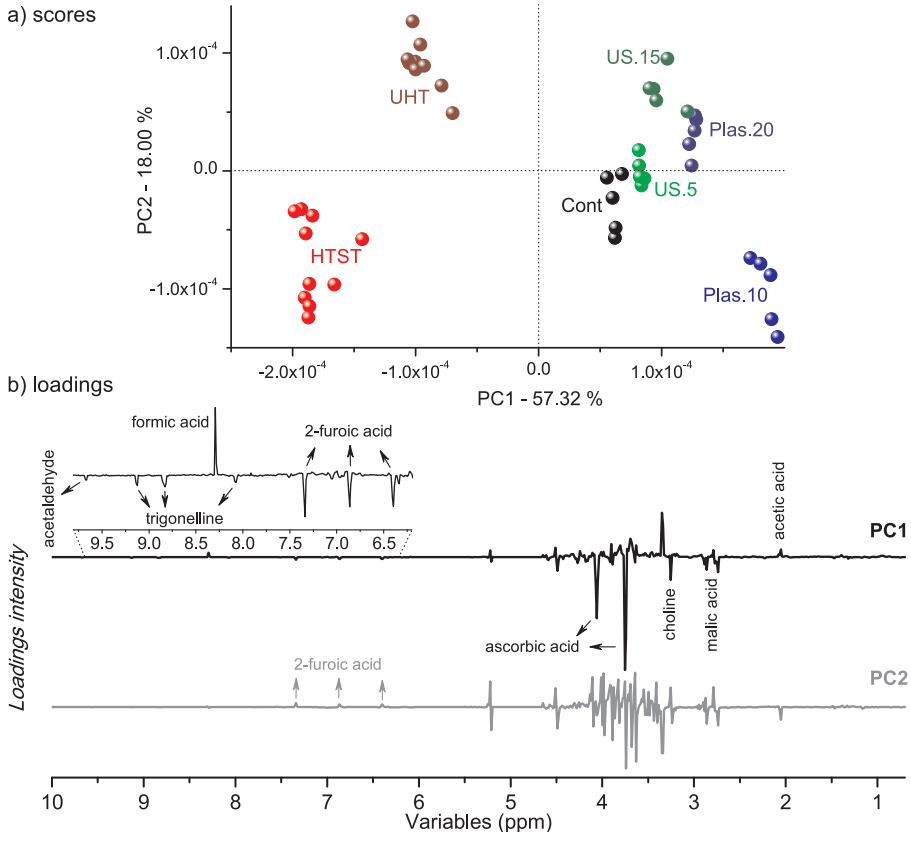


Fig. 3. PC1 × PC2 scores coordinate system (up – a), and loadings (bottom – b) of the pure acerola juices under different processing: Cont – control sample; HTST – pasteurized; UHT – sterilized; Plas.10 – 10 min of plasma treatment; Plas.20 – 20 min of plasma treatment; US.5 – 5 min of ultrasound application; US.15 – 15 min of ultrasound application.

oligosaccharides are available in the Supporting Information.

The acerola juice after thermal processing grouped at negative scores of PC1, and the juices after non-thermal processing clustered at positive scores of PC1 together with the unprocessed juice (control). Negative loadings of the PC1 axis mainly show the increase in the amount of Vitamin C in the juices after thermal processing as shown in the Vitamin C analysis by HPLC-UV. An increase in the Vitamin C concentration was observed in orange juice after thermal processing (Gil-Izquierdo et al., 2002). This increase was assigned to Vitamin C extraction from juice solids. Also, a recent study reported slight increases in the concentration of sucrose, glucose, fructose, and citric acid in processed orange juice using atmospheric cold plasma (Alves Filho et al., 2016). Therefore, the increase in the amount of Vitamin C may be due to its extraction from the suspended pulp. The negative values of PC1 also presented the influence of malic acid, choline, 2-furoic acid, trigonelline, and acetaldehyde loadings. The increase in the amount of furoic acid after thermal processing is related to the degradation of Vitamin C (Louarme & Billaud, 2012), which was represented by PC2 axis.

The PLS method was applied to maximize the separation of the groups previously presented in PCA. Concentrations of Vitamin C previously found using a well-established technique (HPLC-UV) were used as a categorical variable (Y column), since Vitamin C is considered as one of major indicator of nutritional losses (Aguilar, Garvín, Ibarz, & Augusto, 2017). The model was well adjusted (robust groupings) according to the statistical parameters: slope value close to 1; calibration and validation R^2 above 0.98; *SEC* to SEV ratio of 0.99. The model presented a prediction ability of 99.24% with two latent variables (LVs) informing how good a fit can be expected for future predictions.

The same chemometric protocol applied to evaluate the pure acerola juices was used to evaluate the effect of thermal and non-thermal processing in the composition of two types of prebiotic acerola juices (inulin, and *gluco*-oligosaccharides). The respective scores and loadings plots are available in the Supporting Information. The results showed the main clustering tendency of the thermal processing (HTST and UHT) in positive scores of PC2, and non-thermal processing with the control samples at negative scores for both prebiotic ingredients. Particularly for the samples with inulin, the ultrasound juice samples processed for 15 min grouped with the juices after thermal processing in positive scores of PC2. The PC2 loadings plotted for evaluation of the juice containing inulin presented a decrease in the amount of glucose after thermal processing and an increase of 2-furoic acid (a degradation product of ascorbic acid) mainly after UHT processing. The loadings plots for the juices with *gluco*-oligosaccharides only present a slight increase of 2-furoic acid after thermal processing.

Fig. 4 presents the concentrations of Vitamin C (^{1}H signal chosen for this analysis at δ 4.06), choline (δ 3.25), 2-furoic acid (δ 6.40), α-glucose (δ 5.22), β-glucose (δ 4.63), fructose (δ 4.10), malic acid (δ 2.76), alanine (δ 1.48), and ethanol (δ 1.17) with combined uncertainties based on the analytical errors and standard deviation from the quintuplicate of ^{1}H spectra acquisitions determined only for pure acerola juice, since the presence of the prebiotic compounds overlapped most of the signals at carbinolic region (δ 3.0 to 5.5). Although the β-glucose presents resonances near the pre-saturation region of the non-deuterated water (at δ 4.77) the evaluation of the saturation profile (around δ 4.50–5.10) did not show significant influence on these signals. Based on ANOVA single factor, the individual tendencies observed in quantitative evaluation corroborated the results.

The inter-method comparison between ^{1}H *q*NMR and HPLC-UV is a step forward toward Vitamin C quantification in acerola juices by assessing the data from different techniques or approaches. The quantification of Vitamin C using ^{1}H *q*NMR spectroscopy present advantages compared to HPLC-UV technique, where compounds separation is not completely reliable, and quantification requires the use of certified standard compounds. Quantification using ^{1}H *q*NMR also presents its drawbacks in the form of compounds with overlapped resonances. Additionally, HPLC-UV is commonly used as a standard technique for quantification, which requires the construction of calibration curves for each target compound and in the present work, was used to corroborate the NMR quantification. Therefore, both methods present similar results as can be observed at Table S2 of the Support Information file.

An additional NMR experiment was performed to confirm the increase of the concentration of Vitamin C due to thermal effect. The temperature of NMR analysis was increased to from 24 to 90 °C and maintained during 2 min to simulate the HTST processing. After that, the sample temperature was cooled to 24 °C. The spectra acquired before and after the increase of the temperature showed that the concentration of Vitamin C increased around 10% with the temperature increase. The extraction effect of Vitamin C in fruit juices containing suspended fruit pulp (solid particles) due to heat is an unusual result since thermal treatment is usually associated with Vitamin C degradation. In the present study, this behavior was observed by traditional HPLC analysis and confirmed by NMR analysis, and it is in agreement with the previous report for thermally treated orange juice (Gil-Izquierdo et al., 2002).

3.3. Volatile organic compounds variability

Due to the complexity of the GC-MSD data, the chemometric analysis by PCA was applied for evaluation of the influence of thermal and non-thermal processing on the composition of prebiotic and non-prebiotic acerola juice samples. Fig. 5a illustrates the scores graph plotted in two dimensions (PC1 × PC2) representing 83.01% of the total variance. Fig. 5b presents the respective loadings plotted in lines. The PCA graphs for the evaluation of prebiotic juices are available in Supporting Information. Based on loading graphs for prebiotic and non-prebiotic acerola juices, 13 volatile compounds were relevant for the discrimination of the juices (Table 1). The structures of the identified compounds with the respective retention time, retention index, structure, major *m/z* ratio, and percentage of match are available in Supporting Information. It is known that esters, alcohols, aldehydes, and ketones are the dominant classes of volatile compounds in acerola fruit and the amount of esters is an indicative of the maturity stage of the acerola fruit (increase during maturation) (Vendramini & Trugo, 2000). Some studies highlighted the importance of alcohols and esters on the fresh and fruity aroma of acerola (Bicas et al., 2011; Boulanger & Crouzet, 2001; Pino & Marbot, 2001). In the present study, the principal volatile compounds affected manly by thermal processing of the acerola juices were the alcohols 3-methyl 3-buten-1-ol, 3-hexen-1-ol, 1-hexanol, and the ester ethyl hexanoate.

According to the PC1 axis in the scores graph, the thermally processed juices clustered in negative scores, and non-thermal processed juices (ozone and plasma) grouped in positive scores with the unprocessed juice. The respective loadings plotted in lines (Fig. 5b) indicated esters and alcohols as the volatile organic compounds mainly affected by thermal processing. The juices placed in positive loadings of PC1 (non-thermal processing and control) presented the higher amounts of the main alcohols and esters in acerola juice composition. The negative loadings (thermal processing) are related to the higher amounts of some minor alcohols, esters, and aldehydes. The former may have come from thermal degradation of the major alcohols. The juices after thermal processing presented the highest amounts of hexanal, an aldehyde related to the partial oxidation of primary alcohols as 1-hexanol, which was one of the major volatile compounds in the acerola juice. Therefore, non-thermal processing effect on the volatile profile of the acerola juice was not significant. Also, the addition of the prebiotic compounds inulin and *gluco*-oligosaccharides synthesized in the juice decreased the effect of processing on esters and alcohols in the acerola juice composition compared to non-prebiotic juice (Supporting Information). This partial protection was independent of the processing.

Gas chromatography coupled to mass spectrometry (GC–MS) is

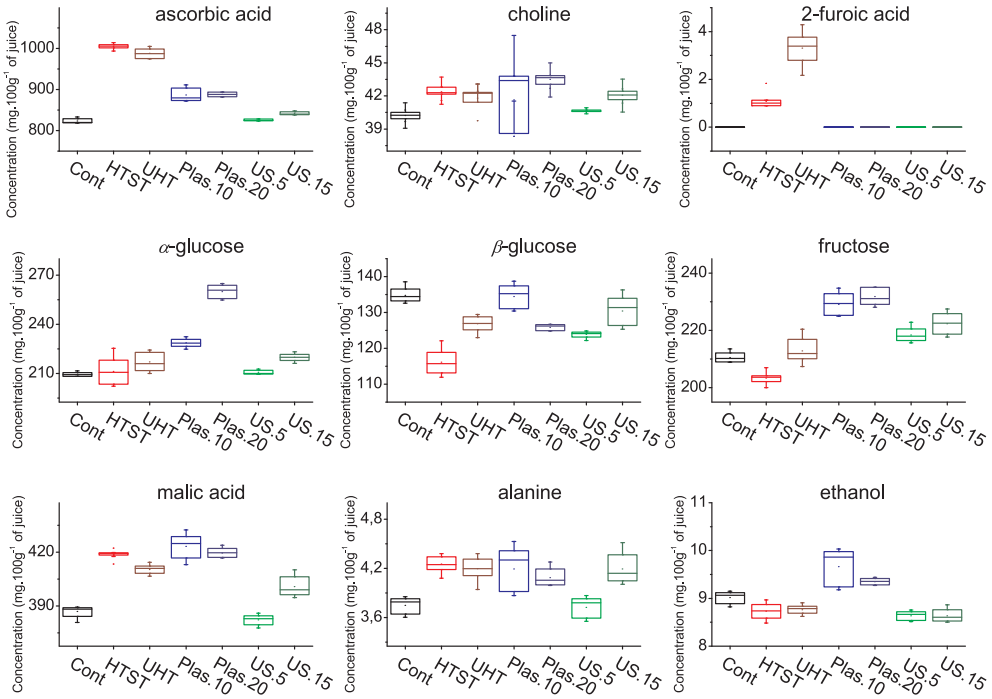


Fig. 4. Concentrations of ascorbic acid, choline, 2-furoic acid, *α* and *β*-glucose, fructose, malic acid, alanine, and ethanol in mg per 100 g of the acerola juices.

widely used to characterize volatile organic compounds in a food matrix, like fruit juices. However, the principal disadvantages reside in the long time required for the analysis (60–90 min) and the necessity of certified standards for quantitative analysis. The ^{1}H *q*NMR analysis allowed the simultaneous detection and quantification of organic compounds in a complex mixture without the use of certified standard compounds, presenting a high dynamic range. On the other hand, the quantification of minor and important compounds was not possible due to the presence of overlapping resonances. The NMR technique is less sensitive than GC–MS. However, this study demonstrated that the complementary use of ^{1}H *q*NMR and GC–MS allowed for a proper exploration of the composition of prebiotic and non-prebiotic acerola

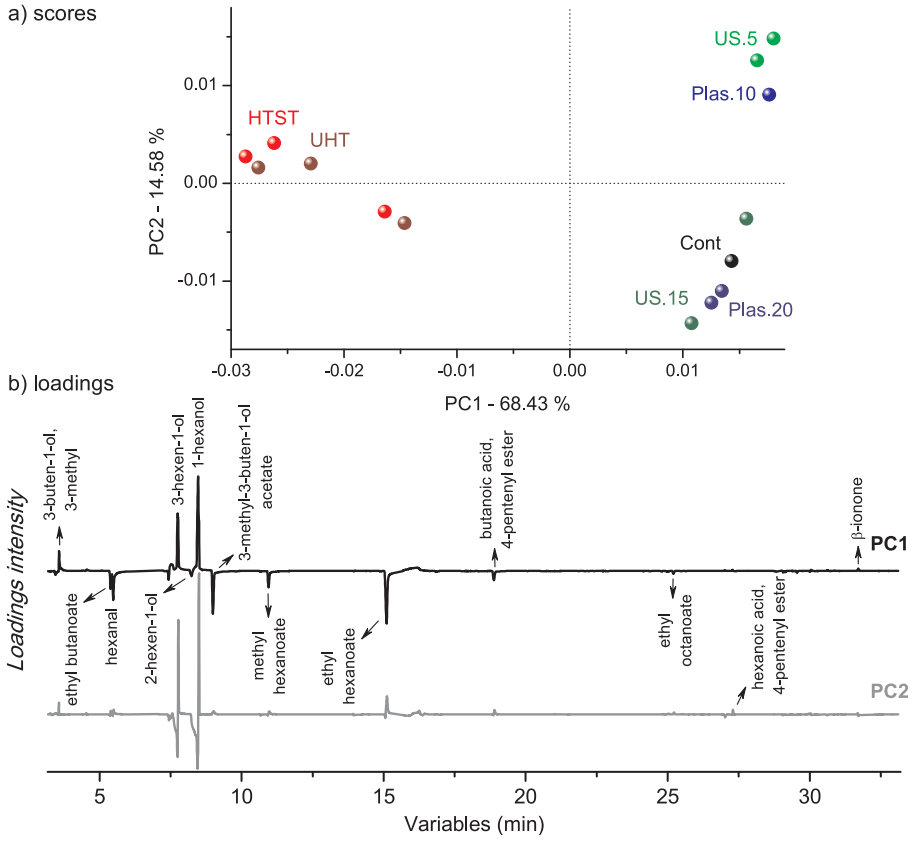
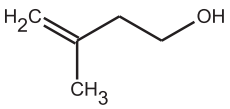
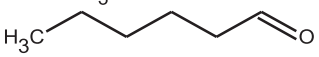
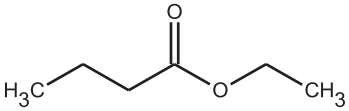
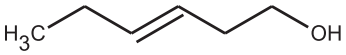
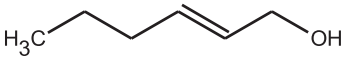
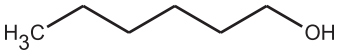
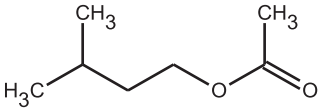
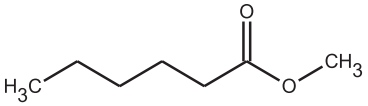
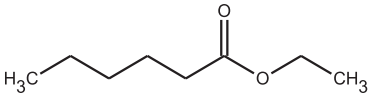
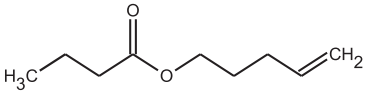
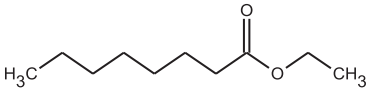
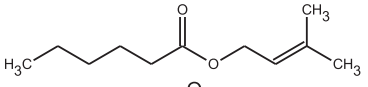
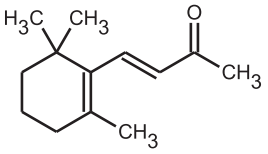


Fig. 5. PC1 × PC2 scores coordinate system (up – a), and loadings (bottom – b) of the acerola juices under different processing: Cont – control sample; HTST – pasteurization; UHT – sterilization; Plas.10 – 10 min of plasma treatment; Plas.20 – 20 min of plasma treatment; US.5 – 5 min of ultrasound application; US.15 – 15 min of ultrasound application.

Table 1
Structures of the relevant volatile flavor components detected in acerola juices according to PCA, with respective retention times (RT), experimental and reference retention index (RI), major *m/z* peak, and percentage (%) of match.

RT (min)	Compound name	Structure	RI* exp.	RI* refer.	Major *m/z*	Match (%)
3.577	3-methyl 3-buten-1-ol		727	723[a]	41	94
5.384	hexanal		801	801[a]	44	86
5.479	ethyl butanoate		804	802[a]	43	92
7.706	3-hexen-1-ol		856	850[a]	41	97
8.021	2-hexen-1-ol		864	859[a]	57	95
8.431	1-hexanol		873	871[a]	56	94
8.972	3-methyl-3-buten-1-ol, acetate		886	880[a]	43	95
10.957	methyl hexanoate		926	921[a]	74	94
15.086	ethyl hexanoate		1001	997[a]	88	95
18.897	butanoic acid, 4-pentenyl ester		1067	1064[b]	68	84
25.208	ethyl octanoate		1199	1196[a]	94	88
27.310	hexanoic acid, 3-methyl-2-butenyl ester		1268	1267[d]	88	68
31.670	β-ionone		1490	1487[a]	177	92

[c]Tentatively identified through the comparison with the retention index and information, reported by (Franco & Janzantti, 2005; Pino & Marbot, 2001)
* RI – Retention index: retention times using *n*-alkenes series (C_7-C_{30}) converted in independent constants.
[a] Retention index reported by NIST and (Adams, 2007).
[b] Retention index reported by (Haken, Madden, & Korhonen, 1985).
[d] Retention index reported at NIST.

juices after thermal and non-thermal processing.

4. Conclusion

Both thermal processing (HTST and UHT) had a pronounced effect on the juices composition compared to non-thermal processing considering the primary metabolites (NMR) and volatile profile (GC–MS). The chemometric results of the NMR data demonstrated that some of the organic compounds found in the processed juices, as carbohydrates and malic acid, are altered according to the type of processing – thermal or non-thermal. The both non-thermal processing did not present significant effect on acerola juice composition. However, the thermal processing presented positive effect on the Vitamin C content of the juices, which highlight that the degradation of Vitamin C might be mainly related to oxygen influence and storage period. This fact might point out how the thermal and non-thermal processing affects the composition of acerola juice.

The insertion of prebiotic compounds (inulin and *gluco*-oligosaccharides) decreased the effect of processing on acerola juice composition, mainly after thermal processing suggesting a protective effect by microencapsulation on aforementioned compounds. The chemometrics evaluation of the ^{1}H *q*NMR and GC–MS dataset was suitable to follow changes in acerola juice under different processing.

5. Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.foodchem.2018.05.038.

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