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Ozone and plasma processing effect on green coconut water

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ABSTRACT

In this study, the effect of plasma and ozone processing on the quality of coconut water was evaluated. For ozone processing, the samples were submitted to different ozone loads and temperatures. For atmospheric cold plasma processing (ACP), samples were exposed to plasma under different frequencies and voltages. The coconut water pH, soluble solids, titratable acidity, color, total phenolic content, and enzymatic activity were determined before and after treatments. The main compounds were also determined by NMR spectroscopy and chemometric analysis. Both processes did not change the pH values, total soluble solids, titratable acidity, and color. Chemometrics analysis of 1H NMR dataset showed no relevant changes after the processing. All ozone treatments promoted complete inactivation of POD activity and did not affect the content of phenolic compounds. After ACP, the smallest POD residual activity was observed when higher frequencies were applied, and slight changes in phenolic compounds content were observed.

1. Introduction

Coconut water is a light, refreshing, and natural drink, and its consumption around the world has increased because the consumers are looking for products with functional properties. A balanced composition of water, sugars, and minerals gives the drink pleasant sensory characteristics and health benefits such as hydration, electrolyte replacement, and digestive problems amelioration (Augusto, Ibarz, Garvín, & Ibarz, 2015; Tan, Cheng, Bhat, Rusul, & Easa, 2014). After extraction, coconut water becomes susceptible to microbiological degradation and oxidation reactions promoted by peroxidases (POD) and polyphenol oxidases (PPO), whose primary function is to oxidize phenolic compounds, leading to negative flavor changes during storage, besides the occurrence of yellow, brown or pink color (Prades, Dornier, Diop, & Pain, 2012; Sanganamoni, Mallesh, Vandana, & Srinivasa Rao, 2017; Surowsky, Fischer, Schlueter, & Knorr, 2013).

Thermal treatments are important conservation methods used to ensure the stability of coconut water, but these processes promote significant sensory and nutritional losses. Moreover, they do not always inhibit the action of enzymes irreversibly. For example, UHT processing did not prevent the appearance of pink color in coconut water (Sucupira et al., 2017). Alternatively, non-thermal technologies represent a promising change in the scenario of food processing. Advances in research related to these technologies have demonstrated the ability to extend the shelf life of food products and obtain better nutrient retention and sensory attributes when compared to thermal methods (Martín-Belloso, Soliva-Fortuny, Elez-Martínez, Robert Marsellés-Fontanet, & Vega-Mercado, 2014). These technologies include the use of ozone and atmospheric cold plasma.

Ozone is a colorless gas with a pungent odor, and it stands out for its high oxidizing power, being a strong sanitizing agent with action on a wide variety of organisms, including bacteria, viruses, and protozoa (Pandiselvam, Sunoj, Manikantan, Kothakota, & Hebbar, 2017). Ozonation may be a promising alternative as it is a relatively simple, fast, and inexpensive application technology. Recognition of ozone as a GRAS substance (Generally Recognized as Safe) in 1997, followed by FDA approval as an antimicrobial agent for direct food use in 2001, expanded the applications of ozone in the food industry (Prabha, Barma, Singh, & Madan, 2016). Bubble column reactors are widely used in industrial-scale for ozone treatment of water and wastewater and may also be an option for liquid food ozonation (Cullen, Tiwari, O'Donnell, & Muthukumarappan, 2009).

Several studies have been carried out on ozone treatment of fruit juices such as apple juice (Torlak, 2014), peach juice (Jaramillo-

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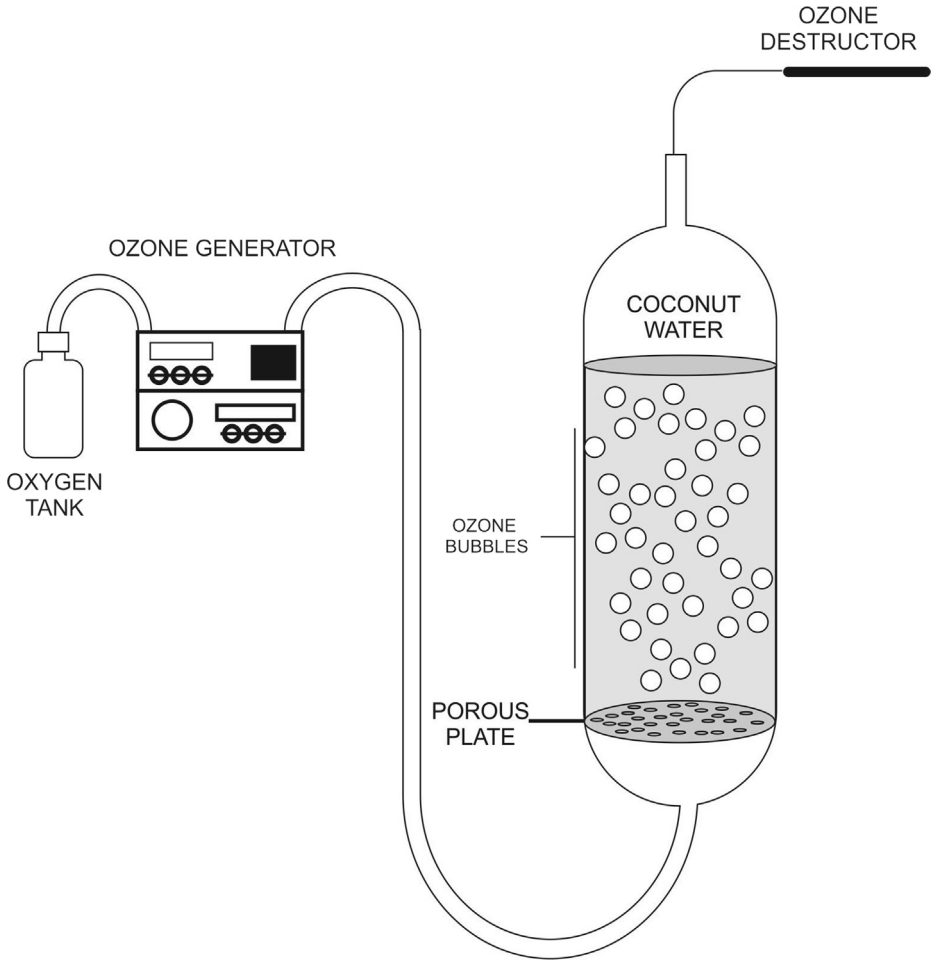

Fig. 1. Operating scheme of ozone processing.

Sánchez, Garcia Loredo, Gómez, & Alzamora, 2017), *Cantaloupe* melon juice (Fundo et al., 2018) and orange juice (Almeida et al., 2015), in order to inactivate or reduce the microbial growth and enzymes activity, and evaluate the effects on quality parameters and bioactive compounds.

Plasma processing is another promising non-thermal technology that consists of the fourth state of matter, which is an ionized gas containing an array of reactive species, such as electrons, free radicals, ions, among others. Studies report the efficiency of cold plasma on inactivation of microorganisms in vegetable food matrices as well as positive results on maintaining quality attributes and nutritional value (Mahnot, Mahanta, Farkas, Keener, & Misra, 2019; Rodríguez, Gomes, Rodrigues, & Fernandes, 2017; Hou et al., 2019). Cold plasma also has been reported to inactivate a range of enzymes such as pectin-methylesterase (PME), peroxidase (POD), and polyphenoloxidase (PPO) (Chutia, Kalita, Mahanta, Ojah, & Choudhury, 2019; Tappi et al., 2014, 2016) in vegetable foods.

Atmospheric cold plasma (ACP) induced by dielectric barrier discharge (DBD) has a high potential for industrial applications. Aside from the use of air and atmospheric pressure, ACP presents advantages such as ease discharge ignition, adaptability to a range of products and matrices, and the possibility of food processing inside the package. The scale-up of the process is still a challenge, and the technology use industrial-scale still needs approval. However, the studies of pilot-scale ACP-DBD systems have demonstrated that larger-scale operation is possible (Ziuzina et al., 2020).

Due to the possible changes in the composition of food matrices that may occur during processing, Nuclear Magnetic Resonance (NMR), a powerful tool for chemical analysis, has been applied to evaluate food processing changes, because it enables the study of chemical composition and molecular dynamics of solid, liquid and semisolid food (Sobolev et al., 2019). Novel applications of NMR in food systems include evaluation of the effect of different preservation processing methods (Alves Filho et al., 2018a) and detection of possible adulteration (Richardson et al., 2019). However, NMR data might generate highly complex matrices with a considerable inherent spectral similarity (Spraul, Schütz, Rinke, et al., 2009). Then, multivariate statistical methods of analysis, as chemometrics, are often necessary (Tang, Vasas, Hatzakis, & Spyros, 2019). In this context, this work evaluated the effect of the atmospheric cold plasma and ozone processing on coconut water composition (by NMR spectroscopy and chemometrics analysis), physio-chemicals parameters and peroxidase inactivation.

2. Materials and methods

2.1. Raw material

Coconut water samples were extracted from green coconuts (*Cocus nucifera*, L.) at the 7th maturation month. Coconuts were sanitized by immersion in chlorinated water (200 ppm of chlorine) for 20 min. The extraction was done manually with the aid of a sterile stainless-steel knife. After extraction, the samples were homogenized and then filtered on standard filter paper. The filtered coconut water was packaged in nylon-polyethylene bags (150 mL portions), vacuum-sealed, and stored at −18 °C.

2.2. Non-thermal processing

Coconut water samples were submitted to two different non-thermal processing: ozone and atmospheric cold plasma (ACP), as described below.

2.2.1. Ozone processing

For the ozone processing, portions containing 100 mL of coconut water were processed into a glass column reactor with 4.5 cm of diameter and 30 cm high. The reactor was built with a gas distributor plate at the reactor bottom with the same diameter of the reactor to improve the gas-liquid mass transfer. The distribution plate was made of sintered porous glass (pore size of 40–50 μm), allowing a uniform distribution of tiny ozone bubbles, which improves the gas-liquid contact and enhances de gas transfer to the liquid phase. The reactor (Fig. 1) was coupled to a portable Model O & L15 Ozone generator (OzoneLife, Sao Jose dos Campos, Sao Paulo, Brazil), which was fed by an oxygen tank with a flow-rate of 125 mL/min. The ozone dosage provided by the generator was set at 29.5 mg/L. The ozone charge applied to the sample ranged from 0.075 to 0.37 mg/mL, obtained changing the processing time. The temperature ranged from 10 to 30 °C, kept by an insulation jacket. Both parameters changed according to a faced centered central composite experimental design (2^3) with 3 repetitions at the central point. Initially, control coconut water samples were thermostated at three different temperatures (10, 20, and 30 °C, named as C10, C20, and C30, respectively) to evaluate the influence of temperature on the enzyme activity. The ozone dissolved in the sample at room temperature (25 ± 2 °C) was quantified by measuring the inlet and outlet ozone concentration according to the iodometric titration method (Rakness et al., 1996).

2.2.2. Atmospheric cold plasma processing

For Atmospheric Cold Plasma (ACP) processing, an Inergiae plasma generator model PLS0130 was used (Inergiae, Florianópolis-SC-Brazil). The system consisted of the generator coupled to a chamber containing two aluminum discs (8 cm diameter) separated at a distance of 15 mm (Fig. 2). Two acrylic sheets (5 mm thick) were used as a dielectric barrier. Aliquots of 20 mL of coconut water were placed in a petri dish, which was closed and subjected to direct plasma exposure. The frequencies applied were 200, 400, 550, and 730 Hz and voltages 15 and 20 kV (Table 1). The processing time was 15 min for all treatments.

2.3. ^{1}H qNMR spectroscopy

The preparation of coconut water for NMR screening was developed following a previous study in order simplify/reduce sample pretreatments preventing the main compounds from degradation (Sucupira et al., 2017). Therefore, an aliquot of 165 μL was directly mixed with 400 μL D_2O (99.9%) and 35 μL D_2O containing 14 mM of EDTA and 1% of sodium-3-trimethylsilyl propionate (TMSP-d_4), and inserted into 5 mm NMR tubes. The 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). The NMR analysis 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 triplicate using the PRESAT pulse sequence for water suppression at δ 4.86 under quantitative conditions (Alves Filho et al., 2018b; Spraul, Schütz, Humpfer, et al., 2009). The inversion recovery pulse sequence was used after 90° pulse calibration (8.10 μs pulse length at 58 dB of power), and probe properly tuned and matched before each sample evaluation to ensure complete relaxation of all nuclei of the samples. It was used a relaxation delay of 30.0 s and an acquisition time of 5.0 s to ensure the full relaxation of all hydrogen present in the samples, with 32 scans, 95 k of time-domain points into a spectral window of 16.0 ppm. The receiver gain value was fixed for all the acquisitions to ensure the same acquisition intensity of the ^{1}H NMR signals among the spectra. 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 to 16 k points. Phase correction was manually performed, and the automatic baseline correction was applied over the entire spectral range.

The identification of the constituents 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., 2013), and literature reports (Richardson et al., 2019; Sucupira et al., 2017). The 2D-NMR data acquisition and processing are available in the Supporting Information.

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

The chemometric analysis was performed to investigate the effect of

Table 1
Frequency and voltage applied in plasma treatment.

Treatments	Frequency (Hz)	Voltage (kV)
Control	0	0
P1	200	15
P2	200	20
P3	400	15
P4	400	20
P5	550	15
P6	550	20
P7	730	15
P8	730	20

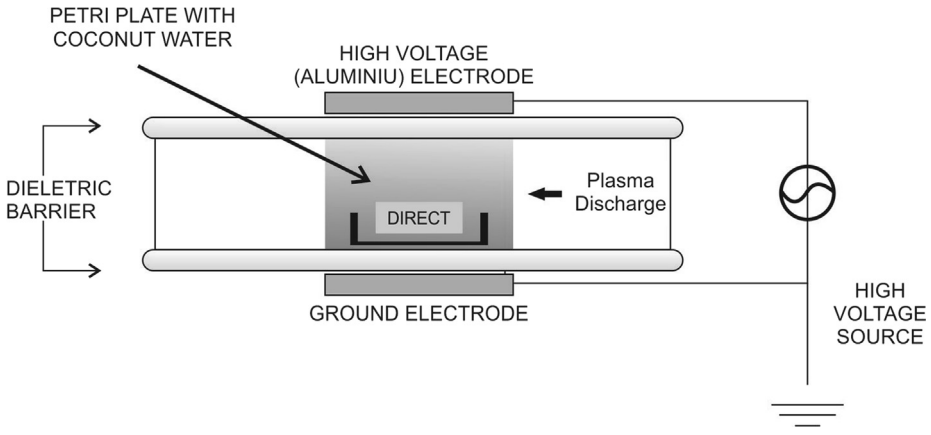

Fig. 2. Operating scheme of cold plasma processing.

different non-thermal processing on coconut water composition. A numerical matrix containing 60 ^{1}H NMR spectra was created using the data acquired in triplicate from 20 different samples. The NMR spectra were converted to American Standard Code for Information Interchange (ASCII) files for numerical matrix construction. To reduce the original data dimensionality and to observe composition trends under a confidence level of 95%, the correspondent matrix was exported for unsupervised chemometric analysis by Principal Component Analysis (PCA) using PLS-Toolbox™ program (version 8.6.2, Eigenvector Research Incorporated, Manson, WA USA). The spectral area influenced by the non-deuterated water suppression based on saturation profiling (δ 4.67–5.06) was excluded. Before the application of the Singular Value Decomposition (SVD) to decompose the matrix in scores, loadings, errors with samples influence on modeling, algorithms for baseline correction, alignment using the Correlation Optimized Warping (COW) with segment length of 50 and slack of 5, and normalization were applied over the variables (spectra signals). The PCA evaluation was carried out after the mean-centered processing over the samples, and the relevant information from the chemical data was obtained using the first two principal components (2 PC).

2.3.2. Quantification by ^{1}H NMR

As the ^{1}H NMR spectra of coconut water were acquired under quantitative conditions (^{1}H *q*NMR) (Spraul, Schütz, Humpfer, et al., 2009), the main organic compounds with no overlapping signals were quantified by the external reference method provided by VnmJ™ program (version 4.2, Agilent). The results were evaluated by the analysis of variance ANOVA single factor using the Statistica 13 (Statsoft) program to statistically certify the differences in the concentrations at the significance level of 0.05 using the Tukey test. The combined uncertainty was based on analytical errors and standard deviation from the triplicate of the spectra acquisition.

2.4. UPLC-HRMS spectrometry analysis

Before the analysis, coconut water samples were previously treated by solid-phase extraction using C18 cartridges to concentrate the phenolic compounds and remove interferents (sugars and amino acids). The analytes of interest were eluted from the SPE cartridge with methanol, which was removed by vacuum concentration (speed-vacuum system). The resulting material was dissolved in water/methanol (1:1v/v) at 1 mg·mL^{-1} concentration and filtered through Millipore™ Teflon membranes (0.22 μm of pore diameter). The ultra-performance liquid chromatography untargeted mixed-mode tandem mass spectrometry (UPLC-HRMS) analysis was performed on an Acquity™ UPLC system (Waters Co., USA) coupled with a quadrupole/TOF system (Waters, USA). A Waters Acquity™ UPLC BEH column (150 × 2.1 mm, 1.7 μm) was used with the temperature set at 40 °C. Mobile phases were water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). The gradient used consisted: 0–15.0 min, 2–95% B; 15.0–17.0 min, 100% B; (17.0–19.0) min, 2% B. The flow rate was 0.400 mL·min^{-1}, and the injection volume of sample was 5.0 μL. Analysis used electrospray ionization (ESI) interface in negative and positive ion modes acquired from 110 to 1180 Da. Source temperature was 120 °C, desolvation temperature was 350 °C, and the desolvation gas flow of 500 L·h^{-1}. Leucine enkephalin was used as lock mass. The acquisition mode was MS^{E} and the instrument controlled by MassLynx 4.1 software (Waters Co., USA). Analyzes were developed in triplicate.

2.4.1. Chemometric analysis of UPLC-HRMS dataset

In order to investigate the effect of non-thermal processing on organic compounds from coconut water, an unsupervised chemometric method by Principal Component Analysis (PCA) was applied on UPLC-HRMS dataset acquired in negative ionization mode. Only relevant processing from NMR analysis was selected to be analyzed by UPLC-HRMS. As developed to ^{1}H NMR spectra analysis, the chromatograms region between 2.0 and 8.0 min were converted to ASCII files and imported by Origin™ 9.4 software, which resulted in a matrix with dimensionalities of 30,105 (45 samples × 669 variables). The PLS Toolbox™ software was used to handle the multivariate data. Pretreatments on variables (retention times) were performed by baseline correction (linear fit algorithm), smooth to improve the signal shape, and normalization. After that, samples were mean-centered since this pretreatment provided better differences between the coconut water processing, with 95% of confidence level.

2.5. Physico-chemical analyses

Analyzes were developed in triplicate. The results were evaluated by the analysis of variance ANOVA and Tukey test at a significance level of 0.05 using the software Statistica 13 (Statsoft).

2.5.1. Enzymatic activity of polyphenol oxidase (PPO) and peroxidase (POD)

The PPO enzymatic activity was measured according to the method described by Wissemann and Lee (1980) with some modifications. An aliquot of 300 μL of coconut water of was added to 1000 μL of 0.1 M potassium phosphate buffer solution (pH 6.0) containing catechol (0.1 M). The reaction was carried out at room temperature (25 ± 2 °C). Absorbance was measured with a spectrophotometer (Thermus Evolution) at 425 nm every 15 s for 30 min. The analysis was performed in triplicate for each sample, and the enzymatic activity was calculated according to Eq. (1), where k is the slope of the line obtained through the absorbance *versus* time plot, and DF is the coconut water dilution factor in the reaction (DF = 4). PPO activity was expressed as enzyme activity unit (U/mL). One enzyme activity unit is the amount of enzyme that causes a change of 0.001 in the absorbance per minute.

$$PPO\ \ Activity = \frac{k}{0.001} * DF \tag{1}$$

The POD enzymatic activity was performed according to the method of Matsuno and Uritani (1972) with some modifications. The 1% (v / v) guaiacol substrate was added directly to the 0.1 M phosphate-citrate buffer solution (pH 5). In a glass tube were added: 825 μL phosphate-citrate buffer containing 1% guaiacol, 75 μL 3% (v/v) hydrogen peroxide and 450 μL of the coconut water sample. The reaction was carried out at room temperature (25 ± 2 °C). Absorbance was measured with a spectrophotometer (Thermus Evolution) at 470 nm every 15 s for 5 min. The analysis was performed in triplicate for each sample, and the enzymatic activity was calculated according to Eq. (2), where *k* is the slope of the line obtained through the absorbance *versus* time plot, and DF is the coconut water dilution factor in the reaction (DF = 3). POD activity was expressed as enzyme activity unit (U/mL). One enzyme activity unit is the amount of enzyme that causes a change of 0.001 in the absorbance per minute.

$$\text{POD}\ \ \text{Activity} = \frac{\text{k}}{0.001} * \text{DF} \tag{2}$$

The residual enzyme activity after treatments was calculated according to Eq. (3).

$$RA(\%) = \frac{Ap}{Ac} * 100 \tag{3}$$

where Ap means the POD activity after each treatment and Ac means the POD activity of control sample.

2.5.2. pH, total soluble solids (TSS) and titratable acidity (TA)

The pH was determined by direct reading in a Marconi potentiometer, model PA 200, calibrated at each use with pH 4.0 and pH 7.0 buffer solutions. Total soluble solids (°Brix) readings were taken by refractometry using a HANNA HI 96801 portable digital refractometer. The apparatus was calibrated with distilled water and measurements

taken at room temperature (25 ± 2 °C). The total titratable acidity was determined by the titration method and the results expressed in g of malic acid/100 mL of the sample.

2.5.3. Color

The color was determined using a Minolta CR300 colorimeter with a protective CAP (Tokyo, Japan). The colorimeter was calibrated using a white calibration plate CR (L = 85.60, a* = 0.36, b* = −2.45) and determined the color parameters: L*, a* and b*. The color was measured, placing 15 mL of the sample in an open, transparent glass petri dish (90 cm of diameter). Five readings of each sample were done against a white background (Fonteles et al., 2013). The differences in color (ΔE) and hue angle (h°) between treated and control samples were calculated according to Eqs. (4) and (5), respectively.

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (4)$$

$$h^\circ = tan^{-1}(b^*/a^*) \quad (5)$$

3. Results and discussion

3.1. Ozone absorbed in the coconut water

As expected, less ozone was absorbed by the sample than provided by the ozone generator. By measuring the inlet and outlet ozone concentration in the glass reactor, we verified that the samples that received the loads 0.075; 0.225 and 0.370 mg/mL absorbed 0.038; 0.140 and 0.210 mg/mL respectively, which corresponds to more than 50% of the ozone provided by the equipment. Besides the ozone solubility in the food matrix, the ozone absorbed by the liquid phase depends on the inlet gas flowrate, ozone concentration in the inlet gas, gas bubble size and the bubble distribution along the reactor. The glass reactor was built with a distributor plate able to produce small bubbles to enhance the gas-liquid mass transfer. The high amount of ozone absorbed by the sample (more than 50% of the ozone loaded) showed that the reactor system used was efficient for ozone processing.

3.2. NMR spectroscopy and molecular identification

To prevent composition variations on coconut water related to samples pretreatment before the analysis (as degradation compounds), a standard protocol for NMR analysis of fruit juices and correlated products was followed to make the samples composition as close as possible of the real (Alves Filho et al., 2018a, 2018b; Sucupira et al., 2017). Fig. 3 illustrates the identified organic compounds in ^{1}H NMR spectra from control coconut water (non-processed sample), where sugars (sucrose, α-glucose, β-glucose, and fructose) was the main class of compound as expected (Sucupira et al., 2017). Other relevant compounds, such as the amino acids alanine, GABA, leucine, proline, and valine, as well as the malic acid and ethanol, were also identified. The molecular structures, ^{1}H and ^{13}C chemical shifts, multiplicity, and constant coupling are described in Table S1 in Supplementary Material.

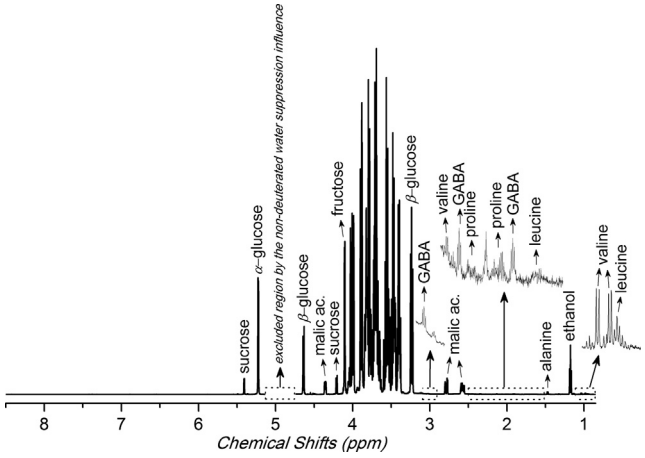

Fig. 3. Representative ^{1}H NMR spectrum from control coconut water.

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

Due to the high number of different non-thermal processing by ozone and plasma, an unsupervised chemometric method by PCA was applied on ^{1}H NMR dataset. This multivariate statistical analysis was applied to investigate the effect of the processing on sample composition. As no compound was detected in ^{1}H NMR aromatic region (between 5.50 and δ 8.50), PCA was developed only for aliphatic (0.85 and δ 3.15) and carbinolic (3.15 and δ 5.50) regions, separately. Fig. 4a and c illustrate scores graphs plotted in two dimensions (PC1 × PC2) from aliphatic and carbinolic regions, respectively, which retained 51.04% and 62.86% of the total variance. Fig. 4b and d present the PC1 loadings plotted as lines since it was the main axis for sample separation based on the coconut water non-thermal processing.

The scores plots from aliphatic (a) and carbinolic (c) regions illustrated the coconut water separation tendency between the ozone (red) and plasma (blue) processing. However, no relevant effect of both non-thermal processing was verified based on the respective loadings independent of the parameters of the treatment (duration and power), since the ^{1}H NMR signals alignment were carefully verified before the multivariate analyses. This no relevant effect was corroborated by the analysis of variance ANOVA single factor of the ^{1}H *q*NMR results of sucrose (referent to the hydrogen chemical shift at δ 5.42), α-glucose (δ 5.21), β-glucose (δ 4.63), fructose (δ 4.10), malic acid (δ 2.76), alanine (δ 1.48), ethanol (δ 1.17), and valine (δ 1.01) (Fig. S1 available in Supplementary Material). Although the β-glucose presented resonance near the suppression region of the non-deuterated water (at δ 4.86) the evaluation of its saturation profile (around δ 4.67 to 5.06) did not show significant influence on the β-glucose signal.

3.3. Organic compounds variability assayed by UPLC-HRMS

The most relevant ozone and plasma processing based on NMR results were selected to be analyzed by UPLC-HRMS system - to evaluate the effect of different non-thermal processing on phenolic compounds from coconut water. Therefore, the PCA method was applied to reduce the dimensionality of the original data in two PC, assisting the interpretation of the multivariate data. Fig. 5 illustrates the scores (a) and loadings (b) from coconut water samples variation according to the non-thermal processing. The respective spectrometric parameters for compounds characterization (*m/z* values and fragmentation profiles) are available in Supplementary Material. The main compounds identified were dimer and trimer procyanidin, caffeoylshikimic and trihydroxy-octadecanoic acids, *trans*-zeatin riboside, and chrysoeriol.

According to the scores, the discrimination of the samples based on non-thermal processing occurred mainly according to PC1 axis. Coconut water samples after ozone processing (independent of the ozone charge) clustered with control samples at positive scores due to the higher amounts of procyanidin dimer, procyanidin dimer (type B), chrysoeriol hexoside I, and an unknown compound at 5.92 min. The PCA indicates that ozone processing was effective in maintaining the contents of coconut water phenolic compounds, preserving the quality of the product - a positive aspect because several studies showed that ozone is associated with deleterious effects on quality characteristics.

The plasma processed coconut water clustered at negative scores of PC1 due to the reduction of the amounts of the compounds above and the increase of *trans*-zeatin riboside, caffeoylshikimic acid, procyanidin dimer (type A) content. The application of cold plasma can promote the increase or decrease of phenolic compounds, depending on the food matrix and other processing conditions. In some cases, the reduction or increase may be related to the interaction of these compounds with the

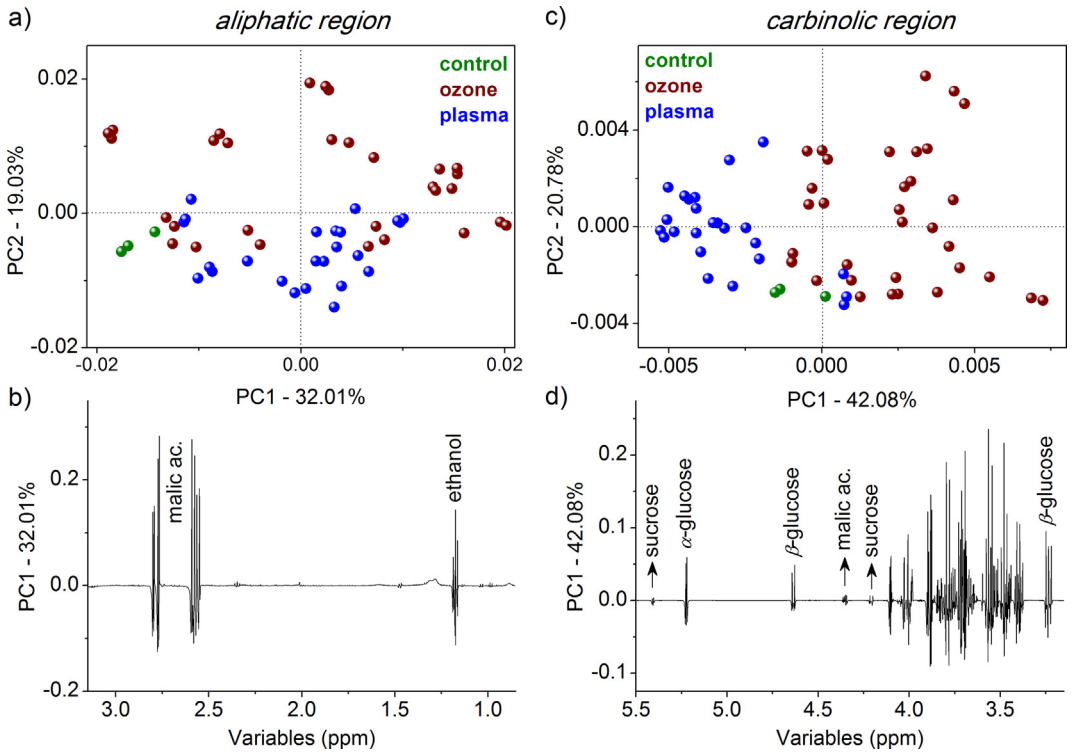

Fig. 4. (a) PC1 × PC2 *scores* coordinate system of the control coconut water (no processed in green), and those after non-thermal processing by plasma (blue) and ozone (red); (b) respective *loadings*. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

reactive species formed during the process, which can promote the molecule linkage disruption or induce the formation of new compounds. The increase of phenolic compounds after cols plasma processing was also reported for pomegranate (Herceg et al., 2016), cashew apple juice (Rodríguez et al., 2017) and cloudy apple juice (Illera et al., 2019).

The results of our study highlighted the lower influence of ozone on organic compounds compared to plasma processing. Also, a particular behavior was detected in samples composition after plasma processing under 200 Hz and 20 kV at positive scores of PC2 due to the increase in amounts of the *trans*-zeatin riboside, procyanidin dimer, procyanidin dimer (type B), chrysoeriol hexoside I, and trihydroxy-octadecanoic acid.

It is important to emphasize that cytokinin is a phytohormone found in coconut water (Kobayashi et al., 1995) and coconut milk (Staden & Drewes, 1975), and this compound is involved in the regulation of plant

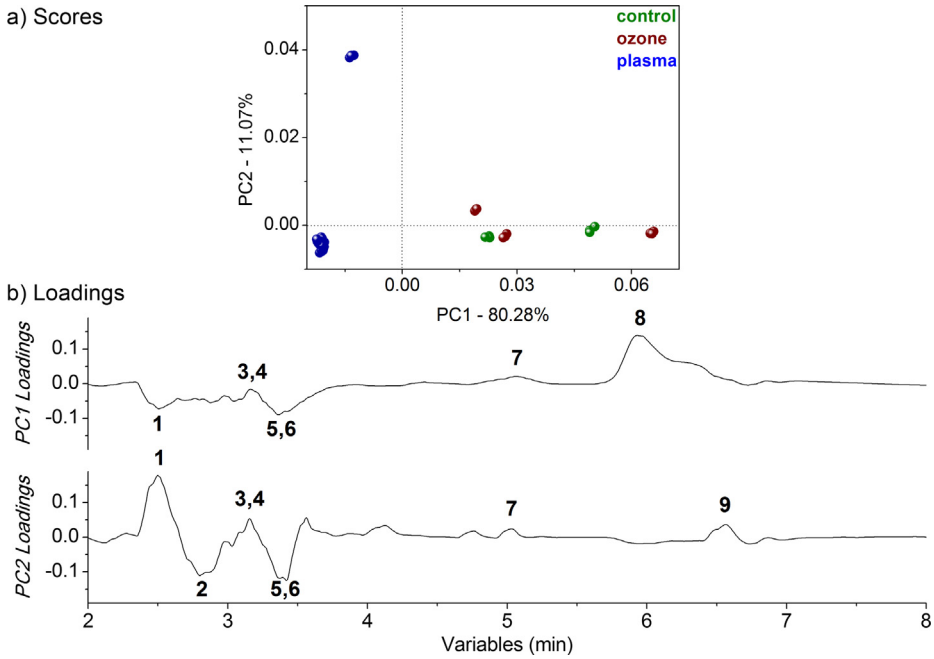

Fig. 5. Two-dimensional space plot for PC1 × PC2 scores coordinate system of the coconut water under negative ionization mode (a) and relevant loading plotted in lines form (b).

growth. The trimer and dimer (type A) of procyanidin may be found in inflorescence of *Cocos nucifera* L. (Padumadasa, Dharmadana, & Am, 2016). Proanthocyanidin is a known flavonoids class that promote human-health benefits due to antioxidant, anti-inflammatory, and anti-atherogenic properties (Chen, Montanari, & Widmer, 1997). The caffeoylshikimic acid is a compound derived from caffeic acid, which intermediates the polymeric phenolics synthesis in plant cells, which was detected in young coconut mesocarp and has been associated to the plant defense due to its antioxidant and antimicrobial properties (Chakraborty & Mitra, 2008). The trihydroxy-octadecenoic acid is an oxidized fatty acid found in fresh food (García, García-Villalba, Gil, & Tomas-Barberan, 2017), and the *trans*-zeatin riboside naturally-occurring phytohormone from coconut (Ge, Yong, Tan, Yang, & Ong, 2004; Kobayashi et al., 1995).

The contents of sucrose, α-glucose, β-glucose, fructose, malic acid, alanine, ethanol and valine (determined by NMR) are presented in Fig. S1 in the Supplementary Material. Fructose and α-glucose presented the higher sugar content in coconut water, while sucrose presented the lowest concentration. The compounds mentioned above presented small changes. Those changes are due to the handling of the sample and not a real processing effect since they present the same intensity and the same pattern for a compound.

3.4. Physicochemical analyses: pH, total soluble solids (TSS), titratable acidity (TA), and color

Supplementary Material presents the values of the parameters pH, TSS, and TA from coconut water before and after ozone and ACP processing. All the statistical analyses presented in the Supplementary Material and commented here were done by Tukey test at a significant level of 0.05 as previously mentioned in the Materials and Methods section.

Ozone processing did not change the pH and acidity of the samples significantly. The slight variations observed in TSS also did not characterize relevant changes. Similar behavior has been reported for ozone processing of prebiotic orange juice (Almeida et al., 2015), açai juice (Oliveira et al., 2018), and peach juice (Jaramillo-Sánchez et al., 2017). Analogously to ozone processing, the pH and TSS values after ACP did not differ significantly compared to the non-processed coconut water (control sample). This result was corroborated by Mahnot, Mahanta, Keener, and Misra (2019) that showed the physicochemical parameters of coconut water did not have any drastic change after plasma treatment. Pankaj, Wan, Colonna, and Keener (2017) also reported no changes in the pH of white grape juice processed by ACP. However, Almeida et al. (2015) reported a small decrease in pH values of prebiotic orange juice after plasma processing.

Color parameters as L*, a*, b*, c* and h°, as well as the total color differences (ΔE), were measured to evaluate the non-thermal processing effect since the ΔE values allows the global evaluation of the differences between the processed and non-processed samples (the data is available in Supplementary material). Slight variations were detected in the individual color parameters, and ACP processing showed the smallest ΔE compared to ozone processing. According to Choi, Kim, and Lee (2002), differences in color parameters are visually noticeable when $\Delta E > 2$. In general, although there is a slight color difference related to the non-processed coconut water, ozone and ACP processing presented $\Delta E < 2$, which is an indication of no noticeable changes and consequently no visual quality loss. These values are lower than those reported for other processing applied in coconut water such as pressure-assisted thermal processing (Chourio, Salais-Fierro, Mehmood, Martinez-Monteagudo, & Saldaña, 2018a) and high-pressure carbon dioxide (Cappelletti et al., 2015). The hue angle, which represents the product characteristic color, was also maintained with small variations.

In the present study, the non-thermal treatments applied (ACP and ozone) were able to maintain the physicochemical parameters of the coconut water. The changes in coconut water observed after ozone and plasma evidence that ozone and ACP did not impart perceptible changes on the product's overall quality. The main concern on coconut water processing is the color change, which is observed after thermal processing. In the present study, the color was preserved for both treatments, with imperceptible changes to the human eye.

3.5. Enzymatic activity

No measurable amounts of PPO enzyme were found in control and treated samples. Thus, PPO results were not shown. To evaluate the influence of the processing temperature on enzyme activity, the non-processed coconut water (control samples) was incubated at three different temperatures (10, 20, and 30 °C) and after that, the enzyme activity was determined at 25 °C as previously. Those samples were labeled as C10, C20, and C30, respectively. The initial activities of the POD enzyme in the control coconut waters C10, C20 and C30 were 621 ± 36, 726 ± 9 and 615 ± 6 U/mL, respectively. The results showed that POD activity in coconut water increased after incubating the sample at 20 °C for 10 min, which means that the processing temperature might affect the enzyme activity.

No detectable activity of POD was found after all ozone treatments, which reveals that the minimum ozone charge applied (0.075 mg/mL) was enough to reduce the activity to undetectable levels. As the enzyme activity after all processing conditions in the experimental domain were the same, the effect of the independent variables on the response (residual activity) was non-significant and the response surface methodology could not be applied. For practical and industrial applications, the results showed that an ozone load low as 0.075 mg/mL of coconut water is enough to inactive the POD. The results highlighted that ozone is a suitable technology for industrial applications considering that the physicochemical parameters were preserved after ozone processing.

Studies have reported relevant inactivation of POD in peach (Jaramillo Sánchez, Garcia Loredo, Contigiani, Gómez, & Alzamora, 2018) and açai (Oliveira et al., 2018) juices. The authors observed a strong correlation between the reduction of the enzyme and the increase in ozone concentration. The decrease of the enzymatic activity can be attributed to oxidative changes in enzyme structure and conformation promoted by direct reaction with ozone or with reactive species derived from its decomposition, associated with varied and complex damage involving interaction with amino acid side chains or protein backbones, causing molecule fragmentation or crosslinking.

The ACP processing promoted the reduction of enzymatic activity of POD in all treatments (Fig. 6). The smallest residual activity (about 28%) was achieved in treatments P7 and P8, where the highest frequency was applied (at 730 Hz). This fact indicates that the reduction in

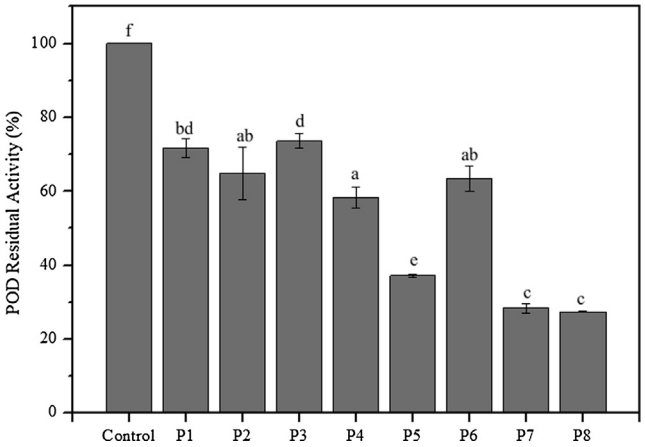

Fig. 6. Residual activity (%) of POD after ACP processing. The overwritten letters represent equality or difference among activities according to the Tukey test (significance level of 0.05).

enzyme activity increased with frequency. The residual activity also seems to decrease with voltage, except for treatment P6, which present higher residual activity (about 68%). However, at the highest frequency, the voltage had no influence on inactivation levels. The study reported by Chutia et al. (2019) demonstrated the application of cold plasma in coconut water under different voltages and concluded that inactivation of peroxidase increases with voltage and time. The same tendency was observed by Pankaj, Misra, and Cullen (2013) with the application of ACP in tomatoes. Although voltage and time are important parameters that influenced the rate and shape of the inactivation curve, our study has shown that frequency is also a parameter that has a significant effect on the reduction of POD activity. The effect of the frequency of the POD inactivation was not reported elsewhere.

The mechanism of enzymatic inactivation by plasma is usually associated with the formation of chemical reactive species (nitrogen oxides, ozone, hydroxyl radicals, superoxide anion radicals, hydroperoxy radicals, among others) during the process, which react with the enzyme causing changes in its structure and loss of activity (Misra, Pankaj, Segat, & Ishikawa, 2016). Han, Cheng, and Sun (2019) evaluated the effect of atmospheric-pressure plasma jet (APPJ) on the activity and structure of horseradish peroxidase (HRP). The HRP residual activity was about 17% after 10 min of processing. Changes in structure and conformation were observed, such as reduction of α-helix and increase of β-sheet, residual aromatic amino acids (TRP, TYR and PHE) and heme degradation at the active site of the enzyme. The inactivation mechanism of APPJ was attributed to the formation of reactive species, including oxygen (ROS) and nitrogen (RNS) species, identified through Optical Emission Spectroscopy.

Some authors reported relevant POD inactivation in coconut water with other technologies, such as pressure-assisted thermal processing (90 °C/400–600 MPa) (Chourio, Salais-Fierro, Mehmood, Martinez-Monteagudo, & Saldaña, 2018b) and thermosonication (655.80 mW/mL) (Ribeiro, Valdramidis, Nunes, & de Souza, 2017). Regarding the superior results for enzyme inactivation keeping the overall quality of coconut water, the ozone process stands out for promoting POD inactivation of coconut water using mild temperatures and maintaining the original color and food matrix composition.

4. Conclusion

This study demonstrates that ozone promoted inactivation of POD in all treatments, indicating that low ozone charges are necessary to reduce enzymatic activity. Moreover, it did not present deleterious effects on phenolic compounds. Atmospheric Cold Plasma presented higher levels of inactivation of POD when higher frequencies were applied, indicating that this is an important parameter that influences enzymatic reduction. Besides, inactivation increased with voltage, except at higher frequencies. ACP promoted the reduction of some phenolic compounds but increased the content of others. No relevant effect of both non-thermal processing was verified based on chemometrics analysis of 1H NMR dataset and quantification of *α*-glucose, *β*-glucose, fructose, malic acid, alanine, and valine. Both processing did not affect pH, total soluble solids, titratable acidity, and color. In the present study, the potential of ozone and cold plasma as a preservation method of coconut water was demonstrated. According to the results presented herein, ozone was the most suitable thermal processing regarding enzyme inactivation without changing the product quality parameters.

CRediT authorship contribution statement

Elaine Porto: 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 Vidal Fonteles:** Methodology, Investigation, Software. **Ronnyely Braz Reis do Nascimento:** Methodology, Investigation, Data curation. **Fabiano A.N. Fernandes:** Conceptualization, Methodology, Investigation, Validation, Funding acquisition. **Edy Sousa de Brito:** Conceptualization, Methodology, Investigation, Validation, Funding acquisition. **Sueli Rodrigues:** Conceptualization, 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.2020.109000.

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