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Cold plasma processing effect on cashew nuts composition and allergenicity

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ABSTRACT

The study investigated the influence of atmospheric plasma processing on cashew nut composition as well as on its allergenicity. The cashew nuts were processed by low-pressure plasma, using glow discharge plasma (80 W and 50 kHz power supply). Anacardic acids and allergens were quantified by HPLC and immunoassay, respectively. Additionally, the overall composition was evaluated by ^{1}H qNMR. Increases in amounts of anacardic acids (15:1, 15:2, and 15:3) and fatty acids (oleic, linoleic, palmitic and stearic) were detected after all process conditions, with 70.92% of total variance captured using 2 LVs. The total amount of anacardic acids increased from 0.7 to 1.2 μg·mg^{-1} of nut. The major change was observed for anacardic acid (C15:3) with an increase from 0.2 to 0.55 μg/mg of nut for the samples treated with a flow of 10 mL·min^{-1} and 30 min of processing. On the other hand, the amount of sucrose decreased, from 33 to 18 mg·g^{-1} of nut, after all processing conditions. Plasma processing of cashew nuts did not affect binding of either the rabbit anti-cashew or human cashew allergic IgE binding. Among the treatments, 10 min of plasma processing at flow rate of 30 mL·min^{-1} of synthetic air followed by 20 min at flow rate 5.8 mL·min^{-1} had the least effect on nut composition as a whole.

1. Introduction

Among healthy foods, cashew nuts (*Anacardium occidentale*, L.) are considered an important nutritional supplement while providing a pleasant taste and flavor. These nuts are accepted worldwide as a source of proteins, fats, and vitamins; and their labor intense production has social and economic impact Moreira et al., 2018). Cashew nuts contain antioxidant capacity due to their phenolic composition, mainly formed by a mixture of anacardic acids (monoene, diene, and triene constituents) and cardol to a lesser extent. It is known that anacardic acids are one of the main bioactive phytochemical compounds found in cashew nuts, having bactericide, fungicide, insecticide, cytotoxic; and anti-inflammation activities (Hemshekhar, Santosh, Kemparaju, & Girish, 2012). However, cashew nut are also classified as an allergenic food, responsible for immune reactions in sensitized or allergic individuals (Mendes, Costa, Vicente, Oliveira, & Mafra, 2016).

Cashew nut allergy is thought to be the second most commonly reported tree nut allergy in the United States (Robotham et al., 2005) (Boyce et al., 2010), and has been often reported to be the cause of severe reactions (Clark, Anagnostou, & Ewan, 2007). The global popularity and consumption of cashew nuts as an ingredient in a variety of processed foods increases the risk of inadvertent exposure. Despite the fact that cashew nuts are considered potent allergen, there is no widely accepted and effective process to reduce allergenicity (Pereira, Rebouças, Ferraz-Carvalho, de Redín, Guerra, Gamazo, Brodskyn, Irache, and Santos-Magalhães, 2018). Three major seed storage proteins have been demonstrated to bind immunoglobulin E (IgE) antibodies, including the Ana o 2 (legumin group) (Wang, Robotham, Teuber, Sathe, & Roux, 2003) and Ana o 3 proteins (albumin group) (Robotham et al., 2005). Both Ana o 2 and Ana o 3 are composed of 2 subunits held together by disulfide bonds. The Ana o 2 protein subunits are in the 31–35 kDa and 20–22 kDa range, whereas the Ana o 3 subunits are lower molecular-weight polypeptides, 5–8 kDa. The Ana o 1 protein has also been demonstrated to bind IgE from cashew allergic patients (Wang et al., 2002), but it is present at low abundance. In cashew nuts, the skin irritation associated with food allergy may also be caused by the presence of urushiol (a mixture of cathecol derivates). Due to modern food processing and regulatory guidelines to avoid contamination of food products with urushiols, it is uncommon to observe cashew nut dermatitis as a result of urushiol in the United States of

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America.

Processing by cold plasma irradiation is an emergent technology for food treatment, especially for temperature sensitive products, which has shown no significant negative effect on food composition (Alves Filho, Almeida, et al., 2016; Alves Filho, Cullen, et al., 2016; Alves Filho et al., 2018). Some processing parameters such as time, flow rate, and intensity of the plasma discharge should be taken into account for maximum efficiency at low cost of operation and, concomitantly, decrease the influence on material composition (Thirumdas, Sarangapani, & Annapure, 2015). Cold plasma owns its effect to reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Surowsky, Schlüter, & Knorr, 2015). The plasma technology has been employed with success on different food products such as for antigenicity reduction of peanut allergen (Venkataratnam, Sarangapani, Cahill, & Ryan, 2019), inhibition of pathogenic bacteria in black pepper surface (Mosovská et al., 2018), physical, structural, volatile and bioactive compounds improvement of a guava flavored whey-beverage (Silveira, Coutinho, Esmerino, et al., 2019; Silveira, Coutinho, Rocha, et al., 2019), functional compounds on siriguela juice (Paixão, Fonteles, Oliveira, Fernandes, & Rodrigues, 2019); and chemical and physical quality of a chocolate milk drink (Coutinho, Silveira, Fernandes, et al., 2019; Coutinho, Silveira, Pimentel, et al., 2019).

In general, a complete analytical investigation of the effect of food processing using spectroscopy and spectrometry techniques generates datasets with elevated dimensionality. In this regard, the application of suitable methods to evaluate the complex matrices variability is essential to achieve comprehensive and reliable results. Accordingly, appropriate protocols for multivariate analyses can enhance the chemical responses of analytical techniques and methodologies (Smilde, Bro, & Geladi, 2005). Therefore, the aim of the study was to investigate the influence of atmospheric plasma processing on cashew nuts composition as well as on its allergenicity.

2. Experimental

2.1. Sampling and plasma processing

Cashew (*Anacardium occidentale*, L.) nuts were purchased from a local supermarket in Fortaleza-CE, Brazil. Groups of samples containing five nuts were created in triplicate to test different plasma processing conditions. The groups of nuts were processed by low-pressure plasma, using a glow discharge plasma generation model Venus PE50 (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) (Alves Filho et al., 2018). Pressure reached within the chamber remained at 300 mbar. The processing of the nuts was carried out in direct contact with the synthetic air. The flow rate of synthetic air plasma (grade FID 4.5, purity 99.95%, White Martins, Brazil) through the chamber and the processing time are described in Table 1. After plasma processing, those nuts were grounded into fine powder, homogenized, and stored at −80 °C until the analysis. The experimental conditions for the synthetic air flow rate ($mL{\cdot}min^{-1}$) and time (min) is described on Table 1.

2.2. Cashew allergen characterization

Samples of control and processed cashew nuts were ground using a coffee grinder and defatted with petroleum ether using Kimble Soxhlet extraction device for 24 h. The defatted nut flour was then mixed (10:1, *v/w*) with 100 mM sodium phosphate buffer (pH 8.0) for 1 h, sonicated three times on ice for 15 s using a Sonic Dismembrator (Fisher Scientific Co., Orlando, FL, USA), and then centrifuged for 30 min at 12,396*g* at 4 °C. The protein concentration of the collected clarified extracts was determined by absorbance at 280 nm using a Pharmaspec UV-1700 (Shimadzu, Kyoto, Japan), and samples were flash frozen in liquid nitrogen and stored at −80 °C.

Table 1
Experimental design for plasma processing of cashew nuts.

Time (min)	Flow rate ($mL{\cdot}min^{-1}$)	Time (min)	Flow rate ($mL{\cdot}min^{-1}$)
None	None	0	0
−1	−1	10	10
+1	+1	30	30
−1	+1	10	30
+1	−1	30	10
−α	0	5.8	20
+α	0	34.1	20
0	−α	20	5.8
0	+α	20	34.1
0	0	20	20
0	0	20	20
0	0	20	20

Comparison of rabbit anti-cashew or human IgE antibody binding to cashew nut extracts was performed by direct ELISA. Cashew nut extract (5 µg) in sodium carbonate buffer (0.015 M Na_2CO_3, 0.035 M $NaHCO_3$, pH 9.6) was used to coat flat-bottom clear-well MaxiSorp immuno nonsterile 96-well plates (Thermo Fisher Scientific, Waltham, MA, USA) overnight at 4 °C. Plate wells were then blocked with phosphate buffered saline (PBS, 10 mM phosphate, 137 mM sodium chloride, pH 7.4) containing 0.1% BSA for 1 h at 37 °C. Rabbit anti-cashew polyclonal antibody diluted 1:5000 or cashew allergic human plasma containing IgE diluted 1:5 was added to plate wells for 1 h at 37 °C. Plate wells were then washed, and 1:10,000 dilution of donkey anti-mouse IRDye 680 labeled secondary antibody or 1:2000 dilution of IRDye 800 labeled mouse anti-human IgE was added and plates were incubated 1 h at 37 °C. After the incubation, plate wells were washed and then scanned on a LI-COR Odyssey CLX for signal quantification. Antibody binding (relative fluorescence units, RFU) is represented graphically as the average of at least four samples. Experimental data underwent analysis of variance (ANOVA) and post-hoc Tukey HSD using an alpha/p value of < 0.01.

2.3. NMR spectroscopy

Firstly, the signal intensity and resolution of the ^1H NMR spectra were investigated by comparison between the aqueous and methanolic extracts (in same concentration) in order to develop a more suitable multivariate and quantitative (^1H *q*NMR) analyses. Despite of the composition similarity and signal intensity (Fig. 1 available in Supporting Information), the methanolic extract presented better spectral resolution, which improves the chemometric evaluation. Therefore, the cashew nut powder, 30 mg was soaked in 700 µL of methanol-d_4 (99.8%). The solutions were automatic mixed (Thermomixer Comfort) during 3 min at 25 °C and centrifuged during 3 min at 806.4*g*. The supernatants were 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 ^1H NMR spectra were acquired using PRESAT pulse sequence for non-deuterated water suppression, since this pulse sequence presented the best irradiation profile (less affected surrounding region according to the analysis of saturation profiling). The spectral data were acquired with 32 scans using 96,000 of time domain points for a spectral window of 16.0 ppm, under quantitative conditions to ensure the complete relaxation of the nuclei Sucupira et al., 2017): RF pulse calibrated to 90° (7.78 µs pulse length at 58 dB of power); acquisition time of 5.0 s and recycling delay of 35.0 s determined by the inversion-recovery pulse sequence; and controlled temperature to 298 K. The spectral processing was performed by applying exponential Lorentzian broadening of 0.3 Hz and zero filling to 16 k points before Fourier

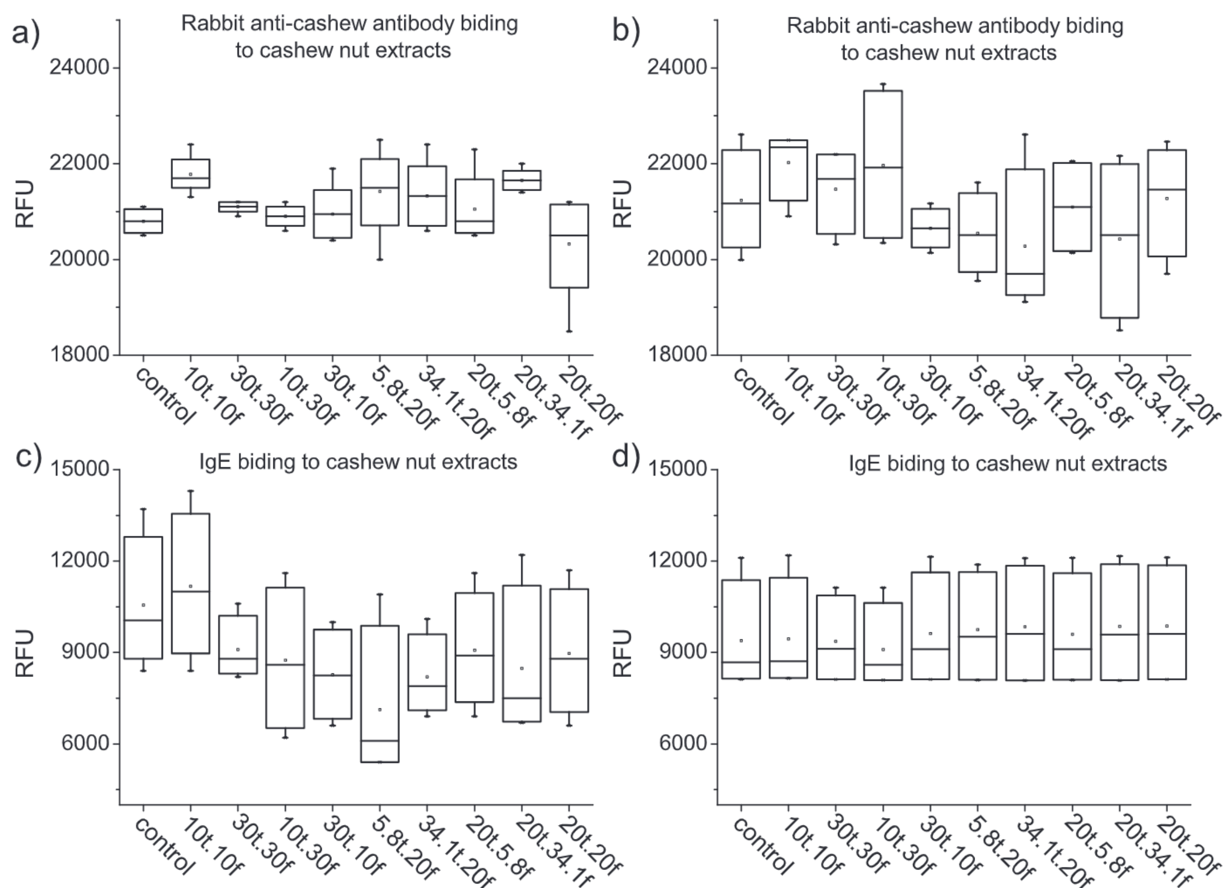

Fig. 1. Replicates 1 (a) and 2 (b) of the rabbit anti-cashew antibody direct ELISA binding to cashew nut extracts. Replicates 1 (c) and 2 (d) of the cashew allergic volunteer IgE antibody direct ELISA binding to cashew nut extracts. The legend "10t,10f" means 10 min of plasma processing at flow rate 10 mL·min^{-1} of synthetic air, and so one.

transformation. The phase correction was performed manually, and the automatic baseline correction was applied over the entire spectral range. The spectra were referenced by methanol-d_4 signal – quintuplet at δ 3.31 for ^{1}H, and septet at δ 49.15 for ^{13}C.

2.3.1. *Chemometric analysis of the NMR dataset*

Multivariate analyses were performed on a numerical matrix constituted by 108 spectra of cashew nuts before (control) and after plasma processing. The spectral region between δ 0.7 and 8.6 was selected, resulting in a matrix with dimensionality of 871,668 data points (108 spectra × 8071 variables).

For matrix construction, the spectra data were converted to American Standard Code for Information Interchange (ASCII) files and imported by Origin™ 9.4 program and then, it was exported for chemometric analysis using the software Matlab™ with PLS Toolbox package (8.6.1 – Eigenvector Research Inc., Wenatchee, USA). Algorithms for baseline correction, variables alignment using COW (Correlation Optimized Warping) with segment of 20 data points and a slack of 10 data points, and normalization were applied over the variables. The samples data were mean-centered and the Singular Value Decomposition (SVD) algorithm was applied to decompose the matrix for an exploratory evaluation by PCA (Principal Component Analysis).

To improve the identification of chemical changes on cashew nut according to different plasma processing and corroborate the marker compounds of the plasma processing, a multivariate regression analysis with variable selection method by iPLS (interval Partial Least Squares) was employed using the autoscaled concentrations of anacardic acids as categorical variables (Y column). The SIMPLS algorithm was applied to build the model with intervals size of 150. The latent variables (LV) were selected in accordance to statistical parameters: RMSEC (Root Mean Square Error of Calibration); RMSECV (Root Mean Square Error of Cross Validation), and the respective correlation coefficients (r^2). The venetian blinds method (5 splits and 1 sample per split) was applied during the cross validation (Wise et al., 2007).

2.3.2. *^{1}H qNMR analysis*

The compounds that stood out in the multivariate analysis with no overlaying signals were quantified using an external reference method. A standard solution of sucrose (5.0 mg·L^{-1}) was used to calibrate the spectrometer and the probe file was updated with the parameters required for determination of the concentrations (Sucupira et al., 2017). Therefore, the signals at δ 5.40 (sucrose), δ 5.34 (linoleic and oleic acids), δ 4.36 (glycerol), δ 2.78 (linoleic acid), and δ 1.61 (linoleic, oleic and palmitic acids) were selected. A simple subtraction among the concentrations provided the individuals concentrations of each compound separately (Barison, Pereira da Silva, Campos, Simonelli, Lenz, and Ferreira, 2010).

The combined uncertainty of the method was estimated based on the analytical errors and the standard deviations of the three replicates of sampling (see Section 2.1). The results were evaluated using the analysis of variance ANOVA single factor with significance level of 0.05, means comparison using Tukey test, and Levene to test the homogeneity of variance to statistically certify the differences among the concentrations.

2.4. *Quantification of anacardic acids by HPLC-UV*

The quantification of anacardic acids monoene (C15:1), diene

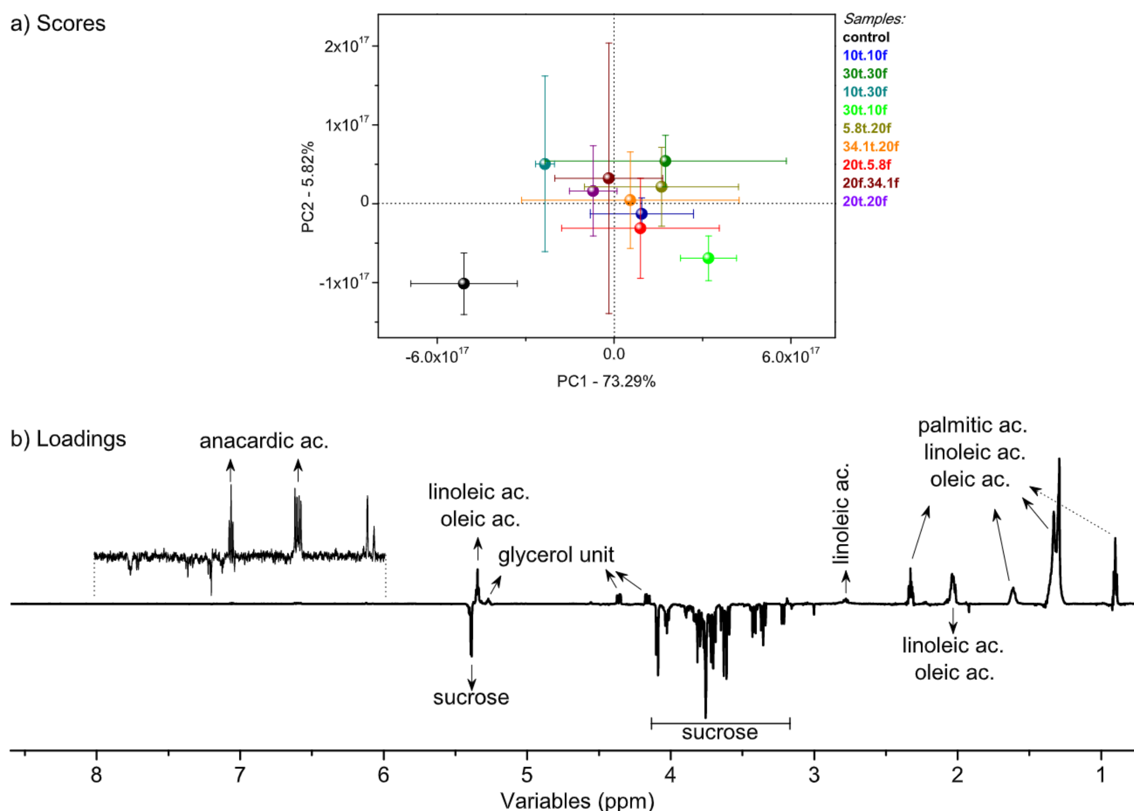

Fig. 2. PCA of the cashew nuts before (control) and after plasma processing: a) PC1 × PC2 scores coordinate system, with error bars representing the combined uncertainty from analytical errors and the standard deviations of the processing replicates; b) relevant loadings plotted in line form. Legend: "10t,10f" means 10 min of plasma processing at flow rate 10 mL·min^{-1} of synthetic air, and so one.

(C15:2), triene (C15:3), and total (monoene + diene + triene) in cashew nuts was performed in triplicate by a validated HPLC-UV method (Oiram Filho et al., 2017). The concentrations were used as categorical variables (Y column) to develop a multivariate regression analysis (as described at Item 2.3.1).

The ground cashew nuts were extracted in methanol (50 mg·mL^{-1}) using ultrasound, and filtered through 0.2 µm filters (PTFE) directly into vials and injected in HPLC-UV system. The chromatographic system consisted of HPLC (Shimadzu LC-20AB Prominence) with binary pump, diode-array detector (Shimadzu SPD-M20A Prominence), reverse-phase C_{18} chromatographic column Shim-pack CLC-ODS (M) (150 × 4.6 mm × 5 µm, Shimadzu), and autosampler Shimadzu SIL-20 AC Prominence (Kyoto, Japan). The mobile phase was composed by water (solvent A), acetonitrile (solvent B), and acetic acid in ratio 20:80:1, and the elution were performed in isocratic mode. The analysis time was 30 min, with a flow of 1.5 mL·min^{-1} at 25 °C, and the injection volume was 20 µL. The chromatograms were monitored at wavelength 280 nm. The software Shimadzu LC Solution was used for instrumental control and chromatographic data processing (Oiram Filho et al., 2017).

2.5. Fatty acids profile

The profile of the fatty acids was obtained by using gas chromatography (GC) coupled to mass spectrometry (MS) and the relative percentage by using GC coupled to a flame ionization detector (FID). This protocol was performed tree times for the control nuts and for those submitted to 30 min of plasma processing at flow rate 30 mL·min^{-1}. The nuts were ground and the extraction was performed by weighting proximately 300 mg of the material, addition of 7 mL of hexane, stirred in ultrasound bath for 5 min and centrifuged at 2000 rpm for 5 min. The extraction protocol was performed 3 times until total fatty acid extraction from the nuts. The supernatant was removed, dried and weighed. Approximately 45 mg of the resultant fatty acids were esterified. To the weighted extract, was added 1 mL of hexane and 1.33 mL of NaOH 0.5 M and kept at 70 °C for 5 min. Then, in the cooled samples was added 1.67 mL of a solution of 0.6 M of NH_4Cl and 0.55 M of H_2SO_4 and the system was agitated for 30 s and heated at 70 °C for 5 min. The system was cooled as fast as possible, added to it 1.33 mL of NaCl 6 M, and stirred. The system was transferred to a separation funnel and the superior phase was transferred to a weighted vial, dried and dissolved in 1 mL of hexane GC grade for injection.

The samples were analyzed in a GC–MS Agilent 5977A equipped with a 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}. A split ratio of 1:100 was used with 1 µL injection and the helium was used as carrier gas at 1 mL·min^{-1}. The injector and the interface temperatures were 250 °C and 280 °C, respectively. The temperature ramp was: 35 °C, increased to 180 °C at 15 °C min^{-1}, to 250 °C at 5 °C min^{-1}. The final temperature (250 °C) was held for 23 min. The peaks were identified on the basis of fragmentation patterns using the NIST Mass Spectral Search Program (Washington, DC, version 2.0 of 2008 – 287,324 compounds). To obtain the relative percentage, the samples were injected on a Shimadzu CG-2010 Plus gas chromatograph equipped with a flame ionization detector (FID) and a RTX-5 methylpolysiloxane column (30 m × 0.25 mm × 0.25 µm). It was employed the same method used in GC–MS.

2.6. Scanning electron microscopy

Dried samples were placed on stubs, sputtered with 20 nm gold (K550X Sputter Coater; Quorum, Laughton, UK) and then observed

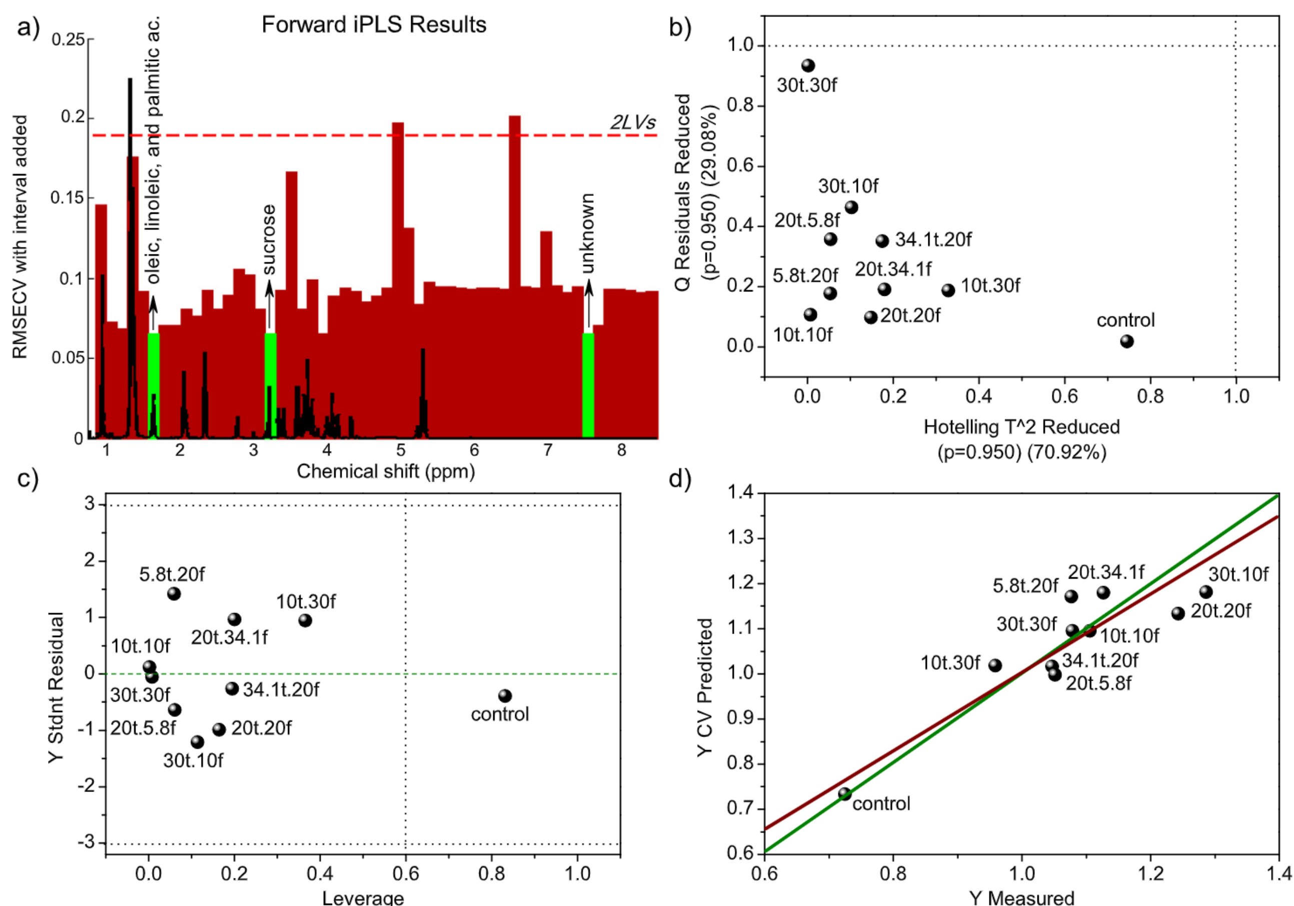

Fig. 3. Regression modeling using the ^{1}H NMR dataset based on anacardic acids concentrations: a) spectral area selected by iPLS (green); b) influence plot of Hotelling T^2 × Q residuals of cashew nuts; c) leverage × studentized residuals; d) Y calibration × cross-validated Y, with 95% confidence limit, real and predicted anacardic acids concentrations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Statistical parameters obtained by multivariate regression modeling by total anacardic acids concentrations.

2LVs[a] (%)	r^2 cal[b]	RMSEC[c]	r^2 CV[d]	RMSECV[e]	RMSEC/RMSECV[f]	Bias[g]	CV bias[h]
70.92	0.898	0.047	0.804	0.065	0.723	0.0	−0.007

[a] Percent variance captured by regression model using 2 LVs in X-block (matrix X).
[b] Coefficient of correlation between the real concentration and the concentration predicted during the calibration.
[c] Root Mean Standard Error of Calibration.
[d] Coefficient of correlation between the real concentration and the concentration predicted during the cross validation.
[e] Root Mean Standard Error of Cross Validation;
[f] Similarity criterion.
[g] Average difference between the estimator and real values in during the calibration.
[h] Average difference between the estimator and real values in during the cross calibration.

with a digital scanning electron microscope (SEM) (EVO 40; Zeiss, Germany; Quanta 450FEG FEI, Czech Republic).

3. Results and discussion

3.1. Effect on allergens

To determine the effect of plasma processing on cashew nut allergens, direct ELISA using protein extracts from processed cashew nuts were used to compare antibody binding. Extracts from processed nuts were first evaluated by SDS-PAGE to determine if there were any visible changes in migration of the proteins. No obvious differences in protein migration pattern were observed among the differently treated cashew nuts (see Supporting Information). A direct ELISA with the processed cashew nut extracts using a rabbit anti-cashew antibody indicated there were only minor differences in binding by the rabbit polyclonal antibodies (Fig. 1a and b). Similarly, only minor differences were observed in human IgE binding to the processed cashew nut extracts (Fig. 1c and d). There was not a statistically significant difference in binding of either the rabbit anti-cashew or human cashew allergic IgE binding to the processed cashew nut extract samples, indicating the plasma processing steps evaluated here would not be useful in the generation of cashew nuts with reduced potential to cause allergic reactions.

Atmospheric cold plasma processing was effective to reduce shrimp tropomyosin allergenicity, with reduction of 76% after 5 min of treatment (Shriver, 2011). Another study showed that the application of plasma processing was efficient to reduce 37% the wheat allergenicity (Nooji, 2011). These allergenicity reductions may be related to interactions of the reactive species formed during the plasma processing with proteins, which can change the conformational structure and cleave peptide bonds; or the protein denaturation by oxidation of amino acids in proteins, or by the reduction of protein solubility due to the aggregates formation; or by crosslinking (Ekezie, Cheng, & Sun, 2018; Shriver & Yang, 2011). The cold atmospheric plasma reduced antigenicity of peanut protein Ara h1 up to 43% for deffated peanut flour

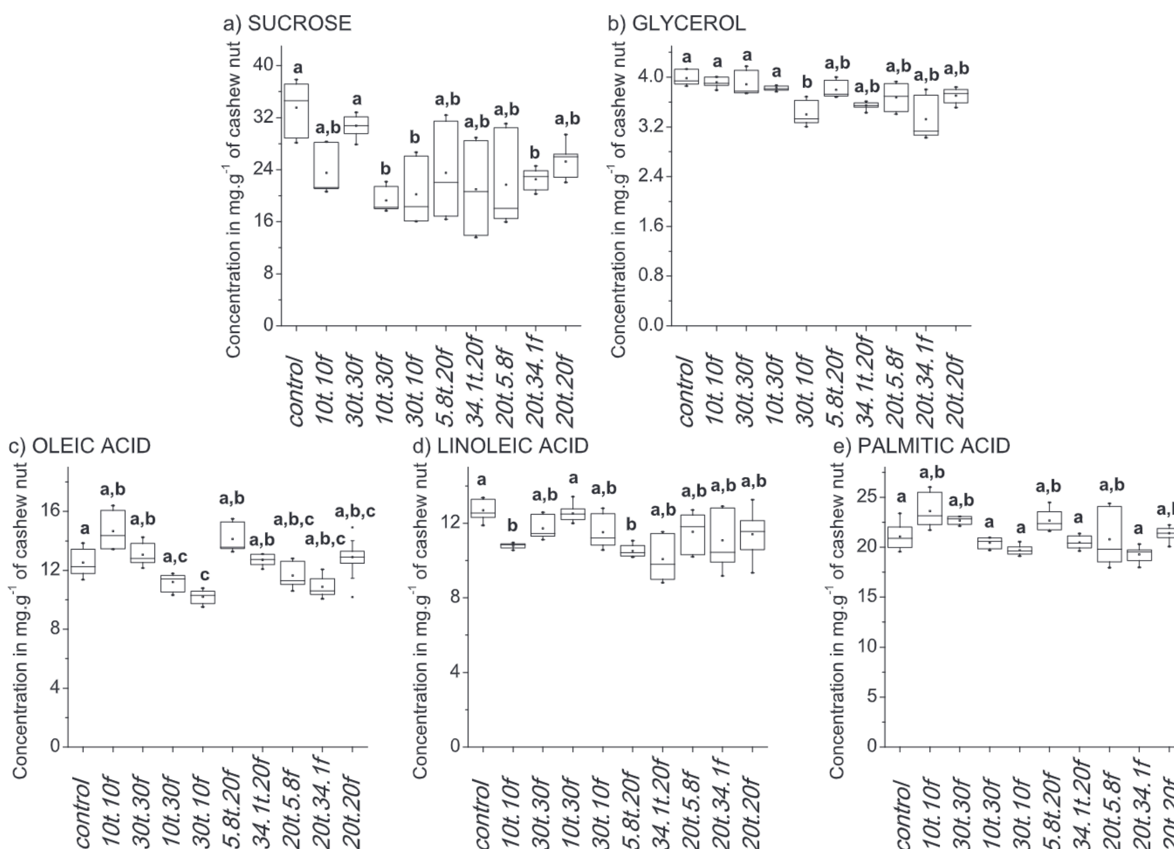

Fig. 4. Concentrations of sucrose, glycerol, oleic acid, linoleic acid, and palmitic acid in mg per g of cashew nut by ^{1}H *q*NMR.

and 9.3% for whole peanut (Venkataratnam et al., 2019), indicating the importance of food matrix composition for plasma effects. The available water is another factor influencing plasma efficiency. According to Tolouie, Mohammadifar, Ghomi, and Hashemi (2018) higher protein inactivation could be seen in solution rather than in dried state. These authors suggest two mechanisms: a) a barrier effect of the protein molecules in the upper layer of dried materials limiting the transition of plasma-generated radical species within the sample, while the solution is exposed homogenously; and b) the interaction of solution components with plasma species, which can contribute to the formation of secondary reactive species, which in turn can cause further protein inactivation. It is known that plasma reactive species can directly react with the external cell membrane and be transported into the cell where internal cell damage can occur through destruction of the DNA, proteins, and other internal components (Surowsky et al., 2015). In addition, some of the plasma species such as hydrogen peroxide, superoxide anions, and perhydroxyl radicals are formed at the plasma–liquid interface, making liquids a suitable matrix for the application of plasma (Surowsky et al., 2015). In the present study reduced pressure plasma was employed, which produces different ROS and RNS, and on different concentrations, from that produced on atmospheric cold plasma. Independently from the plasma source the plasma composition is yet unknown and subject of discussion, making difficult the assumption of mechanisms of action and possible generalizations (Ekezie, Sun, & Cheng, 2017).

3.2. Cashew nuts composition by NMR

The NMR analysis showed that the cashew nuts comprise high levels of sucrose, glycerol, and fatty acids mainly. The identification of these compounds was performed through usual NMR correlations (^{1}H—^{1}H COSY, ^{1}H—^{13}C HSQC, ^{1}H—^{13}C HMBC) as well as assessments using an open access database and literature reports (Alves Filho, Silva, Teofilo, Larsen, & de Brito, 2017; Barison et al., 2010; Morais et al., 2017; Trox et al., 2010). Molecular structures, ^{1}H and ^{13}C chemical shifts, multiplicity, constant coupling, 2D-NMR data acquisition and processing are available in the Supporting Information. The chemical shift from the anomeric proton at δ 5.42 (d 3.7 Hz) was attributed to sucrose, which was confirmed by cross-link with carbon at δ 93.8. For fatty acids characterization, the ^{1}H chemical shifts of the proton at δ 5.38 from oleic and linoleic acids (multiplet) were defined by cross-link with carbons at δ 129.3 and δ 130.9, respectively. The identification of the palmitic acid was performed by proportionality among the signals integration and literature reports (Rico, Bulló, & Salas-Salvadó, 2016; Soares et al., 2013).

It is essential to analyze the effect of individual parameters of plasma processing on composition of cashew nuts. Therefore, in order to investigate the composition variability before and after different plasma processing, an unsupervised chemometric analysis by PCA was applied on ^{1}H NMR dataset to untargeted exploration. Fig. 2 illustrates the PCA results from the evaluation of the whole ^{1}H NMR spectra (δ 0.5–8.6), with an expansion between the chemical shifts at δ 6.0 and 8.0. The bars on scores (Fig. 2a) indicate errors related to the sampling and processing replicates and only the relevant loadings were highlighted at Fig. 2b.

The main separation was related to PC1 axis. In general, the control cashew nuts are located at most negative scores, and the processed nuts at null and positive scores of the same PC. The respective loadings mostly showed the opposite behavior between sucrose and the fatty acids (oleic, linoleic, palmitic) and glycerol. The plasma processing decreased the amount of sucrose and increased the fatty acids and glycerol (made them more available) regardless the time and flow rate of the synthetic air. The PCA results from the aromatic region showed the increase of anacardic acids on the plasma processed cashew nuts.

Fig. 5. Concentrations of anacardic acids in cashew nuts according to the degree of unsaturation: a) total = monoene + diene + triene; b) monoene; c) diene; d) triene. The legend “10 t,10f” means 10 min of plasma processing at flow rate 10 mL·min^{-1} of synthetic air, and so one. The overwritten letters in boxes plot represent equality or difference among the plasma processing parameters.

Table 3
Fatty acid composition of control and plasma processed cashew nuts.

Fatty acid	Control (%)	Plasma treated 20t.20f (%)	Variation (%)
Palmitic acid (16:0)	7.87	7.96	−0.09
Linoleic acid (18:2)	17.30	15.64	1.66
Oleic acid (18:2)	63.03	63.65	−0.62
Stearic acid (18:2)	7.04	8.53	−1.50

The anacardic acids are alkylphenols formed by a salicylic acid unit and a C15 aliphatic chain, which confers lipophilicity to the structure (LogP 7.69–8.35) (Oiram Filho et al., 2017).

A PLS-regression modeling using the interval PLS (iPLS) was developed to maximize the covariance between the independent variables on ^{1}H NMR dataset (X matrix) and the dependent variables – concentrations of total anacardic acids as Y matrix (see Item 3.3). This method performed individual PLS models for each pre-defined spectral interval (equal intervals), which optimized the predictive capacity of

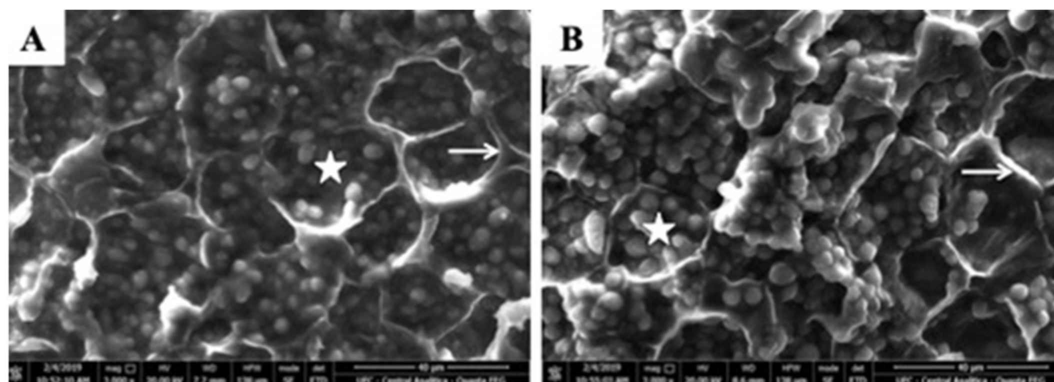

Fig. 6. Scanning electron micrograph of cashew nut. A) control; B) plasma processed at flow rate 20 mL·min^{-1} of synthetic air for 20 min; cytoplasmic granules (★), cell wall (→).

the model while assisted the interpretation by reducing the number of variables and, hence, providing superior prediction capacity than using all the variables in the dataset (Andersen & Bro, 2010). Therefore, new scores related to the content of total anacardic acids were established, since the axes lose their orthogonality to adjust to the higher variations of the concentrations. Fig. 3 describes the merit graphics obtained by the regression modeling using the total concentrations of anacardic acids. Table 2 shows the statistical parameters from the relationship among the anacardic acids, fatty acids and sucrose with 70.92% of total variance captured using 2 LV, low calibration and validation errors, correlation coefficient for calibration and validation above 0.8, no bias model, and relative high similarity criterion between the calibration and validation (Freitas et al., 2018).

Fig. 3a illustrates the relevant intervals chosen for regression modeling using the concentrations of total anacardic acids according to the lowest Root Mean Square Error of Cross Validation (RMSECV): chemical shifts at δ 1.61 related to oleic, linoleic, and palmitic acids; and δ 3.32 from sucrose as the most relevant for regression modeling. Fig. 3b illustrates the influence biplot, with the samples distribution according to Hotelling $T^2 \times Q$ residuals, which clearly showed the absence of outliers (both values above 1, upper left quadrant) (Ballabio, 2015). In addition, the control cashew nuts presented a high influence on regression model (highest T^2). According to Fig. 3c, the leverage plot revealed the influence of each processing condition on the model (also related to the Hotelling T^2), and the studentized residuals (mean zero and unit variance) shows that the samples did not influenced negatively the modeling. Based on ^{1}H NMR data, the control sample presented high leverage and low studentized Y residual; additionally the proximity among the real and predicted anacardic acids concentrations shows the robustness of the model (Fig. 3d). Therefore, the regression model is able to predict the anacardic acid concentration before or after plasma processing.

To clearly observe the variability on the cashew nuts composition, the concentrations of sucrose, glycerol, linoleic, oleic, and palmitic acids in cashew nuts before (control) and after plasma processing were estimated (Fig. 4). Despite the chemometrics have been able to distinguish cashew nuts before and after processing, minor changes were verified in quantitative results, which show a negligible plasma effect on cashew nut composition.

3.3. Anacardic acids

The quantification of anacardic acid in cashew nuts is usually performed using liquid chromatography coupled to UV or MS due to the accuracy of the method (Mattison, Cavalcante, Gallão, & De Brito, 2018). Therefore, a univariate analysis was performed of the concentrations of anacardic acids (total, monoene, diene, and triene) in cashew nuts before (control) and after plasma processing (Fig. 5), by a validated HPLC-UV method (Oiram Filho et al., 2017). Based on ANOVA single factor, a slight increase on anacardic acids concentrations were observed after processing regardless the time or flow rate.

3.4. Fatty acid and SEM

Since the fatty acids were indicated by NMR as the compounds that contribute most to sample separation, a complimentary analysis by GC method was performed on the plasma processed cashew nut (20 t.20f, flow rate 20 mL·min^{-1} for 20 min). The cashew nut fatty acid content was 32.6 and 40.2% for control and plasma treated nuts, respectively. The fatty acid profile of the control and the plasma processed are shown in Table 3. The major fatty acid found was acid oleic acid (18:2) and the primary saturated fatty acid identified was palmitic acid (C16:0) which are in agreement to reported data for cashew nut (Ryan, Galvin, O'Connor, Maguire, & O'Brien, 2006). It was observed no differences among the samples for the fatty acid profile, as previously observed by ^{1}H qNMR. This shows that the plasma treatment does not affect the fatty acid profile, despite the increase on the extractability. Changes in the content of malondialdehyde (indicator of lipid peroxidation) increase, acidity; and pH reduction, were observed on plasma treated wheat seeds, but the mechanisms involved remain unsolved (Los, Ziuzina, Boehm, Cullen, & Bourke, 2019).

The SEM images of cashew nuts are presented on Fig. 6. Plasma treated nuts showed no rupture of the cell walls, but it was observed that the cytoplasmic content of the cotyledonary cells was more evident in comparison to control cashew nuts. Different studies demonstrated that seed germination efficiency was improved by plasma, especially due to surface coat etching and increased wettability (decrease on apparent contact angle), allowing seed embedding (Los et al., 2019; Wang et al., 2017). The evidence of such structural changes could be a possible explanation to the increased fatty acid and anacardic acids extraction on plasma treated cashew nuts.

4. Conclusions

The NMR results demonstrated that it is possible to evaluate the variability of the primary metabolites in cashew nuts under different plasma processing. The unsupervised and supervised chemometric analysis by PCA and iPLS respectively, indicates that plasma processing may present effect on cashew nuts composition, regardless time or flow rate of the processing. However, despite the chemometrics have been able to distinguish cashew nuts before and after processing, minor changes were verified through quantitative results, which show the small effect of the plasma in cashew nut composition. However an increase on oil and anacardic acids content was observed for plasma treated cashew nuts, which was attributed to improved extractability. Such conclusion was possible due to the lack of changes on the fatty acids and anacardic acids profile. In addition, no relevant effect of plasma was detected in the nuts allergenicity.

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