Food Chemistry 240 (2018) 370–376

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Effects of industrial cashew nut processing on anacardic acid content and allergen recognition by IgE

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ARTICLE INFO

Keywords:
Anacardium occidentale
Anacardic acids
Cashew
Food allergen
Food processing
Allergy
IgE

ABSTRACT

Cashew nuts are important both nutritionally and industrially, but can also cause food allergies in some individuals. The present study aimed to assess the effect(s) of industrial processing on anacardic acids and allergens present in cashew nuts. Sample analyses were performed using liquid chromatography coupled with mass spectrometry, SDS-PAGE and immunoassay. The anacardic acid concentration ranged from 6.2 to 82.6 mg/g during processing, and this variation was attributed to cashew nut shell liquid incorporation during storage and humidification. Dehydrated and selected samples did not significantly differ in anacardic acid content, having values similar to the raw sample. SDS-PAGE and immunoassay analysis with rabbit polyclonal sera and human IgE indicated only minor differences in protein solubility and antibody binding following processing steps. The findings indicate that appreciable amounts of anacardic acid remain in processed nuts, and that changes to cashew allergens during industrial processing may only mildly affect antibody recognition.

1. Introduction

Foods that bear nutritional properties are increasingly more attractive to consumers seeking to increase dietary quality, and product quality has become an important consideration in business decisions. Foods are exposed to several factors that may impact their structure and nutritional composition during industrial processing, which can lead to degradation and/or transformation of nutrients and biologically active compounds. These processes may cause a positive impact, such as forming desirable complexes that improve their bioavailability, or a negative impact with loss of nutrients and biologically active potential.

Cashew nut (CN) is one of the main agro-industrial products in African countries, India, Vietnam and Brazil. Its composition has a profile of biologically active amino acids, beneficial fatty acids, alkylphenols, phytosterols, selenium and tocopherols (Melo, Maia, Silva, Oliveira, & Figueiredo, 1998), a high starch content, and a nutritionally and industrially important polysaccharide profile. The nut is consumed fried, with yogurt, as a paste, or used as an ingredient in confectionery and bakery products (Owiredu & Laryea, 2014).

The industrial CN production system employs a thermal-mechanical process. Briefly, harvested CNs are classified by size, followed by humidification by water immersion and equilibration for at least 72 h. The humidification must lead to an 8–10% water content in order to facilitate cutting steps and avoid microbial contamination. The next step is cooking in cashew nut shell liquid (CNSL) at nearly 200 °C for 3 min, which weakens the shell to facilitate cutting and removal of the nutmeat. After removal, nuts with the tegument/skin remaining are separated from the shell and dehydrated at 70–80 °C for 8–10 h, until a uniform moisture content of 3% is reached. This procedure facilitates the next step of tegument removal and classification. Nut classification is assessed based on nut colour, size and integrity. Using heat in industrial processing may favour desirable alterations, such as in roasted coffee, chocolate and bakery products. Additionally, nut proteins may be denatured and amino acids may react with nearby fatty acids or sugars to produce improved sensory qualities. In contrast, there may be undesirable alterations taking place, including reduced solubility of proteins, carbohydrates, or fats which negatively impact the product's sensory and nutritional properties (Fellows, 2006; Oetterer, Reditano-D'Arce, & Spoto, 2006; Ribeiro & Seravalli, 2007).

Diets containing nutmeats, such as walnut, peanut, almond, hazelnut, pistachio, macadamia, cashew nut and Brazil nut, protect the heart, decrease chronic-disease mediators, such as gut fat, stabilize

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http://dx.doi.org/10.1016/j.foodchem.2017.07.146
Received 23 February 2017; Received in revised form 28 June 2017; Accepted 26 July 2017
Available online 27 July 2017
0308-8146/ Published by Elsevier Ltd.

glycemic index, reduce resistance to insulin, and may also decrease the risk of diabetes and cancer (Papanastasopoulos & Stebbing, 2013). Anacardic acid is a phenolic compound present in cashews (Agostini-Costa et al., 2004; Correia, David, & David, 2006; Trevisan et al., 2006) that has been associated with a series of specific pharmacological activities, including anti-microbial, histone acetyltransferase inhibition, anti-cancer and anti-inflammatory (Kubo, Masuoka, Ha, & Tsujimoto, 2006; Stasiuk & Kozubek, 2010; Suo et al., 2012).

Seed storage proteins found in tree nuts are a common cause of food allergy, in some regions of the world. In the United States, CNs are included in a list of tree nuts that commonly cause food allergies and must be clearly labeled when included in foods. Allergic reactions to tree nuts, such as cashews, can often be severe and are rarely outgrown (Fleischer, 2007). Yearly medical costs directly related to food allergy have been estimated at about USD 4 billion annually in the USA (Gupta et al., 2013), and CNs are one of the most frequent causes of severe reactions (Grabenhenrich et al., 2016). Cashew and other tree nuts have 3 conserved seed storage proteins that commonly act as food allergens. These include the 2S albumin, 7S vicilin, and 11S legumin proteins (Radauer & Breiteneder, 2007). In CNs, these proteins have been designated Ana o 1 (Wang et al., 2002), Ana o 2 (Wang, Robotham, Teuber, Sathe, & Roux, 2003), and Ana o 3 (Robotham et al., 2005) and are recognized by the International Union of Immunological Societies (IUIS). Characterization of cashew allergens indicates that they are resistant to digestive enzymes, and in particular Ana o 3 is dependent upon disulfide bonds for stability (Mattison, Grimm, & Wasserman, 2014; Mattison, Desormeaux et al., 2014; Venkatachalam et al., 2008).

Nut processing steps may alter the ability of nut allergens to be detected in food products and/or cause allergy. Numerous studies have found varying results on the effect that processing may have on the immunological state of peanut allergens (Parker et al., 2015; Vissers, Blanc et al., 2011; Vissers, Iwan et al., 2011). Depending upon the region of origin and the size of the producer, industrial CN processing steps can vary. Furthermore, food preparation steps can vary greatly, and heating has been shown to alter the solubility of cashew allergens and can result in their modification (Mattison, Vant-Hull, Vargas, Wasserman, & Grimm, 2016; Mattison et al., 2017). The present study characterized samples from each processing step by SDS-PAGE, as well as immunoblot and ELISA, with rabbit anti-cashew polyclonal sera and human serum IgE. In this context, the present study aimed to assess the effect(s) of industrial processing on anacardic acid levels and allergens in CNs.

2. Materials and methods

2.1. Samples

CNs from 6 industrial processing steps were used. CNs were provided by the Companhia Industrial de Óleos do Nordeste (CIONE), located in Fortaleza, CE, Brazil. Harvested CN were classified by size and humidified by water immersion until equilibration (at least 72 h). Nut humidification was quantified to a tolerance of 8–10% water content in order to facilitate the cut and avoid microbial contamination. Humidified nuts were cooked at 200 °C for 3 min in cashew nut shell liquid (CNSL) to facilitate cutting of the weakened shell. Cut nuts were separated from the shell and dehydrated at 70–80 °C for 8–10 h, until a uniform moisture content of 3% was achieved. The nut tegument or skin was then removed and the nuts were classified based on nut colour, size and integrity. Selected nut samples, from each step, were collected for analysis and their moisture content was quantified in a moisture tester (Steinlite SB900) commonly used in the industry. These selected nut samples were dried in an air-circulation oven for 12 h (overnight) at 65 °C and ground in a food processor (Robot Coupe R201) for 30 s prior to analysis. The processed nut samples were coded as follows: A – raw CN; B – CN stored and selected, calibration; C – CN subjected to humidification; D – CN cooked 2 min in CNSL at 190 °C; E – CN dehydrated, after drying; F – CN selected, after tegument removal.

2.2. Lipid and anacardic acids analysis

The lipid extraction was carried out at a 1:10 ratio (m/v) by immersing the cashew nuts in an Erlenmeyer flask with hexane (Nuclear) at room temperature and stirring for 12 h in a Tecnal TE-420 incubator at 150 rpm. A series of consecutive extractions was performed in triplicate with hexane recovered in a rotary evaporator (Rotavapor R215 – Buchi). The samples were dried at 105 °C before lipid analysis, and lipid content determined gravimetrically.

Anacardic acid content was analyzed based upon a method by Trevisan et al. (2006) in a Varian 250 liquid chromatograph coupled to a 335-diode array detector and a 500-MS IT (Varian) mass spectrometer. The extract was prepared with approximately 1.0 g of the oil diluted in 10.0 ml methanol (Tedia). A Symmetry C18 Waters analytical column (5 μm, 4.6 mm × 250 mm) was used with a flow rate of 600 μl/min, injection volume of 20 μl of the methanolic extract, and a total run time of 40 min. The mobile phase used was water (MilliQ)/0.1% formic acid and acetonitrile (MilliQ)/0.1% formic acid (Sigma-Aldrich) (v/v). Initially 50:50 solvents A and B followed by an increase in solvent B to 100% at 20 min, held isocratically for a further 20 min. The DAD wavelength was set to 270 nm for chromatogram monitoring. The mass spectrum was simultaneously acquired using positive (PI) negative (NI) electrospray ionization at a fragmentation voltage of 80 V for a mass range of 100–2000 uma. Drying gas pressure was 35 psi, nebulizer pressure was 40 psi, drying gas temperature was 370 °C, voltages were 3500 V for both PI and NI, and spray field voltage was 600 V.

The compounds were identified based primarily on the mass spectrum and anacardic acid standards co-injection. The concentration was calculated with the extracted ions *m/z* 341, 343, and 345, and based on an anacardic acid (C15:3) standard curve. The anacardic acid concentration underwent analysis of variance (ANOVA) and Tukey's test at 5% significance.

2.3. Cashew extract preparation

Defatted and ground cashew nut samples from different processing stages were extracted in borate buffered saline (BBS) solution (100 mM H_3BO_4, 25 mM NaB_4O_7, 75 mM NaCl, pH 8.6) at a 1:10 (w/v) ratio for 1 h with constant mixing. Samples were sonicated twice on ice for 15 s using a sonic dismembrator (Fisher Scientific Co., Orlando, FL, USA), and extract solutions were centrifuged for 30 min at 12,000 rpm at 4 °C. The clarified extract solutions were collected using a pipette and protein concentrations were determined using a NanoDrop (ThermoFisher, Pittsburgh, PA, USA) device. Samples were aliquoted and stored at −80 °C prior to use.

2.4. Protein electrophoresis

Extracted protein samples were electrophoresed using a Novex Mini Cell gel rig (Life Technologies, Carlsbad, CA, USA) and pre-stained Precision Plus molecular weight markers (Bio-Rad, Hercules, CA, USA). Sample buffer with reducing agent 4X NuPAGE LDS (Life Technologies, Carlsbad, CA, USA) was added to the protein samples using a 1:4 (v/v) ratio. Samples were heated at 65 °C for 15 min prior to loading, and, after electrophoresis, protein bands were visualized with Safe Stain (Invitrogen, Grand Island, NY, USA). Gel images were captured and normalization of protein load in each lane was confirmed by quantifying the 680 nm channel signal using an Odyssey CLX infrared imaging system (LI-COR, Lincoln, NE, USA).

2.5. Rabbit anti-cashew antibodies and human serum IgE

Rabbit anti-cashew nut sera were generated by Pierce Biotechnology Inc. (Rockford, IL, USA), using pre-screened rabbits and

have been characterized previously (Mattison, Desormeaux et al., 2014). Four rabbits were initially immunized with approximately 1 mg soluble cashew extract and administered booster shots on days 14, 28, and 42 with 0.5 mg of cashew extract for each injection. Test rabbits were phlebotomized and sera were screened for antibodies against total cashew protein. Large-volume phlebotomies were collected from high-titer rabbits (> 1:10,000) and serum was stored at −80 °C for later use. Human sera from cashew allergic patients were obtained from PlasmaLab International (Everett, WA, USA).

2.6. Immunoblotting

Electrophoresed cashew nut extract samples (25 µg) were transferred to a PVDF membrane. The blotted PVDF membrane (Millipore, Billerica, MA, USA) was blocked for 1 h at room temperature with 2% (w/v) nonfat dry milk in phosphate-buffered saline with 0.1% triton X-100 (PBST, pH 7.4). Rabbit anti-cashew antibody was diluted 1:1000 in PBST and membranes were incubated overnight at 4 °C with gentle nutation. Primary antibody was removed; the membranes were washed 3 times, for 5 min, in PBST, and then incubated for 30 min with anti-rabbit IRdye-800 (1:10,000 in PBST) at room temperature. The membranes were washed as above and visualized using an Odyssey CLx (LI-COR, Lincoln, NE, USA) infrared imaging system.

2.7. ELISA

Rabbit IgG and human serum IgE binding to cashew protein was determined in a direct enzyme-linked immunosorbent assay (ELISA) according to the method of Mattison et al. (2016). Briefly, soluble cashew nut samples (1 µg in 50 µl) were adhered to microtiter plate wells overnight at 4 °C. The following morning the wells were blocked with 50 µl of PBS containing 0.1% tween-20 (PBST) and 2% bovine serum albumin for 1 h at 37 °C. The wells were washed 4 times with PBST and then mixed with either rabbit anti-cashew antibodies (1:1000) or pooled human plasma from 6 cashew-allergic donors (1:5) from cashew allergic individuals (PlasmaLab, Everett, WA, USA). Samples were incubated for 1 h at 37 °C and washed as above. Donkey anti-rabbit IRDye 800CW (1:10,000) or anti-human IgE IRDye 800CW (1:5000) secondary antibodies were incubated with sample wells for 1 h at 37 °C and washed 4 times as above. Antibody binding was visualized and quantified using an Odyssey CLx instrument (LI-COR, Lincoln, NE, USA). Antibody binding (IRDye 800 units) is represented graphically as the average of 4 samples with error bars plotted as ± standard deviation. Antibody binding values underwent analysis of variance (ANOVA) and Tukey's test at 5% significance.

3. Results

3.1. Cashew nut moisture and lipid content

The moisture content in the CN collected from the industrial samples ranged between 2.5 and 11.1% in the dehydrated and humidified samples, respectively (Table 1). The CN *in natura* and those stored and selected (A and B) had a similar moisture content (7.8 and 7.2, respectively), an ideal percentage to prevent the proliferation of spoilage microorganisms and keep the CNSL from contacting the nut. The humidification step (C) is used to favour breaking the shell during cooking (D) in order to facilitate cashew nut cooking and CNSL extraction. The moisture content in samples E and F (crude whole and selected whole, respectively) was below 4%, which favours a more effective removal of the shell surrounding the cashew nut. Sample F, selected and ready-to-eat, had a 3.5% moisture content, while the technically recommended percentage for national marketing of cashew nuts is 5% (Brasil, 2009). This final moisture content must be carefully monitored by quality control steps either in the drying or humidification steps given the final product's economic value.

Table 1
Lipid content variation during industrial processing of cashew nuts.

Sample	Moisture (%)	Lipids (g/100 g CN)*	
		Wet basis (WB)	Dry basis (DB)
A	7.8 ± 0.1	42.64 ± 1.01[a]	43.96 ± 1.01[a]
B	7.2 ± 0.1	43.63 ± 2.86[a]	44.78 ± 2.86[a]
C	11.1 ± 0.1	34.12 ± 0.78[b]	35.38 ± 0.79[b]
D	8.6 ± 0.3	43.59 ± 3.55[a]	45.30 ± 3.56[a]
E	2.5 ± 0.2	45.16 ± 3.18[a]	46.11 ± 3.19[a]
F	3.5 ± 0.1	43.38 ± 2.30[a]	44.48 ± 2.30[a]

* Means followed by the same small letters in the columns do not differ at 5% significance according to Tukey's test.

Given the lipid content reported in the literature of 40–52% (wb) CN (Lima, Garruti, & Bruno, 2012; Melo et al., 1998; Ryan, Galvin, O'Connor, Maguire, & O'Brien, 2006), the present study found values within the expected range (Table 1) in all industrial processing steps except for the humidification step, which differed significantly ($p \leq 0.05$). This step increases CN water content, thus causing a decrease in the lipid content in the centesimal composition.

3.2. Anacardic acid content

The chromatograms were integrated and the peaks present in the different samples were aligned according to the retention time and molecular ion (m/z) for anacardic acids (Jerz, Murillo-Velasquez, Skrjabin, Gok, & Winterhalter, 2012; Trevisan et al., 2006). The C15:3, C15:2, and C15:1 anacardic acids were identified and detected in all samples (Fig. 1). The C15:3, C15:2, and C15:1 anacardic acids were quantified (Table 2) and their concentrations were found to vary between 6.2 and 82.6 mg/g during the different stages of processing. The anacardic acid content in the finished product (sample F) was approximately 17.6 mg/kg. Agostini-Costa et al. (2004) and Trevisan et al. (2006) reported variations between 1.8–140 and 1.0–640 mg/kg in fried nut, respectively.

ANOVA demonstrated significant differences ($p \leq 0.05$) between the CN samples, particularly for the high anacardic acid content observed in the humidification step presumably due to the migration of the alkylphenol content present in the cashew nut shell. The low anacardic acid content after cooking (sample D) decreasing by 77% and 84% in the contents of C15:1 and C15:3 anacardic acids, respectively might be caused by their conversion into cardanol through a decarboxylation reaction at high temperatures, around 200 °C (Mazzeto, Lomonaco, & Mele, 2009).

A possible reason for the increase in anacardic acid concentration during storage (A→B) may be the migration of CNSL from the shell to the nut. According to Agostini-Costa et al. (2004), the presence of anacardic acid in the nut is due to the transference of the alkylphenol compounds, which are abundant in the CNSL. This may explain the increase of anacardic acids in sample C, favouring migration through nonpolar affinity. We found no significant differences in anacardic acid content in the dehydrated and selected CN (samples E and F), as compared to those in raw CN (sample A). These results suggest that the degraded and/or transformed content came from the incorporation into the nut during storage (B) and humidification (D).

3.3. Cashew protein solubility and antibody binding

Processing steps can alter the solubility of nut allergens and may influence their ability to cause an allergic reaction. The extractable protein profile from 8 cashew nut processing stages were compared including, *in natura*, stored and selected, humidified, cooked, after shell removal, dried, after tegument removal, and selected nuts in their final consumable state. As shown in Fig. 2A, no appreciable changes were

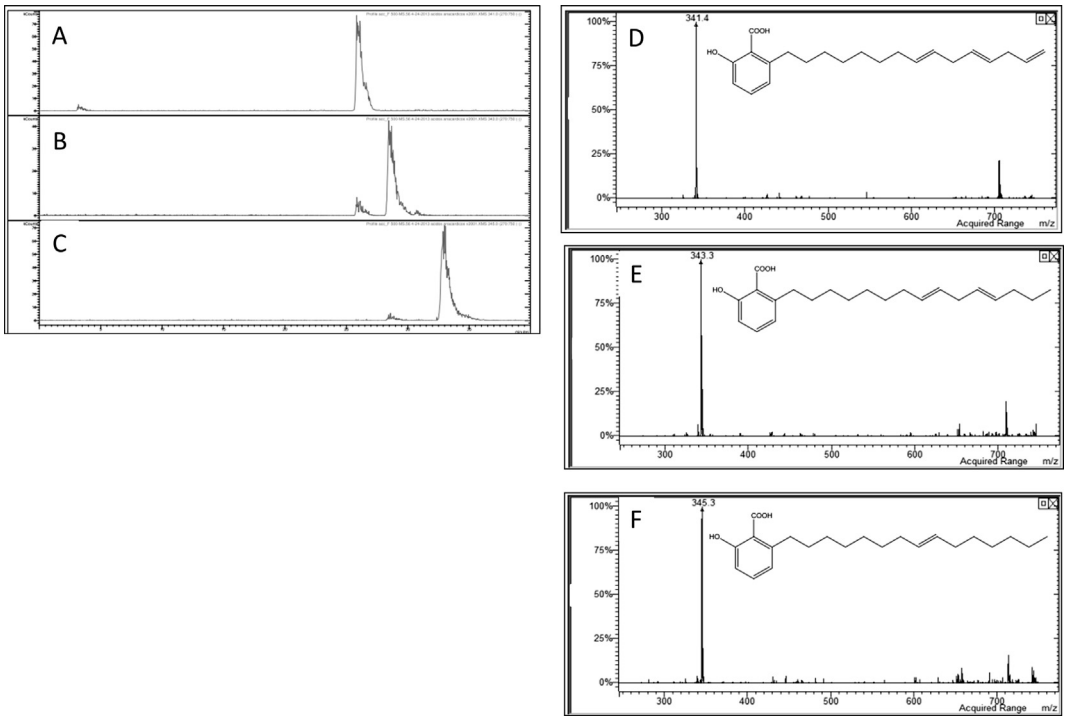

Fig. 1. Quantification of anacardic acids in processed CN samples. Mass spectra chromatograms of C15:3 (A), C15:2 (B), and C15:1 (C) anacardic acids in negative ion mode. Extracted ion chromatogram of anacardic acids C15:3, *m/z* 341 (D), C15:2, *m/z* 343 (E) and C15:1, *m/z* 345 (F) in negative ion mode.

observed by SDS-PAGE in the extractable protein profiles among the nut samples during the processing steps. Among the known allergenic proteins, the intensity of the protein bands for Ana o 1 (50 kDa), the two Ana o 2 subunits (33 kDa and 22–25 kDa), and the large Ana o 3 subunit (8 kDa) remained relatively unaltered during the processing procedure. There were only minor changes in overall protein content within the samples from the various processing stages. Higher molecular weight bands (75–100 kDa) were present in the *in natura*, stored and selected, and dried samples, but were not detectable in the other samples.

Antibody binding to the nut extracts was also compared using rabbit polyclonal anti-cashew antibodies and human serum IgE from cashew allergic patients. Antibody binding appeared slightly reduced in intensity after cooking and in the later steps for most of the proteins in the extracted samples. For example, rabbit anti-cashew antibody binding to the acidic (33 kDa) and basic (22–25 kDa) subunits of Ana o 2 was more pronounced for the *in natura*, stored and selected, and humidified samples, while the cooked, dried, and final selected nut samples appeared to have a reduced signal (Fig. 2B). This slight reduction in binding was consistent among most of the protein bands bound by the antibodies. In contrast, the weak Ana o 3 signal (8 kDa) appeared to be slightly more intense after the cooking step and in subsequent steps.

ELISA was performed as a quantitative measure to assess changes in antibody recognition during cashew nut processing. As shown in Fig. 3A and B, only minor reductions in average rabbit anti-cashew IgG and human sera IgE binding were detected as processing progressed from the *in natura* sample to the final selected nut sample. The present observations suggest there may be only minor changes in cashew allergens during standard industrial processing steps.

4. Discussion

Processed foods are an increasingly common part of the human diet. Food processing steps are aimed at increasing the quality, stability, and sensory-quality of foods, but they may have negative consequences on nutritive value. In the current study, differences in moisture, lipid, and anacardic acid content were observed, in samples collected at different steps within a model of industrial cashew nut processing. The moisture levels within the nuts varied based upon the progression through the processing as expected and increased during the humidification step (Table 1). The lipid content of the nuts dropped, likely as a result of the humidification step, but it was within the expected range of the final product (Table 1).

For the majority of the population, the default pathway to food allergens is tolerance. However, environmental or dietary factors may influence food allergy sensitization, and there is evidence that compounds found naturally in foods or compounds contaminating foods may act as adjuvants. Bacterial products, such as cholera toxin and enterotoxin, have been used experimentally to induce sensitization to peanut allergens in mice (Ganeshan et al., 2009; Li et al., 2000). Dietary

Table 2
Anacardic acids from cashew nut in different industrial processing steps.

Peak	t_{min}	$[M+H]^+/[M-H]^-$	Anacardic acid	Samples Anacardic acid (mg/g CN oil)					
				A*	B	C	D	E	F
1	25.31	343/341	C15:3	12.2 ± 4.9^{a}	41.9 ± 7.5^{b}	82.6 ± 5.3^{c}	13.3 ± 1.2^{a}	19.8 ± 6.6^{a}	12.6 ± 2.5^{a}
2	27.81	345/343	C15:2	8.7 ± 2.1^{ab}	21.0 ± 2.7^{c}	50.6 ± 3.9^{d}	6.2 ± 1.1^{a}	12.4 ± 1.7^{b}	10.6 ± 0.6^{ab}
3	32.02	347/345	C15:1	20.9 ± 2.8^{a}	47.5 ± 1.7^{c}	82.6 ± 7.1^{d}	19.1 ± 2.3^{a}	39.7 ± 3.2^{bc}	28.7 ± 0.4^{ab}

* A – raw CN; B – CN stored and selected, calibration; C – CN subjected to humidification; D – CN cooked; E – CN dehydrated, after drying; F – CN selected, after tegument removal. Means followed by the same small letters in the columns do not differ at a 5% significance level according to Tukey's test.

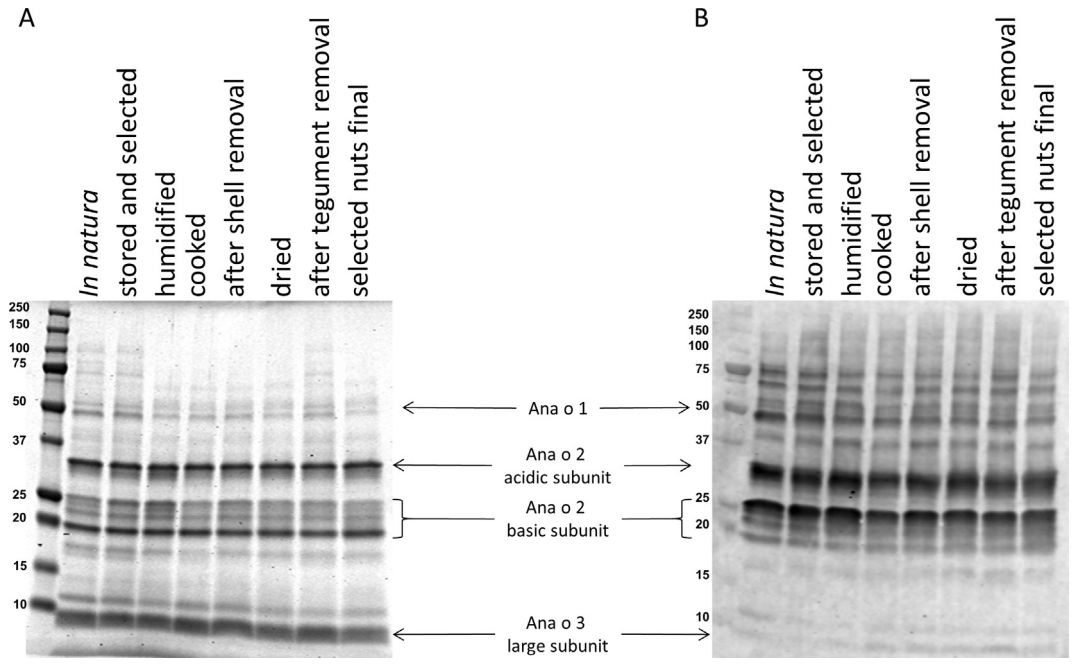

Fig. 2. Changes in protein solubility observed during industrial CN processing steps. CN extract samples from 6 industrial processing steps were evaluated by SDS-PAGE for protein solubility (A) and antibody recognition by using rabbit anti-cashew sera in immunoblots (B).

factors, such as vitamin D deficiency and folic acid supplementation, have been associated with allergic sensitization (Okupa et al., 2013; Sharief, Jariwala, Kumar, Muntner, & Melamed, 2011). Anacardic acid levels varied significantly among the samples during processing and peaked during the humidification step (Table 2). The peak in anacardic acid levels during humidification was transitory and was likely due to passage from the nut shell to the kernel. Collectively, the data show that the storage and industrial processing steps directly impact the anacardic acid concentration in CN and can affect the final anacardic acid concentration within finished nuts. Anacardic acid and other cashew nut shell liquids are under investigation for uses such as cancer therapies, anti-inflammatory agents, and anti-microbials (Hemshekhar, Sebastin Santhosh, Kemparaju, & Girish, 2012). The samples of industrially processed CN evaluated in this study contained appreciable

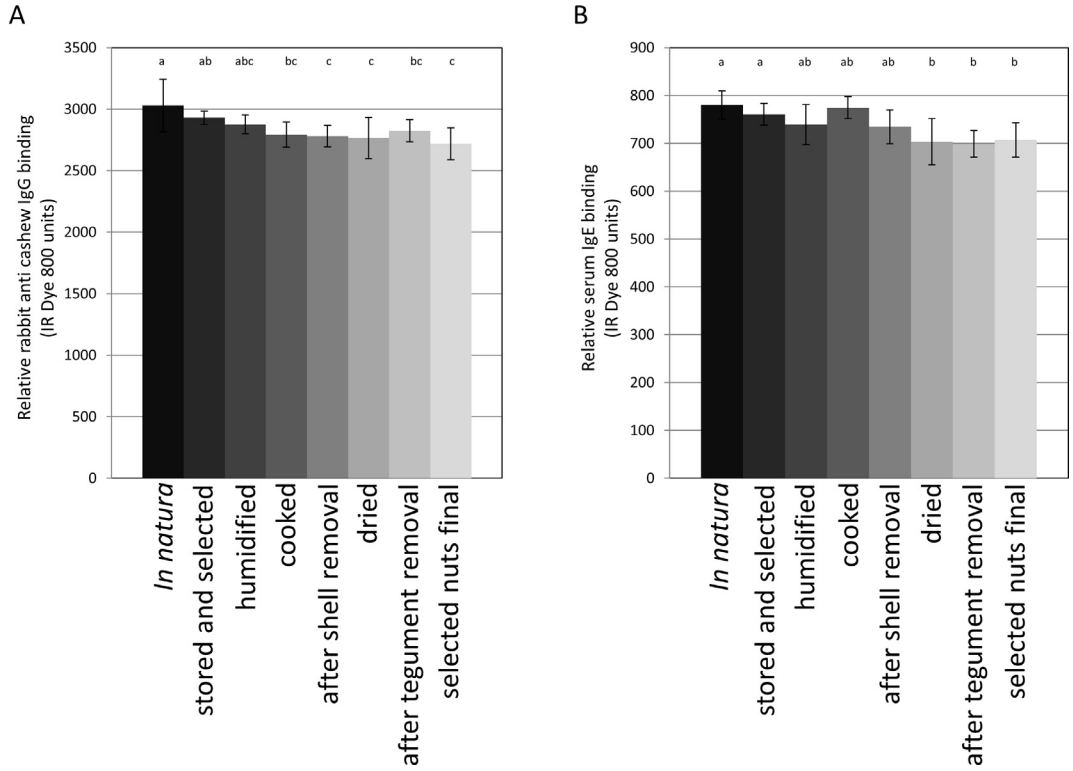

Fig. 3. Changes in antibody recognition observed from industrially processed CN samples. Antibody binding to industrially processed cashew nut samples was evaluated by ELISA using rabbit anti-cashew antibodies (A) and IgE from a pool of cashew allergic patient samples (B). Mean values ± standard deviation are plotted in each graph with small letters indicating significantly different means according to Tukey's test.

levels of anacardic acid in the final product. It seems possible that small amounts of anacardic acid remaining in the final product could act as an adjuvant that may alter the immunological response to cashew nut allergens, but additional research will be needed to establish clinical relevance.

Processing steps are thought to influence the allergenic potency of peanuts. Purified Ara h 1 and Ara h 2/6 peanut allergens treated by dry heat, in the presence or absence of glycating agents, were found to have decreased IgE binding; however, the basophil degranulation capacity of treated Ara h 1 was increased (Vissers, Iwan et al., 2011). Similarly, purified Ara h 2/6 from heated peanuts showed reduced IgE binding (Vissers, Blanc et al., 2011), while Ara h 1 purified from roasted peanuts bound IgE in a manner comparable to Ara h 1 purified from raw peanuts (Blanc et al., 2011).

Several studies have characterized the effects of processing steps on CN, but these reports use CN that have already been through industrial processing steps and are ready for consumption (Masthoff et al., 2013; Mattison et al., 2016). The role of chemical and enzymatic treatments has indicated that they can reduce IgE binding to cashew nut allergens (Mattison, Desormeaux et al., 2014; Mattison, Grimm et al., 2014). Physical and mechanical processing steps, such as roasting, microwaving, boiling, and gamma-irradiation, have been shown to have variable effects on IgG antibody binding to cashew allergens (Su, Venkatachalam, Teuber, Roux, & Sathe, 2004; Venkatachalam et al., 2008). Only mild differences in IgE binding to soluble proteins extracted from industrially processed cashew nut samples were observed using both immunoblot and ELISA assays (Figs. 2 & 3). Although the effect was mild, a slight overall decline in antibody binding to cashew proteins by both rabbit anti-cashew IgG and human IgE was observed as processing progressed. In the future it may be possible to develop modifications to cashew processing steps that result in CN with a reduced ability to cause allergic reactions. The development and application of novel processing steps that can reduce or eliminate the allergenic potency of cashew and other nuts while maintaining their nutritive value and sensory qualities would benefit public health and safety.

5. Conclusion

Industrial processing of CN involves several steps and possibly has significant effects on the quality and sensory characteristics of the final product. Anacardic acids were present in all samples and their concentrations changed during industrial processing. Knowledge of the variation in the anacardic acid content in CN at each step of industrial processing is of crucial importance for quality control of the product. The use of high temperatures during cooking could be of interest to the industry given the 75% reduction in total anacardic acid content that was observed in the current study. No large differences in rabbit IgG or human IgE antibody binding to cashew nut allergens was observed from samples at different stages of processing.

Acknowledgements

The authors are thankful to FUNCAP, UFC, EMBRAPA, INCT-FT, and CIONE. This research was supported by funds from the U.S. Department of Agriculture–Agricultural Research Service. Mention of trade names, commercial products, or companies in this paper is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

References

Agostini-Costa, T. S., Jales, K. A., Garruti, D. S., Padilha, V. A., Lima, J. B., Aguiar, M. J., et al. (2004). Anacardic acid content in cashew apples from Annacardium microcarpum and eight clones of Anacardium occidentale from Northeastern Brazil. *Ciência Rural*, *34*(4), 1075–1080.

Blanc, F., Vissers, Y. M., Adel-Patient, K., Rigby, N. M., Mackie, A. R., Gunning, A. P., et al. (2011). Boiling peanut Ara h 1 results in the formation of aggregates with reduced allergenicity. *Molecular Nutrition & Food Research*, *55*(12), 1887–1894.

Brasil. (2009). Thecnical standards for cashew nut. *Instrução Normativa N° 62, de 15 de dezembro de*.

Correia, S. J., David, J. P., & David, J. M. (2006). Secundary metabolites from species of Anacardiaceae. *Quimica Nova*, *29*, 1287–1300.

Fellows, P. J. (2006). Food processing technology: principles and practices. In Artmed (Vol. Ed.), *Food processing technology*(2nd ed.). *Vol. 1. Food processing technology* (pp. 602–). Porto Alegre: Artmed.

Fleischer, D. M. (2007). The natural history of peanut and tree nut allergy. *Current Allergy and Asthma Reports*, *7*(3), 175–181.

Ganeshan, K., Neilsen, C. V., Hadsaitong, A., Schleimer, R. P., Luo, X., & Bryce, P. J. (2009). Impairing oral tolerance promotes allergy and anaphylaxis: A new murine food allergy model. *Journal of Allergy and Clinical Immunology*, *123*(1), 231–238 e234.

Grabenhenrich, L. B., Dolle, S., Moneret-Vautrin, A., Kohli, A., Lange, L., Spindler, T., et al. (2016). Anaphylaxis in children and adolescents: The European Anaphylaxis Registry. *Journal of Allergy and Clinical Immunology*.

Gupta, R., Holdford, D., Bilaver, L., Dyer, A., Holl, J. L., & Meltzer, D. (2013). The economic impact of childhood food allergy in the United States. *JAMA Pediatrics*, *167*(11), 1026–1031.

Hemshekhar, M., Sebastin Santhosh, M., Kemparaju, K., & Girish, K. S. (2012). Emerging roles of anacardic acid and its derivatives: A pharmacological overview. *Basic & Clinical Pharmacology & Toxicology*, *110*(2), 122–132.

Jerz, G., Murillo-Velasquez, J. A., Skrjabin, I., Gok, R., & Winterhalter, P. (2012). Anacardic acid profiling in cashew nuts by direct coupling of preparative high-speed countercurrent chromatography and mass spectrometry (prepHSCCC-ESI-/APCI-MS/MS). *Recent Advances in the Analysis of Food and Flavors*, *1098*, 145–165.

Kubo, I., Masuoka, N., Ha, T. J., & Tsujimoto, K. (2006). Antioxidant activity of anacardic acids. *Food Chemistry*, 99.

Li, X. M., Serebrisky, D., Lee, S. Y., Huang, C. K., Bardina, L., Schofield, B. H., et al. (2000). A murine model of peanut anaphylaxis: T- and B-cell responses to a major peanut allergen mimic human responses. *The Journal of Allergy and Clinical Immunology*, *106*(1 Pt 1), 150–158.

Lima, J. R., Garruti, D. S., & Bruno, L. M. (2012). Physicochemical, microbiological and sensory characteristics of cashew nut butter made from different nut grades-quality. *Food Science and Technology*, *45*, 180–185.

Masthoff, L. J., Hoff, R., Verhoeckx, K. C., van Os-Medendorp, H., Michelsen-Huisman, A., Baumert, J. L., et al. (2013). A systematic review of the effect of thermal processing on the allergenicity of tree nuts. *Allergy*, *68*(8), 983–993.

Mattison, C. P., Desormeaux, W. A., Wasserman, R. L., Yoshioka-Tarver, M., Condon, B., & Grimm, C. C. (2014). Decreased immunoglobulin E (IgE) binding to cashew allergens following sodium sulfite treatment and heating. *Journal of Agriculture and Food Chemistry*, *62*(28), 6746–6755.

Mattison, C. P., Grimm, C. C., Li, Y., Chial, H. J., McCaslin, D. R., Chung, S. Y., et al. (2017). Identification and characterization of Ana o 3 modifications on arginine-111 residue in heated cashew nuts. *Journal of Agriculture and Food Chemistry*, *65*(2), 411–420.

Mattison, C. P., Grimm, C. C., & Wasserman, R. L. (2014). In vitro digestion of soluble cashew proteins and characterization of surviving IgE-reactive peptides. *Molecular Nutrition & Food Research*, *58*(4), 884–893.

Mattison, C. P. Y. B. M., Vant-Hull, B., Vargas, A. M., Wasserman, R. L., & Grimm, C. C. (2016). Heat-induced alterations in cashew allergen solubility and IgE binding. *Toxicology Reports*, *3*, 244–251.

Mazzeto, S. E., Lomonaco, D., & Mele, G. (2009). Cashew nut oil: Opportunities and challenges in the context of sustainable industrial development. *Quimica Nova*, *32*, 732–741.

Melo, M. L. P., Maia, G. A., Silva, A. P. V., Oliveira, G. S. F., & Figueiredo, R. W. (1998). Chemical composition of the raw and toasted cashew nut (Anacardium occidentale L.). *Ciência e Tecnologia de Alimentos*, *18*, 184–187.

Oetterer, M., Reditano-D'Arce, M. A. B., & Spoto, M. H. F. (2006). Fundamentals of Food Science and Technology. In 1 ed., (pp. 632). Barueri: Manole.

Okupa, A. Y., Lemanske, R. F., Jr., Jackson, D. J., Evans, M. D., Wood, R. A., & Matsui, E. C. (2013). Early-life folate levels are associated with incident allergic sensitization. *Journal of Allergy and Clinical Immunology*, *131*(1), 226–228 e221-222.

Owiredu, I., & Laryea, D. B. J. (2014). Evaluation of cashew nut flour in the production of biscuit. *Nutrition & Food Science*, *44*, 204–211.

Papanastasopoulos, P., & Stebbing, J. (2013). Nuts and cancer: Where are we now? *The Lancet Oncology*, *14*(12), 1161–1162.

Parker, C. H., Khuda, S. E., Pereira, M., Ross, M. M., Fu, T. J., Fan, X., et al. (2015). Multiallergen quantitation and the impact of thermal treatment in industry-processed baked goods by ELISA and liquid chromatography-tandem mass spectrometry. *Journal of Agriculture and Food Chemistry*, *63*(49), 10669–10680.

Radauer, C., & Breiteneder, H. (2007). Evolutionary biology of plant food allergens. *J. Allergy Clin. Immunol. 120*(3), 518–525.

Ribeiro, E. P., & Seravalli, E. A. G. (Eds.), (2007). *Food chemistry* (pp. 196–). (2 ed.). Sao Paulo: Blucher.

Robotham, J. M., Wang, F., Seamon, V., Teuber, S. S., Sathe, S. K., Sampson, H. A., et al. (2005). Ana o 3, an important cashew nut (Anacardium occidentale L.) allergen of the 2S albumin family. *Journal of Allergy and Clinical Immunology*, *115*(6), 1284–1290.

Ryan, E., Galvin, K., O'Connor, T. P., Maguire, A. R., & O'Brien, N. M. (2006). Fatty acid profile, tocopherol, squalene and phytosterol content of Brazil, pecan, pine, pistachio and cashew nuts. *International Journal of Food Sciences and Nutrition*, *57*(3–4), 219–228.

Sharief, S., Jariwala, S., Kumar, J., Muntner, P., & Melamed, M. L. (2011). Vitamin D

levels and food and environmental allergies in the United States: Results from the National Health and Nutrition Examination Survey 2005–2006. *Journal of Allergy and Clinical Immunology*, *127*(5), 1195–1202.

Stasiuk, M., & Kozubek, A. (2010). Biological activity of phenolic lipids. *Cellular and Molecular Life Sciences*, *67*, 841–860.

Su, M., Venkatachalam, M., Teuber, S. S., Roux, K. H., & Sathe, S. K. (2004). Impact of g-irradiation and thermal processing on the antigenicity of almond, cashew nut, and walnut proteins. *Journal of the Science of Food and Agriculture*, *84*(10), 1119–1125.

Suo, M., Isao, H., Ishida, Y., Shimano, Y., Bi, C., Kato, H., et al. (2012). Phenolic lipid ingredients from cashew nuts. *Journal of Natural Medicines*, *66*, 133–139.

Trevisan, M. T., Pfundstein, B., Haubner, R., Wurtele, G., Spiegelhalder, B., Bartsch, H., et al. (2006). Characterization of alkyl phenols in cashew (Anacardium occidentale) products and assay of their antioxidant capacity. *Food and Chemical Toxicology*, *44*(2), 188–197.

Venkatachalam, M., Monaghan, E. K., Kshirsagar, H. H., Robotham, J. M., O'Donnell, S. E., Gerber, M. S., et al. (2008). Effects of processing on immunoreactivity of cashew nut (Anacardium occidentale L.) seed flour proteins. *Journal of Agricultural and Food Chemistry*, *56*(19), 8998–9005.

Vissers, Y. M., Blanc, F., Skov, P. S., Johnson, P. E., Rigby, N. M., Przybylski-Nicaise, L., et al. (2011). Effect of heating and glycation on the allergenicity of 2S albumins (Ara h 2/6) from peanut. *PLoS ONE*, *6*(8), e23998.

Vissers, Y. M., Iwan, M., Adel-Patient, K., Stahl Skov, P., Rigby, N. M., Johnson, P. E., et al. (2011). Effect of roasting on the allergenicity of major peanut allergens Ara h 1 and Ara h 2/6: The necessity of degranulation assays. *Clinical and Experimental Allergy*, *41*(11), 1631–1642.

Wang, F., Robotham, J. M., Teuber, S. S., Sathe, S. K., & Roux, K. H. (2003). Ana o 2, a major cashew (Anacardium occidentale L.) nut allergen of the legumin family. *International Archives of Allergy and Immunology*, *132*(1), 27–39.

Wang, F., Robotham, J. M., Teuber, S. S., Tawde, P., Sathe, S. K., & Roux, K. H. (2002). Ana o 1, a cashew (Anacardium occidental) allergen of the vicilin seed storage protein family. *Journal of Allergy and Clinical Immunology*, *110*(1), 160–166.