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

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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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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

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