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Thermal processing effects on the IgE-reactivity of cashew and pistachio

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ABSTRACT

Thermal processing can modify the structure and function of food proteins and may alter their allergenicity. This work aimed to elucidate the influence of moist thermal treatments on the IgE-reactivity of cashew and pistachio. IgE-western blot and IgE-ELISA were complemented by Skin Prick Testing (SPT) and mediator release assay to determine the IgE cross-linking capability of treated and untreated samples. Moist thermal processing diminished the IgE-binding properties of both nuts, especially after heat/pressure treatment. The wheal size in SPT was importantly reduced after application of thermally-treated samples. For cashew, heat/pressure treated-samples still retain some capacity to cross-link IgE and degranulate basophils, however, this capacity was diminished when compared with untreated cashew. For pistachio, the degranulation of basophils after challenge with the harshest heat/pressure treatment was highly decreased. Boiling produced more variable results, however this treatment applied to both nuts for 60 min, led to an important decrease of basophil degranulation.

1. Introduction

Food allergy has a relevant impact on the quality of life of allergic people, and it is considered as an important lifelong persisting problem. Although the prevalence of food allergy varies depending on the geographical area, study populations analyzed and allergens studied, it is accepted that it affects up to 1–3% of the general population, reaching even 6–8% in children. Tree nuts are among the eight foods that cause the majority of allergic reactions to foods in Europe and the U.S. (Nwaru et al., 2014; Fernández Rivas, 2009). Furthermore, tree nuts are primarily responsible for fatal allergic reactions in the U.S and the U.K (Bock, Muñoz-Furlong, & Sampson, 2007; Pumphrey & Gowland, 2007). Tree nuts are included in the list of the most commonly allergenic ingredients (Regulation EU No 1169/2011/EC, OJEU 2011) and their presence in food must be indicated.

Consumption of tree nuts is on the rise due to their beneficial health effects, especially concerning risk reduction of coronary diseases and due to their rich nutritional composition. Particularly, pistachio nut contains a wide variety of healthy nutritional components, including high amounts of protein, antioxidants, minerals and low content of unhealthy fats (basically from MUFA and PUFA), among others (Bulló, Juanola-Falgarona, Hernández-Alonso, & Salas-Salvadó, 2015). Cashew nut, for its part, is highly energetic and rich in unsaturated fatty acids, fibre, amino acids and vitamins (Rico, Bulló, & Salas-Salvadó, 2015).

Typically, tree nuts allergens are identified as seed storage proteins, among others. In cashew, major allergens are characterized as a vicilin-like protein or 7S globulin (Ana o 1, 50 kDa), legumin-like protein or 11 S globulin (Ana o 2, 53 kDa) and 2S albumin (Ana o 3, 12 kDa) (Robotham et al., 2005; Wang et al., 2002; Wang, Robotham, Teuber, Sathe, & Roux, 2003). It seems that cashew allergy prevalence is increasing over the years and it has been involved in severe anaphylaxis, even exceeding peanut allergy in severity (Clark, Anagnostou, & Ewan, 2007; Van der Valk, Dubois, Gerth van Wijk, Wichers, & de Jong, 2014). Pistachio is also a well-characterized tree nut whose allergens belong to 2S albumin (Pis v 1, 7 kDa), legumin-like proteins or 11S globulins (Pis v 2 and Pis v 5, 32 and 36 kDa), vicilin-like protein or 7S globulin (Pis v 3, 55 kDa) and superoxide dismutase (Pis v 4, 25.7 kDa) (Ahn, Bardina, Grishina, Beyer, & Sampson, 2009; Noorbakhsh, Mortazavi, Sankian,

Abbreviations: BCA, Bicinchoninic acid assay; DBPCFC, double-blind placebo-controlled food challenge; FEIA, Fluor-enzyme-immunoassay; HRP, horseradish peroxidase; MRA, mediator release assay; PVDF, polyvinylidene difluoride; SPT, Skin Prick Testing; TBS, Tris buffered saline; TBST, TBS plus 0.5% Tween-20; TMB, tetramethylbenzidine

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Shahidi, & Assarehzadegan et al., 2010; Willison et al., 2008). Cross-reactivity between cashew, pistachio and mango, all of them members of *Anacardiaceae* family, has been observed (García & Lizaso, 2011; Noorbakhsh et al., 2011). Currently, there is no treatment for cashew and pistachio allergy. Therefore, avoidance is the only effective "therapy" for allergic patients. However, cashew and pistachio presence as traces is sometimes difficult to eliminate, due to cross-contamination in food lines (Taylor & Baumert, 2010).

Thermal (moist heating, dry heating, dielectric heating) and non-thermal (mechanical, enzymatic, irradiation) treatments are mainly carried out in industry to improve food quality, preservation or safety. Moreover, certain thermal processing methods are also used by consumers in order to improve sensorial properties of foods. Food processing can modify the structure and function of food proteins and may alter (by increasing or decreasing) their allergenic properties (Cabanillas & Novak, 2017). In that sense, understanding the potential effects of food processing on the allergenic properties of foods constitutes an active area of research.

In the specific case of nuts, the influence on allergenicity after a wide variety of different treatments has been studied (Jiménez-Saiz, Benedé, Molina, & López-Expósito, 2015; Vanga & Raghavan, 2016; Verhoeckx et al., 2015). Thermal treatments in walnut (Cabanillas et al., 2014), HHP in hazelnut (Prieto et al., 2014), roasting, blanching, autoclaving and microwave heating in almond (Venkatachalam, Teuber, Roux, & Sathe, 2002) and several thermal processing conditions in peanut (Cabanillas et al., 2012, 2015; Maleki, Chung, Champagne, & Raufman, 2000) have been studied with different results, depending on the conditions of the treatments and the material analyzed. Knowledge about the effects of thermal processing on tree nuts such as cashew or pistachio is scarce and based on traditional *in vitro* immunoassays. Only a few studies have analyzed the influence of various treatments including autoclaving (at 121 °C), blanching, pH variation, microwave heating and γ-irradiation over cashew seeds. Although cashew proteins showed high stability to all processing methods used, autoclaving or a combination of γ-irradiation plus autoclaving seemed to cause some decrease in antigen detection (Su, Venkatachalam, Teuber, Roux, & Sathe, 2004; Venkatachalam et al., 2008). Mattison et al. (2014) found that sodium sulphite and heating treatment can modify the structure of specific cashew allergens, decreasing their IgE-binding (Mattison et al., 2014). Interestingly, the same authors also demonstrated by SDS-PAGE and LC-MS/MS that solubility of cashew proteins is modified by heat treatment and the relative amount of peptides from specific cashew allergens was also affected as well as IgE-binding capability of the soluble extracts (Mattison et al., 2016). Oleic acid has been found to bind cashew allergens, reducing the IgE-binding capacity (Chung, Mattison, Reed, Wasserman, & Desormeaux, 2015). In pistachio nuts, a reduced reactivity was observed by western blot and ELISA analysis after soaking in lemon water and steaming, without changes in sensory evaluation (Noorbakhsh, Mortazavi, Sankian, Shahidi, & Maleki et al., 2010).

An altered ability of food allergens to bind IgE using traditional *in vitro* immunoassays is not always directly related to a modified allergenic function (Shi et al., 2013). Therefore, physiologically relevant experiments, such as SPT and mediator release assays (MRA), in which the IgE cross-linking capacity of processed food proteins is analyzed in effector cells of allergy, should constitute an essential part on the research of allergenic properties of processed food. This kind of studies are important preliminary tests to ensure a possible reduction in IgE cross-linking capacity, before performing further clinical studies.

The aim of this work was to elucidate the influence of moist thermal treatments (boiling and heat/pressure) on the IgE-reactivity of cashew and pistachio proteins, by means of traditional *in vitro* immunoassays, and physiological relevant assays as SPT and MRA.

2. Materials and methods

2.1. Plant material, thermal processing and protein extraction

Cashew (*Anacardium occidentale,* type 320) obtained from Productos Manzanares S.L. (Spain) and raw pistachio (*Pistacia vera*, Kerman) from the Germoplasm Bank of Institut de Recerca i Tecnología Agroalimentàries (IRTA-Mas de Bover, Tarragona, Spain) were used for this study. Cashew nuts were not purchased as raw, since they were industrially processed in order to remove harmful oils, shell and the skin.

Nuts were boiled in distilled water (1:5 w/v) for 30 and 60 min (named as "boiled 30′" and "boiled 60′" respectively), or subjected to heat and pressure treatments in distilled water (1:5 w/v) using a Compact 40 Benchtop autoclave (Priorclave, London, UK) at 121 °C (1.18 atm) for 15 min and 30 min (named as "AU 121 °C 15′" and "AU 121 °C 30′") or 138 °C (2.56 atm) for 15 and 30 min (named as "AU 138 °C 15′" and "AU 138 °C 30′").

Untreated and treated nuts were freeze-dried (Telstar Cryodos frezze-drier), ground using a kitchen robot (Thermomix 31-1, Vorwerk Elektrowerke, GmbH & Co. KG, Wüppertal, Germany), defatted with *n*-hexane (34 ml/g of flour) and milled with a sieve of 1 mm (Tecator, Cyclotec 1093, Höganäs, Sweden). The nitrogen contents of the pistachio and cashew flours were determined by LECO analysis, according to standard procedures based on Dumas method. The total protein content was calculated as N × 5.3 (AOAC, 2000).

Proteins from treated and untreated defatted flours from cashew were extracted in a solution of Borate Buffer Saline (BSB) 1:10 w/v (100 mM H_3BO_3, 25 mM $Na_2B_4O_7$ and 75 mM of NaCl, pH 8.4), overnight at 4 °C with constant shaking. After sonication (three times 15 s), centrifugation was carried out at 8250*g* (8500 rpm) at 4 °C for 20 min. Supernatant was collected and sterilized with 0.22 µm filters. The same buffer but adding 1% polyvinylpyrrolindone (PVP) was employed to obtain pistachio protein extract from untreated and treated defatted flours at 1:10 w/v, for 1 h at 4 °C and constant stirring. After centrifugation (27419*g* or 15000 rpm, 20 min at 4 °C), supernatants were dialyzed against distilled water using a membrane with a cut-off point of 3.5 kDa for 24 h at 4 °C, and then they were freeze-dried. Pistachio dry extracts were then resuspended in sterile PBS buffer and sterilized with 0.22 µm filters. The bicinchoninic acid assay (BCA) (Pierce Biotechnology, Rockford, IL, USA) was used for protein extracts quantitation.

2.2. Patients and sera

Sera from six patients with clinical allergy to pistachio and/or cashew, confirmed on the basis of either a convincing history of anaphylaxis with positive SPT and specific serum IgE levels to pistachio and/or cashew measured by means of fluorescent enzyme immunoassay (CAP-FEIA system, Phadia, Uppsala, Sweden), shown in Table 1, or a positive double-blind placebo-controlled food challenge (DBPCFC). The study was approved by the Ethics Committee of the Hospital Universitario 12 de Octubre, Madrid, Spain (Permission No. 0312150129).

Cashew and/or pistachio allergic patients underwent SPT with untreated and treated samples according to standard methods (Malling, 1993). The mean diameters of SPT reactions were expressed in millimetres, and calculated as the sum of the largest diameter and the perpendicular distance, divided by two. SPT was performed in duplicate and a positive (histamine dihydrochloride) and negative (PBS) control were applied. Positive results were considered when wheal size was at least 3 mm greater than that elicited by the negative control. Paired *t*-test was used for comparison of means from untreated with treated samples, and differences were considered significant with $p < .05$. The statistics software GraphPad Prism version 5 for Windows (GraphPAd Software, San Diego, CA, USA) was used.