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Influence of enzymatic hydrolysis on the allergenic reactivity of processed cashew and pistachio

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ABSTRACT

Cashew and pistachio allergies are considered a serious health problem. Previous studies have shown that thermal processing, pressurization and enzymatic hydrolysis may reduce the allergenic properties of food by changing the protein structure. This study assesses the allergenic properties of cashew and pistachio after thermal treatment (boiling and autoclaving), with or without pressure (autoclaving), and multiple enzymatic treatments under sonication, by SDS-PAGE, western blot and ELISA, with serum IgE of allergic individuals, and mass spectroscopy. Autoclaving and enzymatic hydrolysis under sonication separately induced a measurable reduction in the IgE binding properties of pastes made from treated cashew and pistachio nuts. These treatments were more effective with pistachio allergens. However, heat combined with enzymatic digestion was necessary to markedly lower IgE binding to cashew allergens. The findings identify highly effective simultaneous processing conditions to reduce or even abolish the allergenic potency of cashew and pistachio.

1. Introduction

Food allergy affects 6–8% of children and approximately 2% of the population. It has been estimated that food allergy causes approximately 30,000 anaphylactic reactions and 2000 hospitalizations annually in the U.S. (Jerschow, Lin, Scaperotti, & McGinn, 2014). This increase in the prevalence and severity of food allergy has led to growing concerns by consumers and the food industry (Taylor & Hefle, 2001). Peanuts and tree nuts are the leading cause of fatal anaphylactic reactions caused by foods (Bock, Munoz-Furlong, & Sampson, 2001) and have a significant impact on the quality of life (Primeau et al., 2000). The prevalence of allergy to nuts is estimated to be approximately1% in the UK and in the U.S. (Sicherer, Munoz-Furlong, Godbold, & Sampson, 2012). In Spain nuts are ranked second among foods most frequently involved in anaphylactic reactions (Fernandez-Rivas, 2009). Most nut allergens are seed storage proteins such as vicilins (7S globulin subunits composed of approximately 50–60 kDa), legumins (11–13S globulins composed of acidic subunits of 30–40 kDa and basic subunits of 15–20 kDa) and 2S albumins (15–20 kDa) (Crespo, James, Fernandez-Rodriguez, & Rodriguez, 2006). Other allergens of nuts with known biological function, such as lipid transfer proteins (LTP), profilins and pathogenesis-related, class 10 (PR-10) proteins are considered panallergens because they contribute to the allergenicity of a large group of pollen, nuts, seeds, fruits and other plants (Radauer & Breiteneder, 2007). A common feature of the nut allergenic proteins is their resistance to proteolysis and denaturation (Roux, Teuber, & Sathe, 2003).

Cashew (*Anacardium occidentale*) allergy is thought to be the second most allergic nut in the U.S. and may cause more severe reactions than peanuts (Clark, Anagnostou, & Ewan, 2007). Three allergenic proteins of cashew have been identified and characterized: Ana o 1 (7S vicilin, 50 kDa) (Wang et al., 2002), Ana o 2 (11S legumin, 55 kDa) (Wang, Robotham, Teuber, Sathe, & Roux, 2003) and Ana o 3 (2S albumin, 14 kDa) (Robothan et al., 2005). Pistachio (*Pistacia vera*) is another well characterized nut for its allergenic potential and cross-reactivity with cashew and mango (Noorbakhsh et al., 2011). Similar to other nuts, 4 of the five pistachio major allergens identified correspond to seed storage proteins: Pis v 1 (2S albumin, 7 kDa), Pis v 2 (11S legumin, 32 kDa), Pis

Abbreviations: HRP, Horseradish peroxidase; RT, room temperature

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Table 1
The description of the processing treatments and protein content, determined by LECO analysis, for cashew and pistachio samples of this study.

Code	Sample	Treatment	Protein content (g/100gr)
PDF 1	Defatted Flour Pistachio	Untreated (Control)	34.34
PDF 2	Defatted Flour Pistachio	Boiled 30 min	35.51
PDF 3	Defatted Flour Pistachio	Boiled 60 min	37.37
PDF 4	Defatted Flour Pistachio	Autoclaved 120 kPa, 15 min	41.08
PDF 5	Defatted Flour Pistachio	Autoclaved 120 kPa, 30 min	41.92
PDF 6	Defatted Flour Pistachio	Autoclaved 256 kPa, 15 min	43.20
PDF 7	Defatted Flour Pistachio	Autoclaved 256 kPa, 30 min	43.35
CDF 1	Defatted Flour Cashew	Untreated (Control)	33.06
CDF 2	Defatted Flour Cashew	Boiled 30 min	40.17
CDF 3	Defatted Flour Cashew	Boiled 60 min	37.71
CDF 4	Defatted Flour Cashew	Autoclaved 120 kPa, 15 min	36.63
CDF 5	Defatted Flour Cashew	Autoclaved 120 kPa, 30 min	35.41
CDF 6	Defatted Flour Cashew	Autoclaved 256 kPa, 15 min	42.90
CDF 7	Defatted Flour Cashew	Autoclaved 256 kPa, 30 min	37.81

v 3 (7S vicilin, 55 kDa) and Pis v 5 (11S legumin, 36 kDa) and a superoxide dismutase, Pis v 4 (25.7 kDa) (Ahn, Bardina, Grishina, Beyer, & Sampson, 2009; Ayuso, Grishina, & Ahn, 2007; Willison et al., 2008).

Food processing, particularly heat treatment, may alter the allergenicity of foods, and can therefore be useful in controlling allergenic risk (Besler, Steinhart, & Paschke, 2001; Maleki, 2004). Thermal treatment changes the structure, function, digestibility, solubility and immunogenicity of proteins, and therefore the overall allergenicity of the food (Cabanillas, Pedrosa et al., 2012; Liu et al., 2010; Maleki, 2004; Maleki & Hurlburt, 2004; Maleki, Schmitt, Galeano, & Hurlburt, 2014; Mueller, Maleki, & Pedersen, 2014; Nesbit, Hurlburt, Schein, Cheng, & Maleki, 2012; Noorbakhsh et al., 2010; Schmitt, Nesbit, Hurlburt, Cheng, & Maleki, 2010a). These effects depend on the temperature, type and duration of the treatment, the intrinsic characteristics of the protein and the physicochemical conditions of the microenvironment (Davis, Smales, & James, 2001; Maleki, Chung, Champagne, & Raufman, 2000; Nesbit et al., 2012; Schmitt et al., 2010; Wal, 2003). There are no general rules about the effect of processing on allergenicity. Processing can lead to the generation of new allergenic epitopes (neoallergens), as well as abolish the existing epitopes (Cuadrado et al., 2009; Maleki et al., 2000; Nesbit et al., 2012; Álvarez-Álvarez et al., 2005). Several studies have evaluated the changes induced by roasting, boiling, microwave heating, and pressure-cooking on legume and nut allergenicity, showing that processing based on pressure and certain heat treatments seems to have an important impact on in vitro IgE binding capacity (Cabanillas, Maleki et al., 2012; Cuadrado et al., 2009, 2011; Maleki et al., 2000; Schmitt et al., 2010; Álvarez-Álvarez et al., 2005). A study conducted with raw, dry roasted and steamed pistachio showed that immunoreactivity decreases only when roasting is performed with steam (Noorbakhsh et al., 2010). More recently, it has been shown that the multi-enzyme systems that combine the action of endo and exopeptidases cause significant destruction of IgE epitopes in peanut and lentil (Cabanillas, Pedrosa et al., 2012; Cabanillas et al., 2010). Cabanillas et al. (2014) also found that walnuts subjected to high pressure were more susceptible to gastric and duodenal digestion.

The main objective of this study is to assess the digestibility and human serum IgE binding capacity of both soluble and insoluble pistachio and cashew proteins after thermal treatment in combination with high pressure (autoclaving), enzymatic digestion and ultrasound treatments.

2. Materials and methods

2.1. Patients and sera

Sera from 7 patients (P1-P7) with pistachio and cashew allergy, confirmed on the basis of either a convincing history or recently documented reaction after nut ingestion, were used in this study (see Table 1 suppl). The study was approved by the Ethics Committee of Tulane Health Science Center (New Orleans, LA, USA) in accordance with the rules and regulations of the institutional review board.

2.2. Plant material and processing

Cashew (*Anacardium occidentale,* type 320) obtained from Productos Manzanares (Spain) and pistachio (*Pistacia vera*, variety Kerman) from the Germoplasm Bank of Institut de Recerca i Tecnología Agroalimentàries (IRTA-Mas de Bover, Tarragona, Spain) were used in the study. Whole nuts seeds were immersed in distilled water (1:5 w/v) and boiled (100 °C, 30 and 60 min) or autoclaved using an autoclave Compact 40 Benchtop (Priorclave, London, UK) at 121 °C, 120 kPa, for 15 and 30 min and at 138 °C, 256 kPa, for 15 and 30 min. Untreated, boiled and autoclaved nut seeds were ground and defatted with n-hexane (34 ml/g of flour) for 4 h, shaken, and air-dried after filtration of the n-hexane. Defatted flour from untreated cashew and pistachio were the controls for boiled and autoclaved samples. The nitrogen contents of the samples were determined by LECO analysis according to standard procedures based on Dumas method (AOAC, 2003). The total protein content was calculated as N × 5.3 (AOAC, 2003). The analyses were carried out in duplicate and the results summarized in Table 1. The protein content of processed samples was higher than in the raw ones. This could be related to the reduction of dry matter (i.e. carbohydrates) in these processed samples.

Seven food-grade proteases (Amano Enzyme Europe Ltd., Agno, Switzerland) were tested for digestion of cashew and pistachio samples and are referred to as E1-E7 in this study: E1 – Themoase PC10F (endoprotease), E2 – ProteAX (exoprotease), E3 – Protin SD – NY10 (proprietary), E4 – Peptidase R (exopeptidase), E5 – Protin SD – AY10 (alkalase-like), E6 – Protease M “Amano” SD (proprietary), E7 – Protease P “Amano” 3SD (proprietary). All are stable at 50–55 °C and pH 7. The first three were recommended by Amano for the potential to digest allergenic proteins (E1-E3). Digestion of cashew and pistachio protein extracted with buffered saline borate (BSB, 0.1 M H_3BO_3, 0.025 M $Na_2B_4O_7$, 0.075 M NaCl, 1% w/v PVP, pH 8.45) (2 mg/ml) was carried out by incubation with 1 mg/ml PBS pH 7.4 of each enzyme at 55 °C over a period of 19 h while taking samples at various time points (0, 1, 2, 3 and 19 h). After SDS-PAGE analysis, E3 and E5 or E1, E3, E5 and E7 enzymes were selected for further experiments in pistachio and in