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Effects of combined high pressure and thermal treatment on the allergenic potential of peanut in a mouse model of allergy

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ABSTRACT

Allergic reactions to peanut proteins are a worldwide concern. Ara h 2 in peanut (PN) is one of the most important peanut allergens (Koppelman, Wensing, Ertmann, Knulst, & Knol, 2004), which is recognized by serum immunoglobulin E (IgE) from more than 90% of peanut-allergic individuals studied by Stanley et al., 1997. Therefore, this study aimed at determining whether high pressure and thermal treatment could reduce the allergic properties of PN. Peanut protein extracts were treated with high pressure in 100–600 MPa and/or thermal treatment in 25–75 °C for 2.5–30 min, and the immunoreactivity of PN were analyzed by competitive inhibition enzyme-linked immunosorbent assays using pool sera from eight patients with peanut specific IgE. Compared to the boiling treatment, a significant reduction in IgE binding was observed when the samples were treated at 55 °C, 600 MPa for more than 10 min. Furthermore, in a BALB/c mouse model of allergy, significant reductions in specific IgE titers were observed in PN treated with high pressure and thermal (PNH) fed mice, accompanied by reduced histamine levels in serum. Similarly, real-time reverse transcription polymerase chain reaction revealed the decrease in mRNAs encoding interferon-γ, interleukin (IL)-4 and IL-10 in ileum tissues. PNH had almost no allergenicity in the BALB/c mouse model of PN allergy.

Industrial relevance: High hydrostatic pressure (HHP) processing can inactivate microorganisms and enzymes, leaving sensory and nutritional quality almost unchanged. Furthermore, HHP could alter allergen conformation and hence decrease allergenic activity in rice, fish, milk and celery that were reported. As a health concern, peanut allergies have been studied in this work. We determined whether high pressure and thermal could reduce the allergic properties of peanut allergen Ara h 2. In addition, effects of high pressure and thermal treatment on the peanut allergenicity of Ara h 2 (PNH) were characterized using a BALB/c mouse model of allergy.

1. Introduction

Food allergy has a growing prevalence worldwide. Whereas the prevalence among children younger than 4 years is higher at approximately 6–8% and about 4% of the US population older than 10 years (Lee & Burks, 2006). Several food constituents and food products can cause allergy including milk, egg, soy, peanut, tree nuts, fish, shellfish, wheat, and also some fruits like kiwifruit, peach, cherry, and apple (Fernández-Rivas et al., 2006; Zuidmeer et al., 2008). Peanut allergy has become a major health concern worldwide, especially in developed countries, which affects approximately 1% of children under the age of 5 years (Husain & Schwartz, 2012). Peanut and its products are widely eaten by consumers worldwide. Meanwhile, peanut allergies are often associated with severe reactions. Peanut allergy is a typical IgE-mediated immune disease. The early symptoms include pruritus, colicky abdominal pain, vomiting, diarrhea and urticaria. Progressive respiratory symptoms, hypotension and dysrhythmias typically develop in fatal and near-fatal cases. Although, the allergy is not often triggered by skin or air contact with the peanut protein, the peanut must be eaten before life-threatening symptoms will occur (Burks, 2008; Sampson, 2002). In view of the severity of the clinical reactions and because the peanut allergy can be life-long, effective treatments need to be developed.

Seventeen peanut allergens have been identified that are termed as Ara h 1 to Ara h 17. The two major peanut allergens, Ara h 1 and 2, are

Abbreviations: ELISA, enzyme-linked immunosorbent assay; HHP, high hydrostatic pressure; IFN, interferon; IG, intragastric; IL, interleukin; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; SD, standard deviation; SPT, skin prick test; PN, Ara h 2 in peanut; PNH, high-pressure and heat-treated Ara h 2; CT, cholera toxin; mRNA, messenger ribonucleic acid; IgE, immunoglobulin E.

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part of the vicilin and conglutin families of storage proteins, respectively (Burks et al., 1991, 1992; Koppelman, Wensing, Ertmann, Knulst, & Knol, 2004). Several methods have also been used in an attempt to inactivate the allergenicity of peanut, including heat treatment, gamma-irradiation and enzymatic hydrolysis. Curing temperatures between 35 °C and 60 °C gave no difference in the allergenicity of peanut. However, a higher curing temperature (i.e., 77 °C) exhibited a profile of higher levels of IgE binding (Chung, Butts, Maleki, & Champagne, 2003). Beyer et al. (2001) reported compared with roasted peanuts, the relative amount of Ara h 1 reduced in the fried and boiled preparations, resulting in a significant reduction of IgE-binding intensity. In addition, there was significantly less IgE binding to Ara h 2 and Ara h 3 in fried and boiled peanuts compared with that in roasted peanuts. High thermal treatment would affect the quality of the peanut, which was due to change the properties of peanut protein. Boiling followed by γ-irradiation is significantly better in reducing the allergenicity. The IgE binding to boiled and γ-irradiated protein reduced further in peanut (43–68%) as compared to the raw antigens (Kasera et al., 2012). In addition, the allergenicity of peanut extracts could reduce by γ-irradiation in the peanut-sensitized mice. Although properly irradiated food is safe and wholesome, it is not accepted by many consumers who are not yet well informed about the technology (Parlato, Giacomarra, Galati, & Crescimanno, 2014). Enzymatic treatments were regarded as an effective way to reduce the allergenicity of peanut allergens. For example, Alcalase treatment has the potential to reduce the concentrations of major allergens in roasted peanuts and lower the allergenic potential of the peanuts and peanut containing food products (Yu et al., 2015). Similarly, peroxidase can help reduce the allergenic properties of roasted peanut allergens (Chung, Maleki, & Champagne, 2004). However, the newly formed peptides resulting from these enzymatic reactions have shown to affect the sensory qualities of the food products (Shimakura, Tonomura, Hamada, & Shiomi, 2005). Therefore, further research and development of processing technology has been undertaken to inhibit allergen activity or to remove allergens for the preparation of hypoallergenic or non-allergenic food, which may have benefits for food-allergic consumers (van Putten et al., 2011).

As a non-thermal food preservation method, high hydrostatic pressure (HHP) inactivates microorganisms and enzymes at low or moderate temperatures, while maintaining fresh flavor, color, texture, and nutrient content (Buckow, Weiss, & Knorr, 2009; Zhang & Mittal, 2008). Recently, HHP has been applied to a range of food products including fruit juices, jams, guacamole, oysters, and ready-to-eat meat products in several countries (Mújica-Paz, Valdez-Fragoso, Samson, Welti-Chanes, & Torres, 2011; Ramaswamy, Yanwen, & Songming, 2010; San Martin, Barbosa-Canovas, & Swanson, 2002; Schmidgall, Hertel, Bindrich, Heinz, & Toepfl, 2011). In addition, several studies have suggested that HHP can alter the allergen conformation and therefore decreases allergenic potential in foods including rice (Kato, Katayama, Matsubara, Omi, & Matsuda, 2000), fish (Liu & Xue, 2010), milk (Järvinen, Chatchatee, Bardina, Beyer, & Sampson, 2001; Kleber, Maier, & Hinrichs, 2007), and celery (Jankiewicz et al., 1997). In combination with thermal or enzymatic treatments, HHP may be an important tool for reducing or removing the immunoreactivity of β-lactoglobulin in milk and whey proteins in soybeans (Peñas, Préstamo, Polo, & Gomez, 2006). Several studies reported effects of HHP treatment on structural changes and antigenicity of Ara h 2 in peanut. Johnson et al. (2010) showed that no changes in secondary structure were observed following high-pressure treatment at 20 or 80 °C. However, high-pressure microfluidisation treatment changed the secondary structure of Ara h 2 and decreased nearly 50% antigenicity (Hu et al., 2011). With similar study, the immunoreactivity levels of peanuts treated with 600 and 800 MPa pressure for 10 min decreased by 69.2% and 73.3%, respectively (Huang, Yang, & Wang, 2014).

In view of the reports were not consistent. The present study investigated the effects of HHP and thermal treatment on the allergenicity of Ara h 2 from peanut and the role of Ara h 2 treatment on the reduction of peanut allergenicity in an animal model using indirect ELISA, PCR and so on. These data may provide evidence to support the use of HHP and thermal treatment to decrease the allergenicity of food products.

2. Materials and methods

2.1. Preparation of peanut allergen Ara h 2 (PN) extracts

Unshelled raw Xianghua peanuts purchased from local market in Yangling, Shaanxi, were used in this study. Peanut allergen was prepared as described previously (Hu et al., 2011). Peanuts were powdered manually using a mortar and pestle with liquid nitrogen and then defatted by stirring in acetone at a powder/acetone ratio of 1:5 (*w*/*v*) for 3 h at 4 °C. The supernatant was removed by centrifuging three times at 8500 ×*g* for 15 min at 4 °C to obtain the defatted powder. Peanut protein extract was obtained by mixing the defatted powder with 50 mM of Tris–HCl (pH 7.2) at a powder/solvent ratio of 1:5 (*w*/*v*). After 10 h of stirring at 4 °C the supernatant was collected by centrifugation at 8500 ×g for 15 min at 4 °C and ionic salts were removed by subsequent dialysis with the same buffer for 48 h at 4 °C. After that, the anion exchange chromatography was used to purify the peanut allergen Ara h 2. The crude extract was loaded onto DEAE-Sepharose Fast Flow Resin equilibrated with 50 mM of Tris–HCl (pH 7.2). The column was washed until the peak appeared and then proteins were eluted successively with 0–0.2 mM of NaCl gradient in 50 mM of Tris–HCl and the fractions were collected for further analysis.

2.2. High pressure treatment

A high-pressure equipment (FPG 7100: 9/2C, Stansted Fluid Power Ltd., Stansted, UK) was used for HHP treatment, which had a pressure vessel volume of 2 L with 500 mm height and 70 mm diameter. 30% (*v*/v) of propylene glycol of distilled water was used as the pressure-transmitting fluid. The rate of pressure increase was approximately 300 MPa/min and the pressure release was immediate. The temperature increase due to adiabatic heating was approximately 2.5 °C per 100 MPa. The pressure transmitting fluid was also preheated to the desired initial temperature. The unit has three K-type thermocouples (Omega Engineering, CT, USA), two of which are attached to the carrier's top closure and can be used near the samples. An aliquot (2.5 mL) of PN solution was packaged by a vacuum-packing machine (Rishang Co., Ltd., Beijing, China) in a clear Nylon/polyethylene retort pouch. The bags were treated with different pressures (100, 200, 300, 400, 500, and 600 MPa) and temperatures (25, 35, 45, 55, 65, and 75 °C) for various holding times (2.5 5.0, 7.5, 10, 15, 20, and 30 min), producing HHP-treated PN (PNH). Each treatment was carried out in triplicate. Control samples were treated with boiling for the same time.

2.3. Mouse model of intragastric (IG) administration sensitization and sample collection

BALB/c female mice (7 weeks old) were purchased from the Beijing Experimental Animal Center and randomly divided into three groups, i.e., the positive control (control +), negative control (control −), and PNH-fed (PNH) groups, with 60 mice per group. All animals were raised in our animal facility, which was maintained at 20–22 °C under a 14-h (6:00–20:00) light and 10-h (20:00–6:00) dark schedule. Mice were maintained under hygienic conditions with free access to food and water. All procedures were performed in accordance with the guidelines established by the Northwest A & F University Animal Care and Use Committee.

Following a 1-week acclimatization period, the control + group and PNH group were sensitized with 1 mg of intact intragastric (IG) PN extract plus 10 μg cholera toxin (Gentaur, Brussels, Belgium) per mouse as an adjuvant in a total volume of 100 μL of PBS twice per week for a duration of 3 weeks. Control animals received equal amounts of CT in PBS.