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Key factors affecting the immunoreactivity of roasted and boiled peanuts: Temperature and water

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ABSTRACT

Processing time (5–35 min) and temperature (105 °C–150 °C) during thermal processing may affect the structure and immunoreactivity of peanut allergen protein. In the present study, we aimed to find a correlation between heating mode and characteristics of peanut protein, and optimize the heating condition to reduce the allergenicity. Roasted peanut protein under the condition of extremely high temperature (over 130 °C) resulted in high allergenicity, accompanied with reduced concentration and IgE binding capacity of Ara h 1 and Ara h 3, but increased Ara h 2/6 IgE binding capacity. In addition, the structure of roasted protein under the condition of 130 °C for 20 min was the loosest with higher surface hydrophobicity index, more β-fold and the irregular coil, and less α-helix. In contrast, the allergenicity of boiled peanut proteins was decreased as the concentration and IgE binding capacities of all allergen were reduced. Because of the water medium, all bonding energy in the boiling group was lower than that in the roasting group. Hydrophobic interactions and hydrogen bonds were more prominent on the boiling samples. The finding indicates mild roasted temperature or boiling for 10 min can effectively reduce the peanut allergenicity.

1. Introduction

Peanut protein is an ideal raw material for food processing and widely used in meat products, flour products and dairy products. However, peanut allergy has the highest lethality rate which poses a serious threat to human health (Husain & Schwartz, 2012; Finkelman, 2010; Young, Sanders, Drake, Osborne, & Civille, 2005). According to the data of epidemiological studies, 47% of allergy sufferers are allergic to peanuts (Burks, 2008). Around 1% of the population has diagnosed peanut allergy in North America and Europe (Ben-Shoshan et al., 2010; Sicherer, Muñoz-Furlong, Godbold, & Sampson, 2010). Approximately 12% of children at age 8 in the United Kingdom were sensitised to peanut (Nicolaou et al., 2010). Although the per capita consumption of peanuts in China was similar to that of the United States, cases of peanut allergies were extremely rare in China (Beyer et al., 2001). Shek et al. (2010) found the prevalence of peanut allergy was relatively low in Asian countries (0.47%) as compared with the Western-born Asian population (1.62%) of similar genetic decent. Peanuts are commonly boiled or fried in China and the most common method of cooking in the United States is roasting (Beyer et al., 2001). Therefore, it was speculated that peanuts eating form could also impact the sensitivity associated with peanuts.

Each processing had different effects on different types of food proteins which might change the epitope and the binding property of allergenic protein, and subsequently cause the differences in activation of lymphocyte and cytokine release in the process of allergic reactions (Sathe & Sharma, 2009). Thermal processing has the potential to modify the structure, solubility, and immunochemical reactivity of peanut allergen protein, such as effects on the space conformation, binding epitope and hydrophobicity (Mills, Sancho, Rigby, Jenkins, & Mackie, 2009; Shen et al., 2015). However, these changes in different cooking methods which are mainly controlled by heating temperature, time, environment and other addition ingredients were unpredictable. Such alterations were also connected with characteristics of allergens (e.g. chemical bond, molecular internal forces).

To date, 13 peanut allergens have been officially recognized by the International Union of Immunological Societies Allergen Nomenclature Sub-Committee (www.allergen.org). Ara h 1 and Ara h 2, belong to the vicilin and conglutin families of storage proteins, were reported to be highly allergenic in most reactions. Previous

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data showed that the most allergens of peanut such as Ara h 1 and Ara h 2 appeared to be highly thermostable (Koppelman, Bruijnzeel-Koomen, Hessing, & de Jongh, 1999). Thus heat treated had almost no effect on sensitization of peanut. Several studies have indicated that the roasted treatment increase the allergenic activity of peanut protein (Moghaddam et al., 2014; Mondoulet et al., 2005; Vissers et al., 2011). But a result of an animal experiment denied that heating raise the allergenicity (Kroghsbo et al., 2014; Shen et al., 2015). The reason for the inconsistency might due to the experimental conditions of heating temperature and time. In addition, a portion of studies has discussed the effect of temperature on the purified Ara h 1, Ara h 2 or other allergens to explore the mechanism (Blanc et al., 2011; Koppelman et al., 1999; Teodorowicz, Fiedorowicz, Kostyra, Wichers, & Kostyra, 2013). However, these methods often led to inconsistent results with native protein purified from raw peanuts, probably due to the protection effect within the peanut seed.

Therefore, it is necessary to find the effect of different heating mode on peanut allergenicity. In sum, we expect: To find a heating treatment mode (temperature and time) to reduce the immunoreactivity of peanut and be used as a pre treatment for peanut products; to bring a new understanding on the thermal processing on protein immunoreactivity and molecular structure.

2. Method

2.1. Materials

Bovine serum albumin (BSA), goat anti-human IgE-peroxidase conjugate, and Tween 20, were purchased from Sigma Chemical Co. (St. Louis, MO); Immobilon-P membrane was obtained from Millipore Corp. (Bedford, MA). Raw peanut were obtained from Grain Administration of Hebei Province. Seven patients were obtained from the Fourth Hospital of Hebei Medical University, Hebei, China. All the patients had a confirmed peanut allergy on the basis of an extensive history, physical examination, and skin prick testing. All the patient sera were individually tested. The protocol was approved by the local ethical committee of the Fourth Hospital of Hebei Medical University and written informed consent was obtained.

2.2. Protein preparations

Shelled peanuts were (a) in an oven set at 130 °C and collected after 5, 10, 15, 20, 25, 30 and 35 min of roasting; (b) roasted for 20 min at 105 °C, 120 °C, 130 °C, 140 °C and 150 °C, respectively. (c) boiled (100 °C) in water for 5, 10, 15, 20, 25, 30 and 35 min. All samples were defatted using acetone and ether. Protein extraction as followed description, 3 mL TBS (pH 8.0) buffer solution was added to 0.1 g defatted peanut powder with stirring constantly for 12 h at 4 °C. The mixture was centrifuged (15,000 *g* for 15 min) and the extraction was stored at −20 °C. Protein concentrations were determined by using Bradford and BCA analysis.

2.3. SDS-PAGE analysis

All proteins samples were separated by using sodium dodecyl sulfate-polyacrylamidegel electrophoresis (SDS-PAGE). 20 μg proteins samples were loaded into each well. Using a separating gel of 12% acrylamide and a stacking gel of 4% acrylamide for separated. Electrophoresis was performed for 30 min at 80 V for stacking gel and 80 min at 110 V for separating gel. The gels were stained with coomassie brilliant blue (R-250) for 30 min and destained overnight for 12 h. Images for the protein electrophoresis spectrum were taken using an BIO-RAD GelDoc 2000 gel imaging system.

2.4. Determination of intermolecular forces acting on proteins

2.4.1. Determination of free SH (thiol) groups

Methods from Wagner and Anon (1990); and Chan and Bruce (1993) were modified as follows: Forty milligrams of each protein samples were suspended in 0.9 mL of 8 mol/L urea and 1 g/100 mL sodium dodecyl sulphate (SDS) buffer. To make up to 1 mL, 0.1 mL of a 10 mmol/L Ellman's reagent, DTNB, was added to react with both soluble and insoluble proteins and release a soluble chromophore (NTB2). All samples stored at room temperature for an hour with shaking of samples every 15 min to allow solubilization of proteins. Micro-centrifugation (13,600 *g*, at room temperature) for 15 min was done to clarify samples before measuring the absorbance. After centrifugation, 0.1 mL of sample was diluted to 1 mL with 8 mol/L urea buffer and 1 g/100 mL SDS solution. The absorbance was measured at 412 nm by using a micro-plate reader (Spectra max190-Molecular devices, California, USA).

2.4.2. Determination of the secondary bonds

Methods from Pérez-Mateos et al. (1997) and Tan, Lai, and Hsu (2010) were modified as follows: One gram of protein sample was treated with each chemicals which selected for their capacity to cleave certain kinds of bond: (A) 0.05 mol/L NaCl, (B) 0.6 mol/L NaCl, (C) 0.6 mol/L NaCl + 1.5 mol/L urea, (D) 0.6 mol/L NaCl + 8 mol/L urea. The mixtures were stirred at 4 °C for 1 h, and then centrifuged for 15 min at 20,000 *g* in a centrifuge. Protein concentration in supernatants was determined using BCA analysis. The ionic bonds were expressed as the difference between protein solubilized in B and protein solubilized in A. The hydrogen bonds were the difference between protein solubilized in C and protein solubilized in B. Subsequently, the hydrophobic interactions were expressed as the difference between protein solubilized in D and protein solubilized in C.

2.5. Protein surface hydrophobicity (H_0)

Protein surface hydrophobicity (H_0) was determined by the hydrophobicity fluorescence probe according to the method reported by Kato and Nakai (1980). Protein sample were prepared in PBS (0.01 mol/L pH 7.0) and diluted to 0.2, 0.1, 0.05, 0.025 and 0.0125 mg/mL. A volume of 4 mL of each protein solution was then added with 20 μL of 1-anilinonaphthalene-8-sulfonic acid (ANS). The fluorescence intensity of mixture was measured with a Hitachi F2500 fluorescence spectrometer (Tokyo, Japan), at wavelengths of 390 nm (excitation) and 470 nm (emission). The initial slope of fluorescence intensity versus protein concentration plot was used as an index of H_0.

2.6. Fourier transform (FT) spectroscopy

Peanut protein secondary structure was detected by Fourier transform (FT) spectroscopy (Vector 33, Bruker Optics, Ettlingen, Germany). The peanut proteins were extracted from different treatment groups, and adjusted the protein content to the same level before freeze drying. Following the drying, 2 mg protein freeze-dried powder was added a certain amount of potassium bromide to 200 mg and then grinded into uniform powder and pressed into sheets. Each spectrum was vector normalized in the whole range from 400 to 4000 cm^{-1}. Averages of 128 scans at 1 cm^{-1} resolution in a reflective mode were collected from each sample. The baseline was corrected and 1600–1700 cm^{-1} segment was analyzed by using PeakFit v4.12 software. The automatic peak-seeking algorithm was based on the deconvolution and second derivative. And the proportion of all forms of secondary structure was calculated according to the peak area.