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Analytical Methods

Peanut protein sensitivity towards trace iron: A novel mode to ebb allergic response

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ABSTRACT

Peanut is a rich source of plant protein which is inexpensive and abundant in nature. The peanut proteins however cause hypersensitive immunogenic responses in certain individuals. A minute amount of contamination may cause strong allergic reactions and even death. Many chemical pretreatment procedures have been developed and prescribed earlier for removal of this allergenicity. In the present article we have observed trace level Fe(III) and Cu(II) complexation of the protein fractions of peanut at pH 4.8 using different spectral methods. Consequently we studied the allergic response of Fe(III) complex of the protein fractions using competitive enzyme linked immunosorbent assay (ELISA) technique and found that there were considerable losses in allergenicity of conarachin I and arachin fractions upon complexation. Immunoassay of Cu(II) complex was avoided keeping in view the Cu toxicity in living systems. The results bring up a new strategy towards reduction of allergenicity using an inexpensive and simple methodology.

1. Introduction

Peanut allergy is a type 1 hypersensitivity reaction to dietary peanut proteins that causes an over-reaction of the immune system in some people and may cause severe reactions like anaphylaxis and even death. Trace levels (~200 μg) of peanut can cause serious allergic reactions in people who have peanut allergy. Processed food items often contain undeclared amounts of peanuts from cross contamination, particularly in products made in facilities that also process peanuts. It should be beneficial to minimize this cross-contamination and prevent allergic consumers from unintentional uptake of peanuts. Peanut proteins are mostly globulins (saline soluble) (Hefle, 2005). The globulins are made up of two major proteins – arachin and conarachin (Jain, 2004). Arachin and conarachin find a number of applications in diverse fields, the most important being the food industry. They can also be used for detection of allergens (Krska, Welzig, & Baumgartner, 2004).

To date, several protein fractions have been identified as peanut allergens. The most common are Ara h 1 to Ara h 8, among which Ara h 1 (vicilin, also known as conarachin) and Ara h 2 (conglutin) are considered the major allergens because they cause reaction in 70–90% of sensitized individuals (Burks, Sampson, & Bannon, 1998). Ara h 3, a glycinin protein, is considered a minor peanut allergen because only 45% of patients with allergy show specific immunoglobulin E (IgE) in their sera (Rabjohn et al., 1999). Other minor peanut allergens include Ara h 4 (renamed as Ara h 3.02) (glycinin), Ara h 5 (profilin), Ara h 6/ Ara h 7 (conglutinin), and Ara h 8 (Bet v 1) (Mittag et al., 2004). Ara h 3 and Ara h 4 are together known as arachin. Table 1 represents the different peanut allergens with their type and extent of allergenicity (Jedrichowsky & Vichers, 2010). It is believed that solely by removal of the major peanut allergens, the allergenicity of peanuts is reduced. However removal of allergen part from total protein before consumption is not practically achievable. Alternative possibilities are therefore most welcome in the food industry.

Several possibilities have been suggested by the food chemists to minimize this peanut allergenicity. Cerasoli et al. (2014) reported that peroxidase (POD), a catalyst for protein cross-linking, reduces the allergenic properties of peanut. In their report, protein was extracted from raw and roasted defatted peanut meals at pH 8 and incubated with POD in the presence of hydrogen peroxide at 37 °C for 60 min. The samples were then analyzed by SDS–PAGE, western blots, and competitive inhibition enzyme linked

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immunosorbent assay (ELISA). Results indicated a significant decrease in the levels of the major allergens, Ara h 1 and Ara h 2, in roasted peanuts after POD treatment (Chung, Maleki, & Champagne, 2004). The same group also reported that polyphenol oxidase (PPO) and caffeic acid can also reduce the allergenic properties by cross-linking the allergens (Chung, Kato, & Champagne, 2005).

Later it was shown that processing also influences the allergenicity of peanut proteins. The effect of thermal processing on the IgE-binding capacity of whole peanut protein extracts and of the major peanut allergens Ara h 1 and Ara h 2 was studied. Whole proteins, Ara h 1, and Ara h 2 were extracted and purified from raw, roasted and boiled peanuts using selective precipitation and multiple chromatographic steps, and were then characterized by electrophoresis and mass spectrometry. The IgE-binding capacity of the whole peanut protein extracts prepared from boiled peanuts was 2-fold lower than that of the extracts prepared from raw and roasted peanuts. The proteins present in the cooking water were also recognized by the IgE of peanut-allergic patients. The decrease in allergenicity of boiled peanuts results mainly from a transfer of low-molecular-weight allergens into the water during cooking (Mondoulet et al., 2005). In another report a 6-fold reduction in IgE binding or allergenic potency of the peanut extract was observed after treatment with phytic acid. It was concluded that phytic acid formed insoluble complexes with the major peanut allergens, and resulted in a peanut extract with reduced allergenic potency (Chung & Champagne, 2007; Ghatak & Sen, 2013).

Iron plays a crucial role in the cellular functions of a majority of organisms present on earth by the virtue of its protein binding ability. Plasma iron present as Fe^{2+} becomes bound to its transport protein transferrin upon oxidation to Fe^{3+}, transferrin is then imported to the cell by endocytosis (Ba, Doering, Burkholz, & Jacob, 2009). Report on sodium iron chlorophyllin, a water-soluble semisynthetic chlorophyll derivative where the magnesium in the porphyrin ring has been substituted by iron also emphasizes its protein binding affinity (Miret et al., 2010).

This article reports for the first time the role of trace iron in the removal of allergenicity from major peanut proteins. The complexation of the purified proteins conarachin II, conarachin I and arachin were studied with different transition metals at trace concentrations at different pHs. The best condition of complexation was identified and confirmed using spectral studies. The allergenicity of this complex was found to get reduced in comparison to the pure conarachin I and arachin using competitive ELISA.

2. Experimental

2.1. Materials

Sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium acetate and acetic acid (for buffer), CH_3CH_2OH, H_3PO_4, NaOH, CH_3OH (Merck) were used as obtained. Coomassie brilliant blue G (for Bradford), acrylamide, ammonium persulphate, tris base, TEMED (for SDS PAGE) were obtained from HIMEDIA. $(NH_4)_2SO_4$, NaCl (Thermofischer), $MnSO_4{\cdot}H_2O$, $ZnSO_4{\cdot}7H_2O$, $NiSO_4{\cdot}6H_2O$, $CuSO_4{\cdot}5H_2O$, (Merck), $CrCl_3{\cdot}6H_2O$, $CoCl_2{\cdot}6H_2O$, $FeCl_3$ (LOBA Chemie) and all other chemicals used were of analytical grade.

Table 1
List of different peanut allergens.

Peanut allergens	Type of protein	Allergenicity
Ara h 1	Vicilin	Cause of 70–90% peanut allergy reactions
Ara h 2	Conglutin	
Ara h 3	Glycinin	Minor allergens
Ara h 4	Glycinin	
Ara h 5	Profilin	
Ara h 6	Conglutinin	
Ara h 7	Conglutinin	
Ara h 8	Bet v 1 family	

2.2. Apparatus

The UV visible spectra were obtained using an Agilent 8453 diode array spectrophotometer. A double beam Perkin–Elmer LS-55 was used for spectrofluorimetric studies. Circular dichroism (CD) spectra were recorded on a JASCO J-815 spectropolarimeter. A digital pH/ion meter Mettler (S 220-K) was used to measure and adjust the pH of different solutions. LISASCAN II (Erba) was used to determine the allergenicity by comparing the absorbance at 450 nm in the ELISA method. Raman spectra were collected in a micro-Raman set up consisting of a spectrometer (model Lab RAM HR, Jobin Yvon), and a Peltier-cooled charge-coupled-device (CCD) detector.

2.3. Methods

2.3.1. Separation of conarachin II and conarachin I from peanut

Sun dried peanuts were taken and their skins were peeled off. The peanuts were blanched and refluxed with petroleum ether. The lipid portion of peanuts was removed by refluxing in PET ether solvent for 2 h. The fat free mass of peanut was then filtered out and the air dried residue (fat free) was taken as peanut flour or peanut meal. To this meal 0.1% NaOH solution (w/v) was added and stirred well to bring the protein part into solution as water soluble sodium proteionate salt. Dilute H_2SO_4 was then added dropwise with constant stirring till the pH of solution became 5 and the protein precipitated out at this isoelectric pH. White precipitate of proteins was allowed to settle. Then it was centrifuged and the residue containing the protein-part (PP) was washed free from salts. Peanut proteins are mainly globulins. So to the residue 2% NaCl (w/v) solution was added. Conarachin I and II dissolved out in 2% NaCl solution. The 2% NaCl solution containing conarachin part was kept in the refrigerator, saturated to 32% with $(NH_4)_2SO_4$ solution and kept at 4 °C for at least 4 h. This solution was centrifuged at 4000g for 20 min and supernatant was further saturated to 42% $(NH_4)_2SO_4$ and further centrifuged. Supernatant was dialyzed against 0.01(M) phosphate buffer of pH 7.9 in 0.5 M NaCl solution (i.e. extraction buffer (EB)) to remove SO_4^{2-} ions. Pure conarachin solution was fractionated into conarachin I and conarachin II by Superose-6 gel chromatography column using EB. 2 ml samples were continuously taken in eppendorf tubes after pooling out the void volume (Monteiro & Prakash, 1994). UV visible absorption spectrum was studied for each of the 2 ml samples to determine the concentration of the protein. Fractions immediately after void volume containing high molecular weight fraction conarachin II were pooled. Rest of the fractions containing conarachin I was pooled afterwards. The protein solutions were concentrated using concentrating columns (Pall Corporation Macrosep Advance Centrifugal Device) to remove the buffer. Concentration of the resulting protein solutions were measured by the Bradford method (Bradford, 1976).

2.3.2. Separation of arachin from peanut

The defatted peanut meal was homogenized with 0.01 M phosphate buffer (saline) and stirred for 1 h. The solution was centrifuged for 30 min at 27 °C and at a speed of 3500g. To the clear supernatant $(NH_4)_2SO_4$ was added up to 18% saturation and kept at 4 °C for 2 h and centrifuged for 30 min again. The pellet was then dissolved in EB, saturated to 9% with $(NH_4)_2SO_4$ and kept at 4 °C for 1hr. Then the solution was centrifuged again and the pellet was