Beneficial Influence of Short-Term Germination on Decreasing Allergenicity of Peanut Proteins

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Abstract: Most allergenic storage proteins in peanuts are degraded during seed germination. By altering this natural physiological process, it might be possible to reduce peanut protein allergenicity. However, little is known about the change in allergenic proteins and their corresponding immunocreactivity, and the effects of major environmental conditions on their allergenicity during germination. In this study, the influence of different germination conditions (temperature and light) on the degradation of Ara h1 and allergenicity changes of peanut seeds was evaluated by ELISA and Western blotting. The results showed that the 40- and 65-kDa proteins in peanut seeds degraded rapidly during the time course, beginning at 60 (at 25 °C) and 108 h (at 20 °C), and the corresponding immunocreactivity of Ara h1 decreased approximately one-third after 5 to 7 d of germination. Compared with the cotyledons, the embryonic axes had a higher proportion of Ara h1, which was then degraded relatively faster during germination, resulting in a significant reduction in its allergenicity. Although a higher temperature improved the seed germination rate, it affected sprout quality (as did light); therefore, 25 °C and dark surroundings were suitable conditions under which peanut sprouts were processed; neither factor significantly affected the allergenicity of Ara h1. These results provided a theoretical basis for studies using biological methods to reduce peanut allergenicity.

Keywords: allergenic protein, allergenicity, Ara h1, germination, peanut seed

Practical Application: The present study would convince a positive role of germination in improving food safety, which could be an easy way to produce hypoallergenic peanut food.

Introduction

Peanuts have been widely used in food processing because they are rich in fats (45% to 53%) and protein (24% to 29%; Il-Ho and others 2007); however, they are also 1 of the 8 major food allergens causing approximately 0.5% to 2% of all food allergies, a proportion that increases every year (Cabanos and others 2011). Peanut allergy is an IgE-mediated adverse immune reaction with symptoms ranging from mild oral allergy syndrome to anaphylactic reactions and sometimes even death (Cabanos and others 2011). In addition, peanut allergy not only occurs in patients at an early age, but lasts a lifetime (DeLong and others 2011). Thirteen peanut allergens have been identified, including the Ara h1-11, with official recognition by the International Union of Immunology Societies, and the recently identified agglutinin and 18-kDa oleosin (Mari and others 2006; Cabanos and others 2011). Most peanut allergens are seed storage proteins (Breiteneder and Ebner 2000).

Ara h1, Ara h2, and Ara h3 are characterized as major peanut allergen proteins because they are recognized by the sera from 40% to 90% of patients with peanut allergy (Clarke and others 1998; Jong and others 1998; Kleber–Janke and others 1999; Rabjohn and others 1999). The 65-kDa Ara h1 (Szymkiewicz 2010), also named conarachin, is a seed storage globulin with the highest concentration in peanuts, comprising 12% to 16% of the total peanut protein (Jong and others 1998). Ara h1 has a 40% similarity with the soybean (*Glycine max*) and pea (*Pisum sativum*) vicilins at the protein level, and a 64% similarity with broad bean (*Vicia faba*) and pea vicilins at the DNA level (Lycett and others 1983; Burks and others 1995). By using the IgE serum pool from peanut-allergic patients, 23 linear epitopes have been identified in Ara h1, and most of them are placed in the hydrophobic regions of the monomer binding sites (Cabanos and others 2011). Ara h2 is a glycoprotein with a molecular weight of 17 to 19 kDa and that inhibits the activity of trypsin (Burks and others 1992; Maleki and others 2003). Ara h6 and Ara h7 exhibit 60% and 40% to 50% sequence identity, respectively, with Ara h2, and the former shows inhibitory effects on trypsin and chymotrypsin (Szymkiewicz 2010). Ara h3 and Ara h4 are classified into the 11S cupin superfamily and are isomers. After sodium dodecyl sulfate (SDS) electrophoresis (under reducing conditions), Ara h3 extracts produced a series of proteins with a molecular weight ranging from 14 to 45 kDa (Koppelman and others 2003).

Ara h5, Ara h8–11, agglutinin, and 18-kDa oleosin are non-seed storage proteins. Ara h5 is a 14-kDa protein with high thermal stability, and has an 80% similarity in amino acid sequence with actin (Kleber–Janke and others 1999). Ara h8 is a 17-kDa monomeric protein belonging to the Bet v 1 family (Szymkiewicz 2010); Ara h9 is a lipid transfer protein; Ara h10–11 are 16- to 17-kDa oleosins, and agglutinin is a lectin associated with cellular recognition (Szymkiewicz 2010; Cabanos and others 2011).

Food processing will affect the activity of allergenic proteins differently. For example, heating will cause structural alteration of allergenic proteins, such as inactivation and bond breaking or forming (Shriver and Yang 2011), and might lead to the loss of conformational or linear epitopes (Thomas and others 2007; Cabanillas and others 2012) or make them more susceptible to hydrolysis by digestive enzymes (Jiménez-Saiz and others 2011).

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Apart from this, thermal processing is able to induce the Maillard reaction between allergenic proteins and reducing sugar, thereby increasing the activity of allergens (Maleki and others 2000; Nakamura and others 2006). Other major components in the food, such as sugar and fat, can also influence the activity of allergenic proteins. It has been reported that fat increases the immunoreactivity of allergenic proteins in peanuts (van Wijk and others 2005), and pectin exerted a protective role for allergic proteins in kiwifruit (Polovic and others 2007).

No effective clinical treatment has been developed for peanut allergy, and avoiding allergen exposure might appear to be a simplistic approach to reduce this allergy (Schmitt and others 2010); however, the wide use of peanuts in food processing makes nearly impossible to completely avoid all allergens, and studies have shown that as high as 75% of consumers suffer an allergy from accidental ingestion of peanut protein (Kagan and others 2003). Thus, reducing the levels or activity of allergenic proteins in peanuts could significantly protect consumers from this allergy. Gene technology, enzymatic degradation, and autoclaving are commonly used to reduce allergenicity in food. The immune activity of food materials can be reduced using gene technology by regulating the expression or modifying the structure of allergic proteins (Herman and others 2003; Chu and others 2008). Enzymatic hydrolysis or microbial fermentation can also reduce the allergenicity in food. Yu and others (2011) treated peanuts with trypsin and α-chymotrypsin, and the peanuts nearly completely lost their IgE-binding capacity. Autoclaving reduces allergenicity in food by causing structural alterations in proteins. Studies suggested that heating damages the conformational epitopes of the allergenic protein in strawberries and apples (Scheurer and others 2004; Bohle and others 2006), and autoclaved peanuts and lupine also displayed significantly reduced allergenicity (Álvarez-Álvarez and others 2005; Cabanillas and others 2012). Although genetic engineering and biological or physical treatments can reduce protein allergenicity, these methods have disadvantages related to the food safety issue and their high cost.

Recent studies have shown that during germination, some seed storage proteins, including allergenic proteins, are degraded into peptides or amino acids to supply the nitrogen needed for seed growth (Yamada and others 2005; Il-Ho and others 2007; Wu and others 2012). Il-Ho and others (2007) suggested that the levels of the major allergens Ara h1–2 decrease significantly after germination of peanut seeds for 48 h. Wu and others (2012) and Yamada and others (2005) reported a significant degradation of storage proteins and decreases in the allergenicity in rice and soybeans after short-term germination. Legume sprouts are very popular in Eastern countries as traditional vegetables, and are becoming increasingly accepted by Western consumers. Importantly, after short-term germination, the content of vitamins, minerals, and antioxidant polyphenols increases significantly in legume seeds (Khalil and Mansour 1995; Bains and others 2014; Li and others 2014), thereby improving the nutritional value of seed sprouts. Although previous studies have indicated that the allergenic proteins could degrade dramatically during seed germination, but most of them were conducted from the angle of genetic engineering, so did not detect the change in allergenicity of seed during germination nor study the effects of germination condition on allergenic proteins. In this study, the influence of different germination conditions (temperature and light) on the degradation of Ara h1 and allergenicity changes of peanut seeds was evaluated by enzyme-linked immunosorbent assay (ELISA) and Western blotting.

Materials and Methods

Seeds and germination

Dry peanut seeds were purchased from a local supermarket in Wuxi, Jiangsu Province, China. The seeds were first soaked in boiled water for 10 min at 50 °C, and then for 12 h at 20 °C. Imbibed seeds were transferred into aseptic plastic trays and different germination conditions were applied as follows: (1) 20 °C in darkness; (2) 25 °C in darkness; (3) 30 °C in darkness; (4) 25 °C and 12-h illumination (fluorescent light intensity, 500 lx); (5) 25 °C and given 24-h illumination (fluorescent light intensity, 500 lx). These seeds were germinated for up to 132 h (25 °C and 30 °C) and 252 h (20 °C) in thermostat incubators (±1.0 °C) regulated to 90% to 100% relative humidity. The seeds were rinsed every 12 h with boiled water at 20 °C during germination. Germinated seeds, cotyledons, plumules, hypocotyls, and radicles were separated and weighed, packed and frozen in liquid nitrogen, and kept at −80 °C for analysis. Dry seeds soaked in boiled water at 50 °C for 10 min were considered to be the 0-h time point and were used as a control. Fresh weight, water content, and soluble protein levels were measured for seeds from each time point.

Preparation of peanut protein extract (PE) and soluble protein levels

For obtaining PEs, peanut samples were ground and suspended in 0.01 mol/L phosphate-buffered saline (PBS), pH 7.4 (solid-to-solvent ratio 1:10 w/v). After agitation for 5 min at room temperature, samples were extracted at 4 °C for 12 h and centrifuged for 30 min at 4000 × g (Mondoulet and others 2005). The supernatants were collected and stored at 4.0 °C for protein determination. Protein concentrations were determined using the Bradford analysis with a bovine serum albumin (BSA) protein assay kit (Sangon Biotech Co., Ltd, Shanghai, China).

Purification of Ara h1

Ara h1 was separated and purified using a combination of chromatographic methods (Mondoulet and others 2005) as follows: (1) DEAE Fast Flow, an anion exchange column (GE Healthcare Life Sciences, Piscataway, N.J., U.S.A.), equilibrated with 0.01 mol/L PBS buffer (pH 7.4), elution 0.01 mol/L PBS buffer (pH 7.4, 1.0 mol/L NaCl) at 5.0 mL/min; and (2) high-performance liquid chromatography on an AKTA purifier system (GE Healthcare Life Sciences) on the Superdex^{TM} 200 10/300GL gel filtration column (GE Healthcare Life Sciences), equilibrated with 0.01 mol/L PBS buffer (pH 7.4), elution 0.01 mol/L PBS buffer (pH 7.4, 1.0 mol/L NaCl) at 0.5 mL/min. The purity of the proteins was estimated to be >95%, as estimated using a densitometric scan of an SDS-polyacrylamide gel electrophoresis (PAGE) gel (under reducing conditions) stained with Coomassie Brilliant Blue. The protein band excised from gel was identified as Ara h1 by tandem mass spectrometry (executed by Institutes of Life and Health Engineering in Jinan Univ.).

Preparation of polyclonal rabbit antibodies against Ara h1

The purified Ara h1 was injected into rabbits (male New Zealand white rabbits) for production of polyclonal antibodies through subcutaneous and intramuscular quadruple immunization at per-week intervals (Il-Ho and others 2007; Troszyńska and others 2007). Standard guidelines of Jiangnan Univ. for the care and use of animals were followed. Note that 1 mL of a solution containing 10 mg of protein was used for the immunization. The 1st immunization was performed in the presence of a complete

Freund's adjuvant (Sigma-Aldrich Corporation, St. Louis, Mo., U.S.A.), and the subsequent in the presence of an incomplete Freund's adjuvant (Sigma-Aldrich Corporation). The sera containing reliable polyclonal antibodies against Ara h1 were used without purification in our studies.

Immunoreactivity of allergenic protein

The immunoreactivity of the Ara h1 in raw and germinated peanut seeds was analyzed using competitive inhibition ELISA according to Schmitt and others (2004). Briefly, microtiter plates were coated with Ara h1 at a concentration of 2.5 μg/mL. Fifty-fold dilutions of peanut sample extracts (0.064 g dry weight samples in 1.0 mL, 0.01 mol/L, pH 7.4, PBS) were mixed with equal volumes of polyclonal rabbit anti-Ara h1 (1:300000 dilution) and incubated for 30 min at 37 °C. Each mixture was added in triplicate to the ELISA wells and incubated at 37 °C for 1.0 h. The horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (Sigma Aldrich Corporation) were added to each well (diluted 1:5000 v/v) of ELISA plate and incubated at 37 °C for 30 min. Purified proteins of Ara h1 (100, 50, 40, 30, 20, 10, 8.0, 4.0, 2.0, and 0.5 μg/mL) were used to construct a standard curve. The ELISA results were read at 450 nm using the Thermo Multiskan MK3 microplate reader (Thermo Scientific, Shanghai, China).

Electrophoresis

Whole peanut PEs (0.064 g dry weight samples in 1.0 mL, 0.01 mol/L, pH 7.4, PBS) were analyzed using 12% SDS-PAGE as described by Schmitt and others (2010). A 10-μL sample solution (10-μL sample extracts under reducing conditions in 40 μL SDS sample buffer) was loaded per lane of SDS-PAGE. The SDS-PAGE was stained using Coomassie Brilliant Blue R250 solution and photographed.

Western blotting

To detect Ara h1 or protein fragments of it, immunoblotting analysis were performed (Mondoulet and others 2005; Il-Ho and others 2007; Cabanillas and others 2012). After SDS-PAGE, the separated proteins were electrophoretically transferred to 0.45-μm nitrocellulose membranes (Boster Biological Technology Co., Ltd, Pleasanton, Calif., U.S.A.). The membranes were blocked overnight with 0.01 mol/L PBS (pH 7.4) containing 3.0% BSA at 4.0 °C and subsequently incubated for 3.0 h at 37 °C with the specific polyclonal antibodies for Ara h1 diluted 1:500 in 1.0 mol/L Tris-HCl, pH 7.5, 0.15 mol/L NaCl, and 0.1% Tween 20 (TBST). After 3 washes of 10 min each with TBST, membranes were incubated for 2.0 h at 37 °C with HRP-conjugated goat anti-rabbit IgG (Sigma-Aldrich Corporation) that was diluted 1:2000 in TBST. After washing again with TBST, the membranes were colored using the 3,3′-diaminobenzidine HRP color development kit (Sangon Biotech Co., Ltd, Shanghai, China) and then photographed.

Data analysis

Data on soluble protein levels and immunocreactivity of Ara h1 are presented as means ± SD of triplicate measurements. All values reported were calculated on a dry-weight basis.

Results and Discussion

Soluble protein levels

Soluble protein contents in the peanut sprouts deceased rapidly during 12-h imbibition, but then increased gradually in the middle stages (12 to 84 and 12 to 156 h) of germination before declining slowly in the late-germination period (84 to 132 and 156 to 252 h, Figure 1A). During seed germination and growth, storage compounds such as fat, starch, and protein are degraded into smaller molecules and transport to specific locations for the synthesis of new cell structures and functional substances. In the early phase (0 to 12 h) of peanut seed germination, the lack of cell membrane integrity causes the extravasation of intracellular proteins and amino acids (Bewley 1997), resulting in a rapid decrease in the levels of soluble proteins (Figure 1A). Later, the integrity of the membrane system is quickly restored following seed imbibition (Bewley 1997). At the same time, activated endogenous proteases synthesize a series of new enzymes that lysate storage proteins into peptides and amino acids (Il-Ho and others 2007), thereby significantly increasing the soluble protein levels (Figure 1A). Slightly earlier or simultaneously to the degradation of storage proteins, new proteins are synthesized from peptides and amino acids (Bewley 1997). Then, in the middle phases (12 to 84 and 12 to 156 h) of germination, when synthesis of new substances exceeds degradation of storage proteins, the soluble protein levels in peanut sprouts again decreased and finally reached a balance in the late phases of germination (Figure 1A). In addition, seed respiration consumes a large portion of the amino acids that are derived from the storage proteins (Lehmann and Ratajczak 2008).

Under adequate water supply, temperature and lighting are the 2 most important environmental factors that affect seed germination (Nasser and others 2013). Temperature directly influences seed germination rate and speed (Gairola and others 2011). This study showed that under higher temperatures, the soluble protein levels in peanut sprouts peaked at an earlier time point (Figure 1A). Soluble protein content reached a plateau at 60, 84, and 156 h of

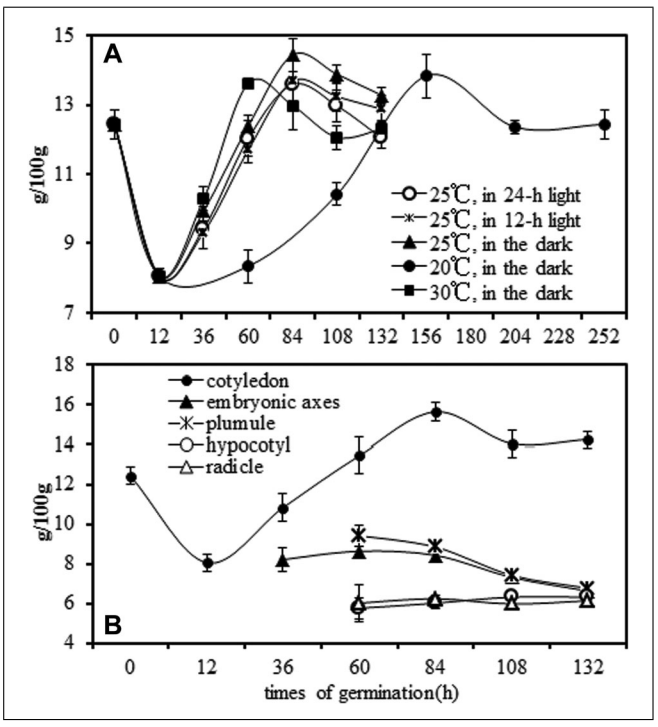

Figure 1–Soluble protein levels of peanut sprouts under different germination conditions (A) and different tissues germinated at 25 °C in the dark (B). Vertical bars indicate ±SD.

germination at 30 °C, 25 °C, and 20 °C, respectively. However, high-temperature (30 °C) germination will render sprouts vulnerable to disease (data not shown), and if seeds are germinated at 20 °C, they might require a longer generation period and a rough mouthfeel (data not shown) will affect taste sensory evaluation. This study also found that the peanut cotyledons became green when sprouted under light, and the sprouts had a disagreeable taste (data not shown). Hence, a temperature of 25 °C under dark conditions is suitable for peanut sprouts processing.

Soluble protein contents in the cotyledons were markedly higher than that in the embryonic axes, and the values in plumules were also obviously higher than that in the hypocotyls and radicles (Figure 1B). After 84-h germination, the soluble protein levels in cotyledons and plumules reached 15.62 and 8.85 g/100 g, respectively, higher than the corresponding values in embryonic axes and hypocotyls or radicles about 50% and 30%. These results agree with a previous study indicates that during seed development, higher transcript levels are detected in the cotyledons compared to the embryonic axes for some storage proteins (Il-Ho and others 2007).

SDS-PAGE of peanut sprouts

SDS-PAGE revealed large changes in protein composition of peanut seeds during germination. The results indicated that 3 bands with molecular weights of approximately 65, 40, and 22 kDa were the most abundant PE in ungerminated peanut seeds (Figure 2A). The protein bands of approximately 65 and 40 kDa

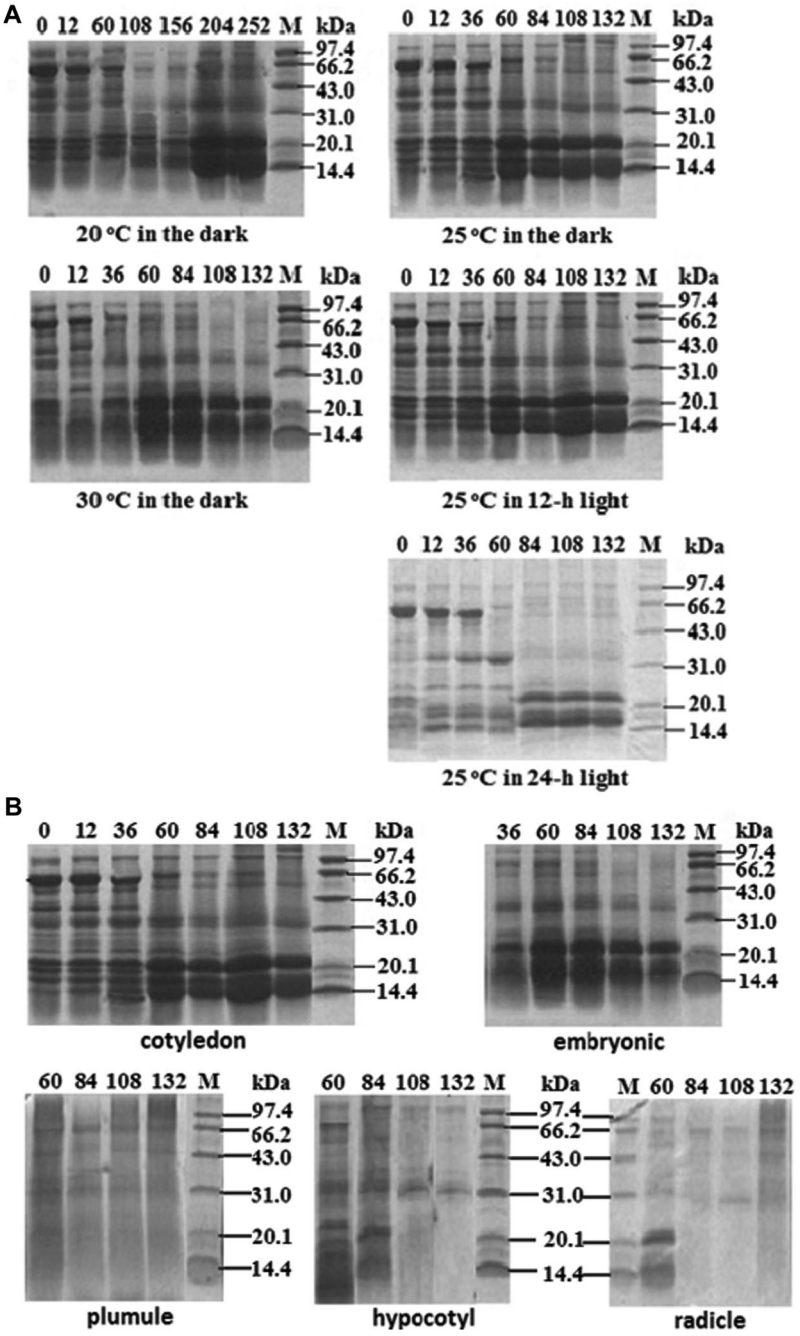

Figure 2–SDS-PAGE of protein extracts from peanut sprouts under different germination conditions (A) and different tissues germinated at 25 °C in the dark (B). 0, 12, 36, 60, 84, 108, 132, 156, 204, and 252, harvesting times after imbibition (hours). For the 0 time point, seeds without treatment. Protein molecular weight marker (M) is indicated.

degraded rapidly during the time course, beginning at 60 and 108 h; however, others with molecular weights ranging from 19 to 17 kDa simultaneously became increasingly clear (Figure 2A). And some interesting results were observed: the levels of approximately 22- and 19-kDa proteins were unchanged or increased over the time course in germinated peanut seeds (Figure 2A).

The major allergenic proteins Ara h1 and Ara h3 are within the protein mass range of approximately 65 and 40 kDa. Il-Ho and others (2007) also demonstrated that allergenic proteins Ara h1 (63 kDa) and Ara h3 (44, 40, and 36 kDa) were degraded during germination. These results indicated that high molecular weight proteins, including allergenic proteins, are significantly degraded during peanut seed germination. Such degradation is directly associated with a significant increase in protease activity (Mondoulet and others 2005). The Cys proteinase is considered the most important protease that degrades storage proteins during seed germination (Wilson and others 1986; Shutov and Vaintraub 1987).

There was not much different from samples germinated under different temperatures and lighting conditions in the SDS-PAGE results with the exception of double protein degradation times slower for the seeds treated at a low temperature (20 °C) than others germinated under different temperatures and lighting conditions (Figure 2A). Large protein (approximately 65 and 40 kDa) degradation was easily observed beginning at 60 h in the samples treated at 25 °C and 30 °C, but the seeds germinated at 20 °C needed 108 h. The lighting condition was no obvious effect on protein degradation time.

To examine the germination process in greater detail, different tissues of peanut sprouts were collected at 12, 36, 60, 84, 108, and 132 h after germination at 25 °C in the dark. Because peanut seeds showed obvious embryonic axis growth and differentiation (with plumules, hypocotyls, or radicles) at only 36 and 60 h, respectively, this study assessed embryos at 36- or 60-h germination. As shown in Figure 2(B), protein distribution in the embryonic axes was not

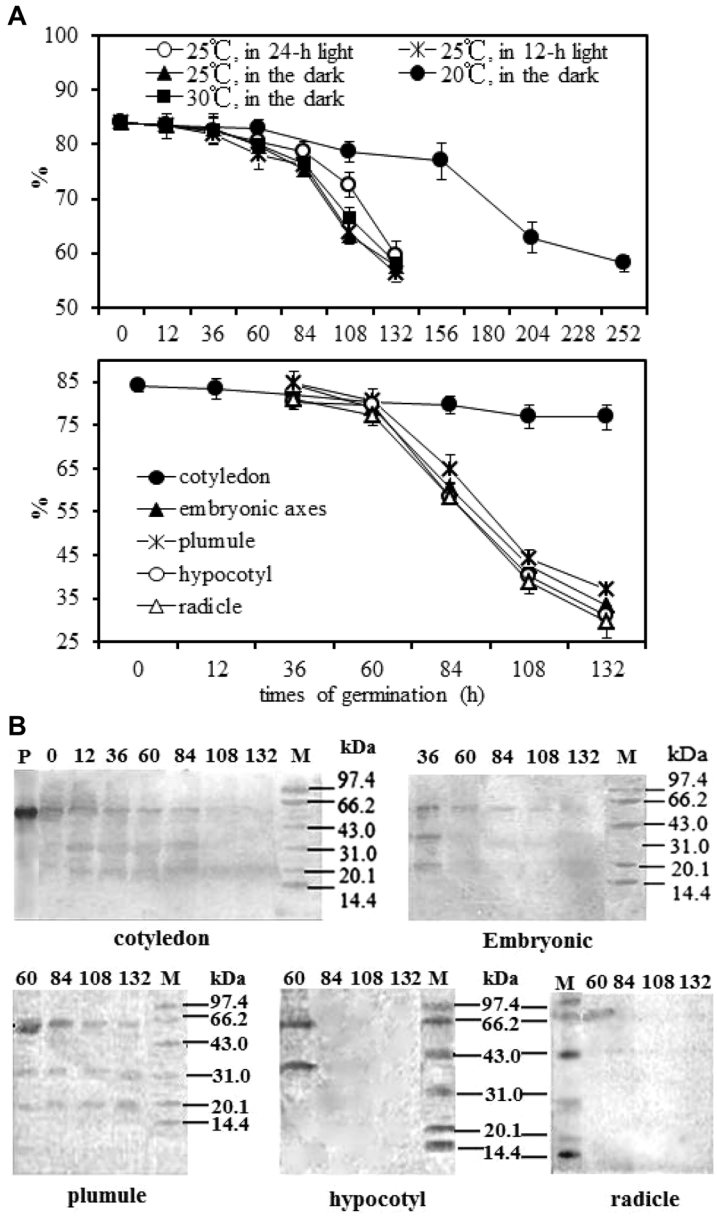

Figure 3–Polyclonal rabbit antibodies against Ara h1 were used to detect Ara h1, and its immunoreactivity and protein fragments. 0, 12, 36, 60, 84, 108, 132, 156, 204, and 252, harvesting times after imbibition (hours). For the 0 time point, seeds without treatment. Immunoreactivity (inhibition rate) of Ara h1 in peanut sprouts under different germination conditions and different tissues at 25 °C in the dark (A); vertical bars indicate ±SD. Ara h1 and its fragments were detected by Western blotting, and the protein extracts from different tissues of peanut sprouts germinated at 25 °C in the dark (B). Lane P contains purified Ara h1 from raw peanut. Protein molecular weight marker (M) is indicated.

as rich as that in the cotyledons, but it displayed a similar degrading pattern, with approximately 65- and 40-kDa proteins degrading while the level of small molecular weight proteins (<19 kDa) increased during germination; however, the degradation rate of 65-kDa proteins was higher in the embryonic axes—its band disappeared from the SDS-PAGE gels after 108-h germination—yet in the cotyledons did not disappear until 132 h after germination. Our study also showed that the fresh weight of embryonic axes increased dramatically, while that of cotyledons increased slowly after 36 h. At 84 h after germination, the fresh weight of cotyledons increased by only 40%, while that of the embryo increased by 80% (not shown). The decrease in soluble proteins in the embryo was also more rapid (Figure 1B); and the SDS-PAGE detected a faster degradation rate of the 65-kDa protein in the embryonic axes (Figure 2B), which is consistent with the results of Il-Ho and others (2007). These findings suggest that the mobilization and transformation of soluble proteins, including allergenic protein Ara h1, is more rapid in the embryos than in the cotyledons.

Protein degrading patterns in plumules, hypocotyls, and radicles were also different. The 65-kDa proteins showed the highest degradation rate in hypocotyls (disappeared after 84-h germination) but no significant variation in plumules or radicles, and the 40-kDa proteins showed a reverse pattern of degradation (Figure 2B). Proteins with different molecular weights show varying rates of degradation in different parts of the peanut embryo, which may be explained by the different functional requirements of the plumules, hypocotyls, and radicles.

ELISA and Western blot analysis of allergenic protein

To investigate the variation of Ara h1 allergenicity during germination, total immunoreactivity and the distribution of immunoreactive fragments were detected using ELISA and Western blotting, respectively. As Western blotting patterns of peanut sprouts under different germination conditions were all similar to that in the cotyledons germinated at 25 °C in the dark, only the data for cotyledons were shown (Figure 3B).

As shown in Figure 3(A), immunoreactivity of Ara h1 decreased with the increase in germination time, and a rapid reduction was observed at the end of germination period (>84 and 156 h). After 132 h (25 °C and 30 °C) and 252 h (20 °C) of germination, the inhibition rate of Ara h1 (positively correlated with immunoreactivity) in peanut sprouts decreased by approximately one-third compared to that at 0 h (Figure 3A). The decline of immunoreactivity is closely related to degradation of allergenic proteins in germinated peanut seeds. Previous studies have reported that the levels of allergenic proteins were dramatically reduced and no allergen transcripts were detected during germination and seedling growth (Il-Ho and others 2007). However, the variation in Ara h1 immunoreactivity in the cotyledons differed from that in the embryos. The inhibition rate of Ara h1 decreased only slightly in the cotyledons (by only 8.0%), but declined rapidly in the embryonic axes as germination continued, with the inhibition rate decreasing by 60% at 132 h compared to that at 36 h (Figure 3A). As shown in Figure 3(B), Ara h1 was degraded into immunoreactive fragments of 40 and 20 kDa, and the fragments in embryonic axes could barely be detected after 60 h of germination. These results further confirmed that as allergenic protein Ara h1 is degraded, its immunoreactivity decreases by approximately 30% after short-term germination, and the immunoreactive fragments include mainly 40- and 20-kDa proteins with the degradation being more pronounced in embryonic axes. Il-Ho and others (2007) and Jensen and others (2008) observed similar results, and they concluded that this result may be a consequence of a slower turnover of these proteins in cotyledon than in embryonic axes or a dilution of allergenic storage proteins caused by *de novo* synthesis of new proteins.

Higher inhibition rates of Ara h1 were detected in plumules compared to hypocotyls and radicles (Figure 3A). Western blot analysis provided more detailed results for degradation of Ara h1. The immunoreactive fragments of Ara h1 in the hypocotyls and radicles disappeared after 84-h germination, but in the plumules did not disappear until 132 h after germination (Figure 3B). The variation of Ara h1 immunoreactivity in the plumules was quite similar to that in the embryonic axes, which indicated that the immunoreactivity of Ara h1 in the embryonic axes came mainly from the plumules. Meanwhile, a high initial accumulation explains why immunoreactivity of the embryo comes mainly from the allergenic proteins located in the plumules.

Conclusion

PEs from peanut seeds, including allergenic proteins, are significantly degraded into smaller molecules during a short-term germination. A significant reduction of immunoreactivity of Ara h1, approximately one-third, was noted after 5 to 7 d of germination. Short-term germination could be an easy way to produce hypoallergenic peanut food. Further studies are needed to assess the effects of germination on other major peanut allergens and the clinical relevance of the study. It will be beneficial to convince a definite role of germination in improving food safety.

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Compliance with Ethics Requirements:

The study was approved by the local ethics committee (Experiments on Animals at the Jiangnan Univ. in Wuxi, resolution no. 20141111–1215).

Reference

Álvarez-Álvarez J, Guillamón E, Crespo JF, Cuadrado C, Burbano C, Rodríguez J, Fernández C, Muzquiz M. 2005. Effects of extrusion, boiling, autoclaving, and microwave heating on lupine allergenicity. J Agr Food Chem 53:1294–8.

Bains K, Uppal V, Kaur H. 2014. Optimization of germination time and heat treatments for enhanced availability of minerals from leguminous sprouts. J Food Sci Technol 51:1016–20.

Bewley JD. 1997. Seed germination and dormancy. Plant Cell 9:1055–66.

Bohle B, Zwölfer B, Heratizadeh A, Jahn-Schmid B, Antonia YD, Alter M, Keller W, Zuidmeer L, van Ree R, Werfel T, Ebner C. 2006. Cooking birch pollen–related food: divergent consequences for IgE- and T cell–mediated reactivity in vitro and in vivo. J Allergy Clin Immun 118:242–9.

Breiteneder H, Ebner C. 2000. Molecular and biochemical classification of plant-derived food allergens. J Allergy Clin Immun 106:27–36.

Burks AW, Williams LW, Connaughton C, Cockrell G, O'Brien TJ, Helm RM. 1992. Identification and characterization of a second major peanut allergen, Ara h II, with use of the sera of patients with atopic dermatitis and positive peanut challenge. J Allergy Clin Immun 90:962–9.

Burks AW, Cocrell G, Stanley JS, Helm RM, Bannon GA. 1995. Recombinant peanut allergen Ara h 1 expression and IgE-binding in patients with peanut hypersensitivity. J Clin Invest 96:1715–21.

Cabanillas B, Maleki SJ, Rodríguez J, Burbano C, Muzquiz M, Jiménez MA, Pedrosa MM, Cuadrado C, Crespo JF. 2012. Heat and pressure treatments effects on peanut allergenicity. Food Chem 132:360–6.

Cabanos C, Urabe H, Tandang-Silvas MR, Utsumi S, Mikami B, Maruyama N. 2011. Crystal structure of the major peanut allergen Ara h 1. Mol Immunol 49:115–23.

Chu Y, Faustinelli P, Ramos ML, Hajduch M, Stevenson S, Thelen JJ, Maleki SJ, Cheng H, Ozias-Akins P. 2008. Reduction of IgE binding and nonpromotion of aspergillus flavus fungal growth by simultaneously silencing Ara h 2 and Ara h 6 in peanut. J Agr Food Chem 56:11225–33.

Clarke K, Hourihane O'B, Dean W, Dean K. 1998. Serological characteristics of peanut allergy. Clin Exp Allergy 28:1251–7.

DeLong JH, Simpson KH, Wambre E, James EA, Robinson D, Kwok WW. 2011. Ara h 1–reactive T cells in individuals with peanut allergy. J Allergy Clin Immun 127: 1211–8.e3.

Gairola KC, Nautiyal AR, Dwivedi AK. 2011. Effect of temperatures and germination media on seed germination of Jatropha Curcas Linn. Adv Biores 2:66–71.
Herman EM, Helm RM, Jung R, Kinney AJ. 2003. Genetic modification removes an immunodominant allergen from soybean. Plant Physiol 132:36–43.
Il-Ho K, Srivastava P, Ozias-Akins P, Gallo M. 2007. Temporal and spatial expression of the major allergens in developing and germinating peanut seed. Plant Physiol 144:836–45.
Jensen LB, Pedersen MH, Skov PS, Poulsen LK, Bindslev-Jensen C, Andersen SB, Torp AM. 2008. Peanut cross-reacting allergens in seeds and sprouts of a range of legumes. Clin Exp Allergy 38:1969–77.
Jiménez-Saiz R, Belloque J, Molina E, López-Fandiño R. 2011. Human immunoglobulin E (IgE) binding to heated and glycated ovalbumin and ovomucoid before and after *in vitro* digestion. J Agr Food Chem 59:10044–51.
Jong DE, Van Z, Spanhaak K, Pellegrom P. 1998. Identification and partial characterization of multiple major allergens in peanut proteins. Clin Exp Allergy 28:743–51.
Kagan RS, Joseph L, Dufresne C, Gray-Donald K, Turnbull E, Pierre YS, Clarke AE. 2003. Prevalence of peanut allergy in primary-school children in Montreal, Canada. J Allergy Clin Immun 112:1223–8.
Khalil AH, Mansour EH. 1995. The effect of cooking, autoclaving and germination on the nutritional quality of faba beans. Food Chem 54:177–82.
Kleber–Janke T, Crameri R, Appenzeller U, Schlaak M, Becker WM 1999. Selective cloning of peanut allergens, including profilin and 2S albumins, by phage display technology. Int Arch Allergy Imm 119:265–74.
Koppelman SJ, Knol EF, Vlooswijk RAA, Wensing M, Knulst AC, Hefle SL, Gruppen H, Piersma S. 2003. Peanut allergen Ara h 3: isolation from peanuts and biochemical characterization. Allergy 58:1144–51.
Lehmann T, Ratajczak L. 2008. The pivotal role of glutamate dehydrogenase (GDH) in the mobilization of N and C from storage material to asparagine in germinating seeds of yellow lupine. J Plant Physiol 165:149–58.
Li YC, Qian H, Sun XL, Cui Y, Wang HY, Du C, Xia XH. 2014. The effects of germination on chemical composition of peanut seed. Food Sci Technol Res 20:883–9.
Lycett GW, Delauney AJ, Gatehouse JA, Gilroy J, Croy RR, Boulter D. 1983. The vicilin gene family of pea (Pisum sativum L.): a complete cDNA coding sequence for preprovicilin. Nucleic Acids Res 8:2367–80.
Maleki SJ, Chung SY, Champagne ET, Raufman JP. 2000. The effects of roasting on the allergenic properties of peanut proteins. J Allergy Clin Immun 106:763–8.
Maleki SJ, Viquez O, Jacks T, Dodo H, Champagne ET, Chung SY, Landry SJ. 2003. The major peanut allergen, Ara h 2, functions as a trypsin inhibitor, and roasting enhances this function. J Allergy Clin Immun 112:190–5.
Mari A, Scala E, Palazzo P, Ridolfi S, Zennaro D, Carabella G. 2006. Bioinformatics applied to allergy: allergen databases, from collecting sequence information to data integration. The Allergome platform as a model. Cell Immunol 244:97–100.
Mondoulet L, Paty E, Drumare MF, Ah-Leung S, Scheinmann P, Willemot RM, Wal JM, Bernard H. 2005. Influence of thermal processing on the allergenicity of peanut proteins. J Agr Food Chem 53:4547–53.
Nakamura A, Sasaki F, Watanabe K, Ojima T, Ahn DH, Saeki H. 2006. Changes in allergenicity and digestibility of squid tropomyosin during the Maillard reaction with ribose. J Agr Food Chem 54:9529–34.
Nasser AM, Barakat HF, Kabeil AK, Hegazy NSS. 2013. Synergetic action of light and temperature on seed germination of some solanaceae members. J Stress Physiol Biochem 9:85–100.
Polovic N, Blanusa M, Gavrovic-Jankulovic M, Atanaskovic-Markovic M, Burazer L, Jankov R, Velickovic TC. 2007. A matrix effect in pectin-rich fruits hampers digestion of allergen by pepsin in vivo and in vitro. Clin Exp Allergy 37:764–71.
Rabjohn P, Helm EM, Stanley JS, West CM, Sampson HA, Burks AW, Bannon GA. 1999. Molecular cloning and epitope analysis of the peanut allergen Ara h 3. J Clin Invest 4: 535–42.
Scheurer S, Lauer I, Foetisch K, Moncin MSM, Retzek M, Hartz C, Enrique E, Lidholm J, Cistero-Bahima A, Vieths S. 2004. Strong allergenicity of Pru av 3, the lipid transfer protein from cherry, is related to high stability against thermal processing and digestion. J Allergy Clin Immun 114:900–7.
Schmitt DA, Hsiaopo C, Maleki SJ, Burks AW. 2004. Competitive inhibition ELISA for quantification of Ara h 1 and Ara h 2, the major allergens of peanuts. J AOAC Int 87:1492–8.
Schmitt DA, Nesbit JB, Hurlburt BK, Cheng H, Maleki SJ. 2010. Processing can alter the properties of peanut extract preparations. J Agr Food Chem 58:1138–43.
Shriver S, Yang W. 2011. Thermal and nonthermal methods for food allergen control. Food Eng Rev 3:26–43.
Shutov AD, Vaintraub IA. 1987. Degradation of storage proteins in germinating seeds. Phytochemistry 26:1557–66.
Szymkiewicz A. 2010. Peanut (*Arachis hypogea*) Allergens. In: Jedrychowski L and Wichers HJ, editors. Chemical and biological properties of food allergens. 1st ed. Fla.: CRC Press. p 267–74.
Thomas K, Herouet-Guicheney C, Ladics G, Bannon G, Cockburn A, Crevel R, Fitzpatrick J, Mills C, Privalle L, Vieths, S. 2007. Evaluating the effect of food processing on the potential human allergenicity of novel proteins: International workshop report. Food Chem Toxicol 45:1116–22.
Troszyńska A, Szymkiewicz A, Wołejszo A. 2007. The effects of germination on the sensory quality and immunoreactive properties of pea (Pisum Sativum L.) and soybean (Glycine max). J Food Quality 30:1083–100
van-Wijk F, Nierkens S, Hassing I, Feijen M, Koppelman SJ, de-Jong GAH, Pieters R, Knippels LMJ. 2005. The effect of the food matrix on in vivo immune responses to purified peanut allergens. Toxicol Sci 86:333–41.
Wilson KA, Rightmire BR, Chen JC, Tan-Wilson A. 1986. Differential proteolysis of glycinin and β-conglycinin polypeptides during soybean germination and seedling growth. Plant Physiol 82:71–6.
Wu YM, Guan RX, Liu ZX, Li RZ, Chang RZ, Qiu LJ. 2012. Synthesis and degradation of the major allergens in developing and germinating Soybean Seed. J Integr Plant Biol 54: 4–14.
Yamada C, Izumi H, Hirano J, Mizukuchi A, Kise M, Matsuda T, Kato Y. 2005. Degradation of soluble proteins including some allergens in brown rice grains by endogenous proteolytic activity during germination and heat-processing. Biosci Biotech Bioch 69:1877–83.
Yu J, Ahmedna M, Goktepe I, Cheng H, Maleki S. 2011. Enzymatic treatment of peanut kernels to reduce allergen levels. Food Chem 127:1014–22.