

Effect of Processing on the Structure and Allergenicity of Peanut Allergen Ara h 2 Roasted in a Matrix

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ABSTRACT: Peanut allergy is the leading pediatric food allergy. Many attempts have been made to reduce its allergenicity by processing. After roasting, Ara h 2 and its derivatives in the matrix were isolated by immunoaffinity chromatography (IAC). The structure and allergenicity of Ara h 2 were analyzed by circular dichroism, mass spectrometry (MS), western blotting, the enzyme-linked immunoassay, and cell modeling. Our results showed that a large portion of Ara h 2 was fragmented and cross-linked. Ara h 2 monomers accounted for only 13% of the total proteins after IAC purification. In addition, the structure of Ara h 2 changed after roasting. In addition to methylation and oxidation modification, the disulfide bonds of Ara h 2 were found to be rearranged after roasting. In the conformational structure of Ara h 2, the content of the α -helix decreased from 27.1 to 21.6% after roasting, while the content of the random coil increased from 29.1 to 34.3%. Six cleavage sites of trypsin were exposed, while three were covered. In terms of allergenicity, most of the cross-linking products were not recognized by patients' sera. Only one faint band around 40 kDa was observed in our blotting. For Ara h 2 monomers, roasting enhanced their IgE binding capacity and ability to stimulate the degranulation of basophils. The potential allergenicity increase of Ara h 2 monomers did not reflect the allergenicity change of Ara h 2 in the matrix due to the amount and property of its derivatives after roasting.

KEYWORDS: peanut allergen, Ara h 2, allergenicity, immunoaffinity chromatography, roast

1. INTRODUCTION

As the leading pediatric food allergy, the peanut (*Arachis hypogaea* L.) allergy (PA) can cause anaphylaxis, and its prevalence has increased dramatically.^{1,2} Although it is lifelong in most instances, disease-modifying treatments for the PA are lacking.³ Many attempts have been made to reduce the potential allergenicity of peanut allergens.^{4–10} Previous studies have shown that the allergenicity can be changed by altering the structure and other physicochemical properties of food allergens.^{11–15}

Seventeen allergens have been identified in the peanut (<http://www.allergen.org>), among which Ara h 1, Ara h 2, Ara h 3, and Ara h 6 are the main allergens.¹⁶ A previous study has shown that Ara h 2 ranges from 5.9 to 9.3% of the total protein content.¹⁷ The structure-related allergenicity of Ara h 2 is of great importance for the allergenicity of the peanut.^{18–20} Due to the difficulty in the separation of individual allergens after the roasting process, most studies were performed on purified allergens.^{11,20} However, the effect of the roasting process on the allergenicity of Ara h 2 in the matrix remains not fully understood.^{4,21}

Although previous studies on processed pure allergens may have been able to discover the relationship between the structure and allergenicity, the actual changes of allergens in the matrix induced by processing are much more complicated.^{6,22,23} We have developed an immunoaffinity chromatographic method for Ara h 2 isolation and isolated the thermally processed products containing Ara h 2 from the peanut

matrix.²⁴ This method can be used to extract Ara h 2 and its derivatives to determine the effects of processing on Ara h 2.

In the present work, after the peanut kernel was roasted, immunoaffinity chromatography (IAC) was performed to separate the products of Ara h 2 in order to determine the effects of roasting on Ara h 2 in the matrix.

2. EXPERIMENTAL SECTION

2.1. Sample Preparation. Fresh peanuts (Ganhua 7) were purchased from a local supermarket (Nanchang, China), washed, and drained. In the roasted group, the peanuts were roasted in an oven at 170 °C for 20 min.⁵ The roasted samples after cooling in air and fresh peanuts were used to produce defatted peanut powder (DPP) following the methods previously described by Zhang et al.⁵ Briefly, the kernels of fresh or roasted peanuts without the skin were finely milled using a grinder (YouQi Co., Ltd., Taiwan, China). Then, the powder was defatted by acetone with 0.07% β -mercaptoethanol (Sangon Biotech, Shanghai, China) at a 1:5 (w/v) ratio three times. After the defatting, the milled powder was air-dried and stored at –20 °C until use.

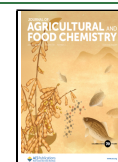
2.2. Total Protein Extraction. A 20 mmol/L Tris–HCl buffer (pH 8.2) was used to extract the total protein from the DPP at a ratio of 1:20 (w/v). After stirring at 4 °C for 3 h, the extract was

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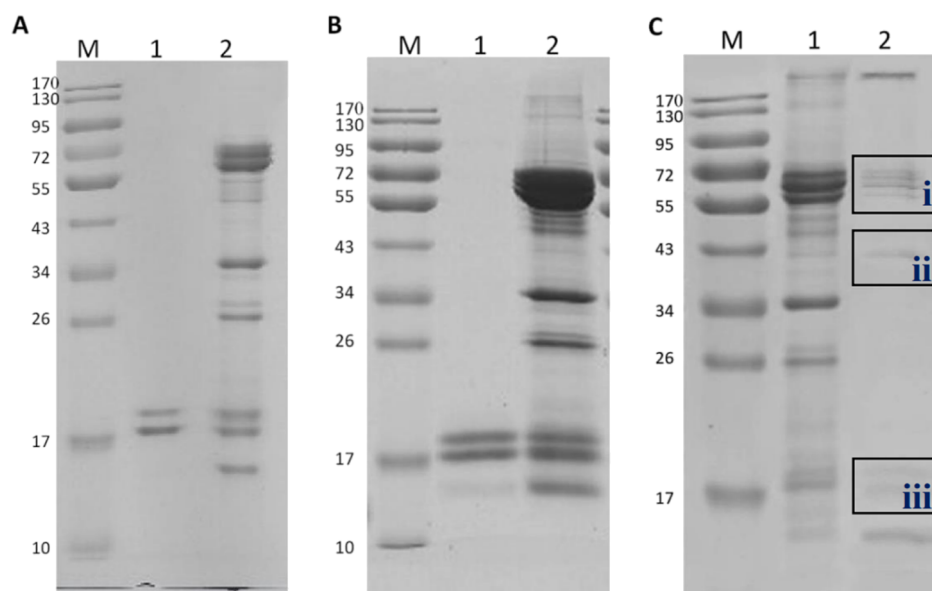


Figure 1. Characterization of the extracted total proteins and purified Ara h 2 from raw and roasted peanuts. (A): Native total peanut protein and Ara h 2 purified by IEC. M: marker; 1: Ara h 2 (IPN Ara h 2); and 2: total protein of the raw peanut. (B): Native total peanut protein and Ara h 2 purified by the homemade affinity column. M: marker; 1: purified products of the affinity column (mainly APN Ara h 2); and 2: total protein of the raw peanut. (C): Total protein extracted from the roasted peanut and products purified by the homemade affinity column. M: marker; 1: total protein of the roast peanut; and 2: purified products of the affinity column (including APR Ara h 2). In lane 2, three areas (i, ii, and iii) were chosen for analysis by MS.

centrifuged at 20 000g for 30 min and then filtered through a 0.45 μ m membrane. The clarified extraction solution was stored at -20°C until use.

2.3. Affinity Column Purification. A homemade IAC column was used to purify Ara h 2 from the peanut products.²⁴ The column for specific extraction was constructed by coupling polyclonal antibody anti-Ara h 2 IgG on CNBr-activated Sepharose 4B. Peanut extraction (1 mg/mL, 2 mL) was loaded on the IAC column and then washed with 10 bed volumes of 20 mM PBS (pH 7.4). Three bed volumes of the glycine–HCl elution buffer (0.1 M, pH 2.7) was used to elute Ara h 2 and its derivatives at a flow rate of 1 mL/min.

2.4. Purification of Ara h 2. Ara h 2 in the roasted peanut was purified from specific elution products through affinity chromatography using an ultrafiltration membrane (a cutoff of 50 kDa). This sample was named APR Ara h 2 (affinity chromatography-purified roasted Ara h 2). The Ara h 2 in the specific eluent purified from the fresh peanut by affinity chromatography was named APN Ara h 2 (affinity chromatography-purified native Ara h 2). As the control, IPN Ara h 2 [ion-exchange chromatography (IEC)-purified native Ara h 2] was prepared following the methods of Hu et al.²⁵

2.5. Sample Identification. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed to analyze the protein patterns of these samples. The density of certain band(s) in each lane on the image was quantified using Quantity One (Bio-Rad, Hercules, CA, USA). Bands of interest were cut off for mass spectrometry (MS) analysis following the methods of He et al.²⁶ The proteins within each band were digested in gel with trypsin (1:100 ratio, 37°C overnight). The digested peptides were analyzed using reverse-phase liquid chromatography and MS at a high resolution in a Q-Exactive mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). The MS result was analyzed using MaxQuant software (Ver. 1.5.3.17) to find the origin of the peptides.²⁷

2.6. Conformational Structure Characterization. The structure of Ara h 2 was characterized following the methods of Zhang et al.⁶ The secondary structure of purified Ara h 2 was analyzed by circular dichroism (CD) using an MOS-450 spectropolarimeter (French Bio-Logic SAS, Claix, France), while the UV absorption was evaluated using a UV spectrophotometer (Purkinje General, Beijing, China), scanned from 260 nm to 350 nm at 25°C at a rate of 120 nm/min.

2.7. Allergenicity Assessment. **2.7.1. Western Blot.** Following electrophoresis, proteins from gels were transferred onto a nitrocellulose membrane using a gel transfer device (EPS 601, GE, USA). Membranes were blocked with phosphate-buffered saline/Tween containing 2% BSA and then incubated with diluted (1:10) human sera pooled from 11 patients with a confirmed PA (Chongqing Manuik Technology Co., Ltd., Chongqing, China, details can be seen in the Supporting Information) overnight with gentle shaking. After washing, the membranes were incubated with biotinylated goat IgG–anti-human IgE, and the color was developed using horseradish peroxidase and imaged on a Bio-Rad imaging system (GS-800, USA).²⁸

2.7.2. IgE Binding Capacity. The IgE binding experiment was performed by a competitive enzyme-linked immunoassay (ELISA), as described previously.²⁹ In short, microplate wells were precoated with IEC-purified native Ara h 2 (2.5 $\mu\text{g/mL}$) overnight and washed on the 2nd day. Ara h 2 and anti-Ara h 2 IgE (human sera pool used in 2.7.1) were added into the wells after blocking and incubated for 1 h. Unbound IgE was then removed from microplate wells, followed by incubation with 100 μL of horseradish peroxidase-conjugated goat anti-human IgE for 1 h. After the color development was terminated, the absorbance was determined at 490 nm by using a microplate reader (model 1860, Bio-Rad, USA).

2.7.3. Cell Model Analysis. The human basophil leukemia (KU812) cell line was maintained in RPMI 1640 and cultured at 37°C with 5% CO_2 . For the induction of Fc ϵ RI expression, KU812 cells were preincubated with specific sera of PA patients ($v/v = 30:1$) for 12 h and then seeded in a 48-well round-bottom microtiter plate. The Ara h 2 samples (1 μg in each well) were added and incubated for 4 h.⁶ The contents of histamine and TNF- α were measured using a commercial human His ELISA kit (Fusheng Industry Co., Shanghai, China) and a commercial human TNF- α ELISA kit (Xinbosheng, Beijing, China). The level of β -hexosaminidase (β -HEX) was measured as described by Zhu et al.³⁰

3. RESULTS

3.1. Purification of Products by an Affinity Column. In our previous study, we tried to purify Ara h 2 from either raw or roasted peanuts by IEC. However, the majority of Ara h 2

and its derivatives after roasting in the matrix could not be captured by IEC.³¹ In this study, we used IAC to isolate Ara h 2 from both raw and roasted peanuts. As a comparison, IEC was also employed in our experiments. As shown in Figure 1, proteins isolated by IEC and IAC were analyzed by SDS-PAGE. Both IEC and IAC can purify Ara h 2 from raw peanut. Ara h 2 displayed two bands around 17 kDa, which indicated two isoforms for Ara h 2 in peanuts. For the contamination of Ara h 6, the purity of APN Ara h 2 (88.9%) was lower than that of IPN Ara h 2.

As seen in total protein, high-molecular-weight polymers were formed during roasting, and some of them were also found after the purification. After the roasting, the content of monomer Ara h 2 decreased from 5% (Figure 1B lane 2) to 2.5% (Figure 1C lane 1). The profiles of proteins isolated by IAC from roasted peanuts appeared to be different from those of raw peanuts. Besides Ara h 2 and Ara h 6, several bands with the molecular weight more than 170, around 70 kDa, and about 40 kDa appeared. The density of monomer Ara h 2 constituted only 13% for this lane. These results indicated that a major part of Ara h 2 was aggregated or cross-linked during roasting.

3.2. Identification of Polymers. To determine whether the detected bands in SDS-PAGE represent Ara h 2 aggregates with other proteins, we cut the bands of interest off for MS detection. As shown in Table 1, the bands in areas i and ii of

Table 1. Protein Identification on Samples in Area i and Area ii of Figure 1C by MS

sample	protein	accession	coverage	unique peptides	total peptides
i	Ara h 1	A0A6B9VGI9	48.50	10	34
		Q6PSU3	47.59	8	32
	Ara h 2	A0A445BYI5	32.50	1	5
		Q6PSU2	36.05	2	6
	Ara h 3	A0A445CPR0	33.58	3	15
		A0A445CPR7	33.90	4	15
		A1DZF0	30.62	5	14
		O82580	22.09	1	10
		Q647H4	29.29	6	14
		Q6IWG5	30.98	12	14
	Ara h 6	A0A444Y5J5	53.10	6	6
ii	Ara h 1	A0A6B9VGI9	24.58	3	14
		Q6PSU3	27.41	5	16
	Ara h 2	A0A445BYI5	14.37	1	2
		Q6PSU2	19.19	2	3
	Ara h 3	A0A445CPR0	17.36	3	8
		A0A445CPR7	17.61	2	7
		A1DZF0	17.20	2	7
		Q647H4	17.16	3	7
	Ara h 6	Q6IWG5	10.00	3	4
		A0A444Y5J5	23.45	3	3

Figure 1C contained fragments from Ara h 1, Ara h 2, Ara h 3, and Ara h 6. Notably, the proteins in area ii had lower molecular weights than those in area i. In addition, the numbers of peptides found in this area were also less than those in area i. This finding indicated that the aggregates in area ii might be constituted by small fragments of these allergens. The molecular weights of cross-linked products were only about 40 kDa, while Area i included bands around 70 kDa. The appearance of proteins with higher molecular

weights indicates aggregation among larger or more fragments after roasting. Moreover, many kinds of modification, including methylation, oxidation, and C₆H₁₀O₅ on allergens, were found by MS in aggregations.

3.3. Characterization of the APR Ara h 2 Structure. As indicated by the identified sequence of Ara h 2 (Q6PSU2)³² (Table 2), the enzymatic sites of trypsin greatly changed after

Table 2. Protein Identification on the Ara h 2 Monomer before and after Roasting by MS^a

no	sequence of peptides	APN Ara h2	APR Ara h 2
1	²³ QQWELQGDR ³¹	+	+
2	²³ QQWELQGDRR ³²	+	+
3	³² RCQSQR ³⁹	+	+
4	³³ CQSQR ³⁹	+	+, ○
5	⁴⁰ ANLRPCEQHLMQK ⁵²	+	+, ◇
6	⁴⁰ ANLRPCEQHLMQKIQR ⁵⁵	+	-
7	⁵³ IQRDEDSYGR ⁶²	+	-
8	⁵⁶ DEDSYGR ⁶²	+	+
9	⁹² RGAGSSQHQR ¹⁰²	+	-
10	⁹³ GAGSSQHQR ¹⁰²	+	+
11	¹⁰³ CCNELNEFENNQR ¹¹⁵	+	+, ○
12	¹¹⁶ CMCEALQQIMENQSDR ¹³¹	+, ◇	+, ◇
13	¹³⁶ QQEQQFK ¹⁴²	+	-
14	¹³⁶ QQEQQFKR ¹⁴³	+	-
15	¹⁴⁴ ELRNLPQQCGLR ¹⁵⁵	+	-
16	¹⁴⁷ NLPQQCGLR ¹⁵⁵	+	+
17	¹⁵⁶ APQRCDLEVESGGR ¹⁶⁹	+	-
18	¹⁶⁰ CDLEVESGGR ¹⁶⁹	+	+
19	¹⁶⁰ CDLEVESGGRDR ¹⁷¹	+	-
20	¹⁶⁰ CDLEVESGGRDRY ¹⁷²	+	-

^a "+" indicates detected peptides, "-" indicates not detected peptides, ○ indicates methyl modification, and ◇ indicates oxidation modification; the red italics section indicates complete linear epitopes; and the red bold section indicates incomplete linear epitopes.

roasting. The proteins in area iii of Figure 1C (IPN Ara h 2 monomers) were identified by MS by using native Ara h 2 as a comparison. The MS results showed 20 peptides detected in the native Ara h 2, while only 11 of them were detected in the roasted Ara h 2 after trypsin digestion. These peptides contained several linear epitopes.³³ Some of them were intact, including ⁴²LRPCEQHLMQ.⁵¹ No difference was found between raw peanuts and roasted peanuts. Some linear epitopes became fragmented in the roasted sample. Fragment ⁵³IQRDEDS⁵⁹ from epitope ⁵²KIQRDEDS⁵⁹ was detected in the raw peanut, but only ⁵⁶DEDS⁵⁹ was found in the roasted peanut.

As a comparison to the raw peanut, more modifications including methylation and oxidation were found on the roasted peanut. In the raw sample, the only oxidation was located on M125. The oxidation on both residues M125 and M49 was detected in roasted samples. In addition, two extra methyl groups were found in the roasted samples, namely, R39 and R115.

Although the peptides found in roasted APR Ara h 2 were less than those in APN Ara h 2, the trypsin digestion profile of Ara h 2 changed after the roasting.⁶ For example, peptides ⁴⁰ANLRPCEQHLMQK⁵² and ⁴⁰ANLRPCEQHLMQKIQR⁵⁵ were found in the raw peanut, while only the former was found in the roasted peanut. It was possible that K⁵² was

exposed to trypsin after roasting, but this site was buried in the raw peanut. This also happened on other cleavage sites, including R55, R92, R146, R159, and R169. Some of the cleaving sites were exposed after the roasting. In fragment $^{136}\text{QQEQQFKRELRLNPQQGLR}^{155}$, four peptides were found in the raw peanut, while only one was found in the roasted sample. Cleaving sites R135, K142, and R143 seemed to be buried after processing.

The cleavage sites affected by roasting are labeled in Figure 2. All three masked sites and one exposed site were located on

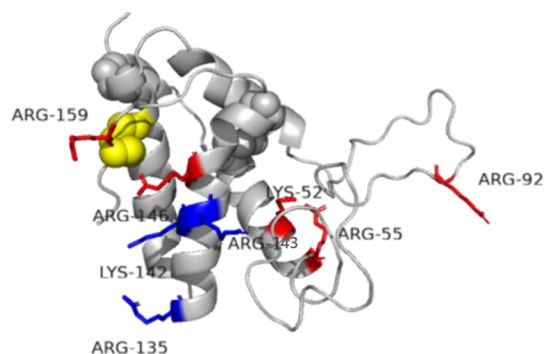


Figure 2. Difference of trypsin cleavage sites between raw Ara h 2 and APR Ara h 2. The cleavage enzymatic sites differed are marked as sticks, those covered after roasting are colored in blue, and exposed ones are colored in red and the disulfide bond effected cleavage is marked in yellow.

helix 5, indicating that the conformational structure of this fragment greatly changed. For the other exposed sites, two were located on helix 2 and two on random coil regions. R169 was not shown in the model.²⁰ Interestingly, R159 was located next to a disulfide bond between C118 and C160. This site might have been missed by trypsin in Ara h 2 from the raw peanut, but after roasting, the breakage of this disulfide bridge exposed this cleavage site of trypsin.

We also characterize the conformational structures of Ara h 2 before and after roasting (Figure 3). The CD and UV spectra of native Ara h 2 were similar, regardless of the purification method. Therefore, the secondary structures of IPN Ara h 2

and APN Ara h 2 might be the same. However, the structure of APR Ara h 2 differed from them. Its α -helix content decreased from 27.1 to 21.6% (APN Ara h 2), while the random coil content increased from 29.1 to 34.3% (APN Ara h 2). This finding indicated that the protein after the roasting tended to be disordered. The increase in UV absorption also indicated that its overall structure became more stretched than that of Ara h 2 from the raw peanut.

3.4. Potential Allergenicity of Ara h 2 after Roasting.

We performed the western blot (WB) test to assess the allergenicity of Ara h 2 and its roasted products (Figure 4). Before the IAC, the total peanut proteins between raw and roasted samples showed little difference in the WB. After the isolation of Ara h 2, the bands in the same position contained proteins with different immunological properties. All the polymers that were bound to anti-Ara h 2 IgG contained pieces of Ara h 2, as verified by MS, but their IgE recognition seemed different. As shown in lane 6 in Figure 4A,B, the concentration of proteins on the band above 170 kDa was quite high. However, it was not recognized by IgE in the WB. Meanwhile, the band of Ara h 2 below 40 kDa monomers could be recognized clearly in a low concentration. Similar to the band about 40 kDa, the band above 170 kDa showed no blotting, indicating no IgE binding. For the blotting around 70 kDa, only one narrow band was observed and had a weak signal. IgE recognition on the polymers was much weaker than that on the Ara h 2 monomer.

The potential allergenicity of the Ara h 2 monomer was also assessed by the ELISA with competitive inhibition and in the KU812 cell model, as shown in Figure 5. In the competitive inhibition ELISA, the IC_{50} values of native Ara h 2 monomers obtained by different purification methods were 0.156 and 0.158 $\mu\text{g/mL}$, which indicated that they had the same IgE binding capacity. The IC_{50} value of the Ara h 2 monomer in the roasted peanut was 0.098 $\mu\text{g/mL}$, showing that roasting enhanced the IgE binding capacity of Ara h 2, especially when the concentration was low.

The KU812 cell model showed that APR Ara h 2 showed the increased release of both β -HEX and $\text{TNF-}\alpha$, but the release of histamine was decreased. This finding indicated that the protein sample from the roasted peanut had greater ability

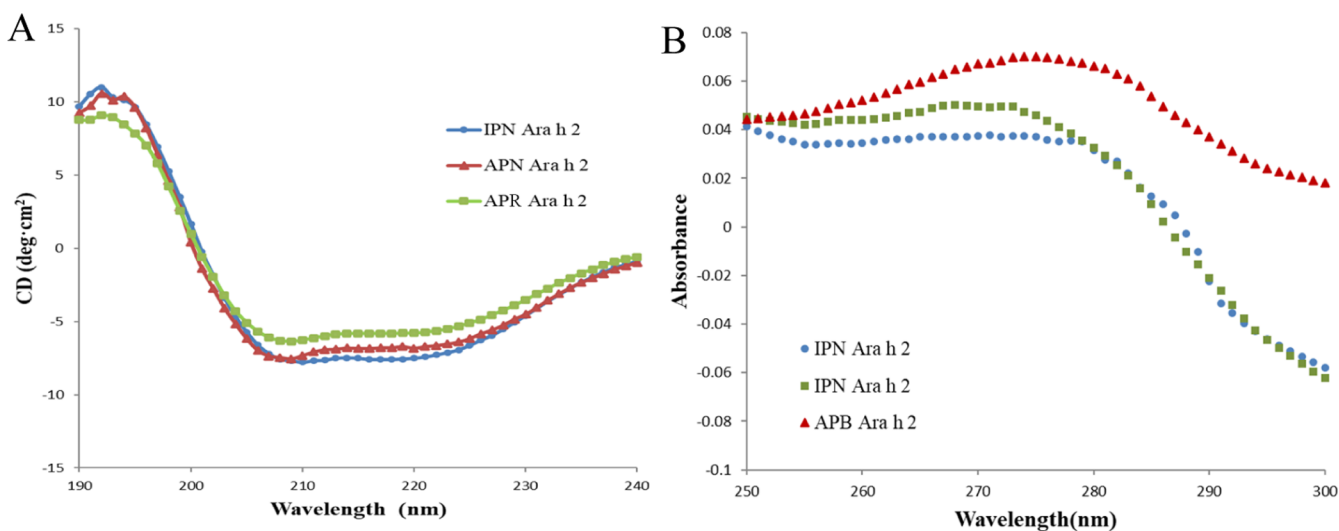


Figure 3. Secondary structure and UV spectra of Ara h 2.

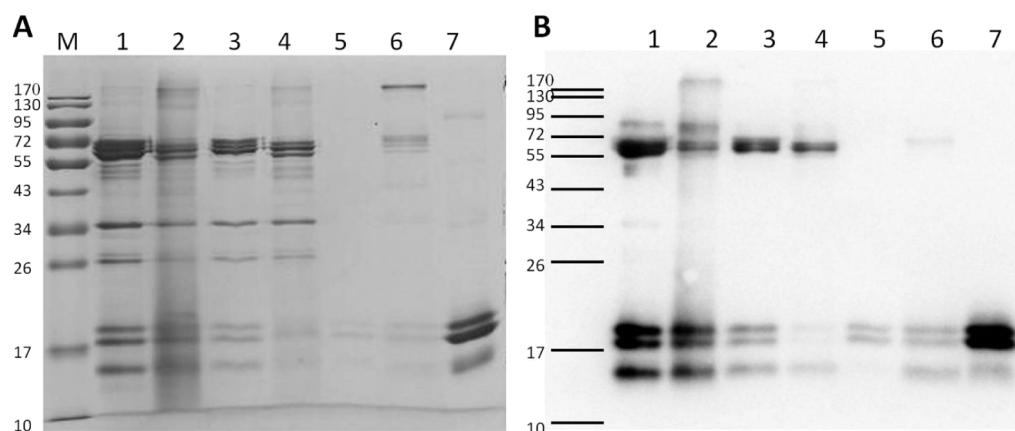


Figure 4. WB on the peanut protein. (A) SDS-PAGE profile. (B) WB of patients' sera. M: marker; 1: raw peanut protein; 2: roasted peanut protein; 3: nonspecific eluent of the raw peanut in IAC; 4: nonspecific eluent of the roasted peanut in IAC; 5: APN Ara h 2; 6: affinity-purified products from the roasted peanut in IAC; and 7: IPN Ara h 2.

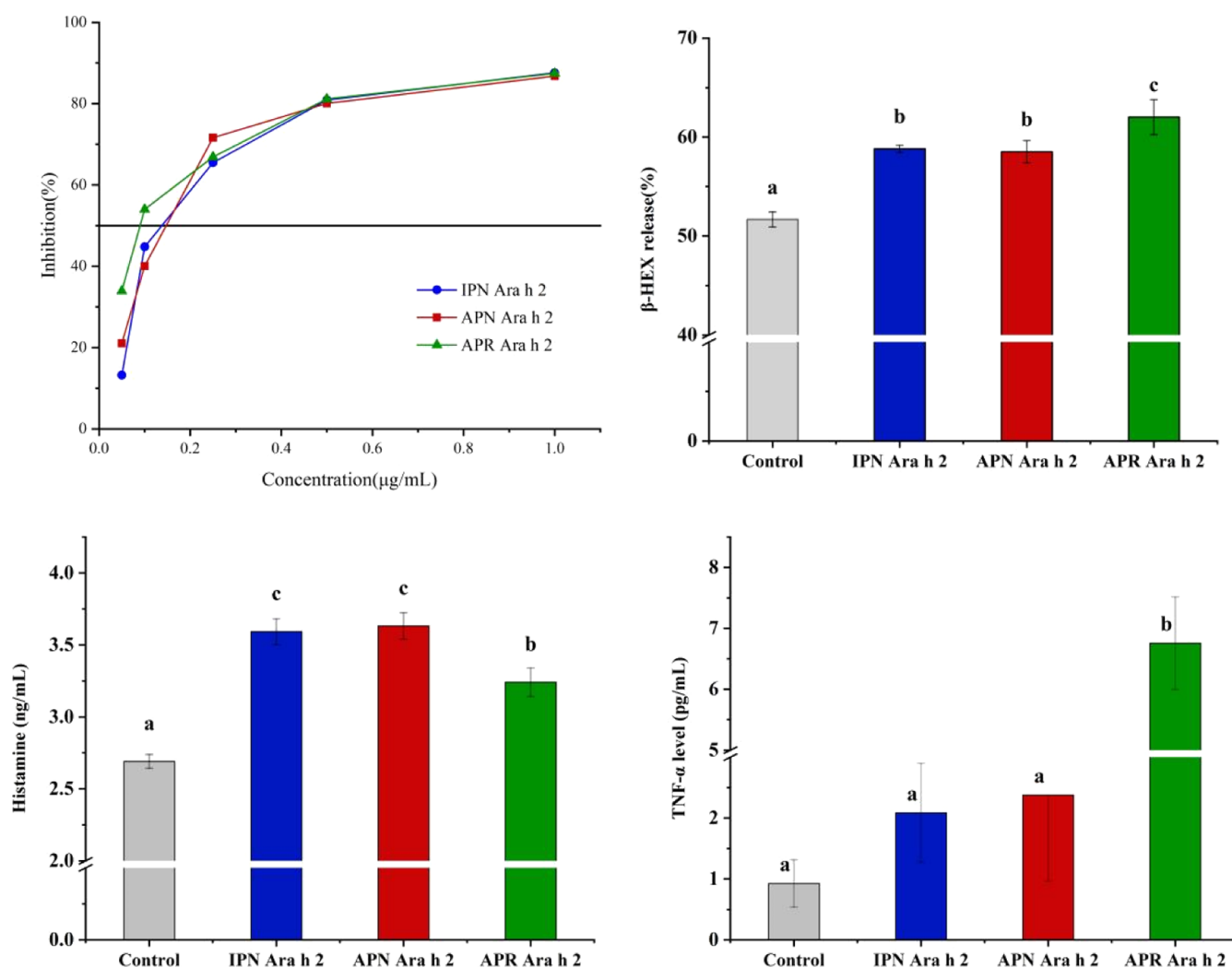


Figure 5. Potential allergenicity assessment on Ara h 2 by Ig E binding and the KU812 cell model. (A) Ig E binding capacity; (B) release ratio of β -HEX; (C): release of histamine; and (D): release of TNF- α . Different letters indicate significant differences ($p < 0.05$).

to stimulate the degranulation of human basophils but lesser

ability to recruit granulocytes to proinflammatory factors.

4. DISCUSSION

4.1. Whereabouts of Ara h 2 after Roasting. The effect of processing on purified Ara h 2 has been broadly studied,^{4,20,21} but the change of Ara h 2 roasted in the matrix could not be imitated in the purified protein. As shown in

Figure 1C, bands with Ara h 2 were mixed in roasted total proteins. No significant difference from the total native protein was found. After the IAC separation, Ara h 2 was traced.

Among the derivatives, the monomer of Ara h 2 decreased,³⁴ which meant that a significant part of Ara h 2 was broken down and/or aggregated, appearing at bands of higher molecular weight or disappearing as small fragments. In the matrix, the intermolecular cross-linked proteins containing Ara h 2 fragments induced by roasting^{35,36} were found and characterized. In the SDS-PAGE, bands of 40, ~70, and >170 kDa separated by IAC should contain the antigen of Ara h 2 or its fragments, which was proven by MS. The MS result also demonstrated that the Maillard reaction of allergens was enhanced by the heat treatment,^{37,38} but only two modifications, oxidation and methylation, were found on Ara h 2 monomers.

Not all proteins were extracted. Thermal processing of peanuts alters the solubility of proteins,³⁴ which might extract and test only a specific protein group.^{6,39} After the roasting, the protein extraction ratio of the Tris–HCl buffer decreased dramatically,⁶ indicating that some proteins including Ara h 2 were not present in the solution.

4.2. Structural Change of Ara h 2. Thermal processing may cause the degradation, aggregation, and cross-linking of allergens.³⁴ We found that the structure of Ara h 2 significantly changed. For the primary structure, some peptide fragments of Ara h 2 were broken in pieces during the roasting. These pieces, if aggregated with other peptides/proteins,⁴⁰ could be detected in SDS-PAGE (as seen in Figure 1C lane 2). In addition, some Ara h 2 molecules were modified, as oxidation and methylation on Ara h 2 were observed by MS. Some Ara h 2 molecules remained as monomers, but their disulfide bonds might have been rearranged.⁴¹ Our MS results indicated that the high cleavage efficiency of R159 relied on the breakage of the disulfide bond on C160. The aggregation of Ara h 2 was not found, but the high-molecular-weight band (e.g., above 170 kDa) might contain the Ara h 2 polymer.

The conformational structures of Ara h 2 monomers were characterized after roasting, which were changed.⁴² The decreased α -helix content of Ara h 2 and the increased random coil content after roasting were found, as previously reported.⁴³ In fact, the low content of the α -helix was also found on thermally processed purified Ara h 1.⁴⁴ For Act d 2, another heat-stable protein, thermal stress significantly affected its secondary structure, losing the α -helix and obtaining more random coils.⁴⁵ In our UV spectra experiments, the absorption maximum wavelength was increased, as seen on heated purified Ara h 2.⁴² The absorbance value at 280 nm increased for Ara h 2 extracted from the roasted peanut.⁴³

The structural changes of Ara h 2 have been shown to change its digestibility.^{5,46} Ara h 2 is a trypsin inhibitor, and roasting was reported to decrease its digestibility.³⁶ However, recent studies have shown that the digestibility of Ara h 2 increased after roasting.^{6,43,46} Our MS result showed that the cleavage sites increased on Ara h 2 monomers, mainly due to the conformational change of the protein.

4.3. Effect on Potential Allergenicity. Previous studies on the allergenicity of Ara h 2 after the processing were mainly focused on Ara h 2 monomers only.^{21,34,40} It has been demonstrated that the IgE binding capacity of Ara h 2 increased after roasting.^{34,40,47,48} For example, the Maillard reaction had been reported to enhance the IgE binding capacity of Ara h 2.^{35,49} In the present work, the IC₅₀ value of

Ara h 2 monomers decreased after roasting, but no glycosylation was found. In other words, the roasting-induced conformational structural change on Ara h 2 enhanced its IgE binding capacity. In Act d 2, conformational changes significantly reduced its IgE binding capacity, too.⁴⁵ The alteration in the protein structure and other physicochemical properties changed the allergenicity of the protein,^{11,13} which may due to changes in the IgE binding capacity.

The potential allergenicity of Ara h 2 after roasting showed different results in cell and animal models. In a rat basophilic leukemia (RBL) cell model, the β -HEX release increased,⁴³ which is accordance with the present results in the KU812 cell model. In the RBL assay, thermal treatment had no impact on the potential allergenicity of the unhydrolyzed allergen,⁴⁶ while the roasted peanuts showed higher allergenicity on BALB/c mice.⁴³ After the roasting, the Ara h 2 monomers occupied only 13% of all the derivatives in the SDS-PAGE lane. Thus, the allergenicity of other products is of greater importance, which has not been well-studied.

The extraction and assessment methods greatly influence the allergenicity,⁶ possibly because the content of protein changed in different extracts. After the processing in the matrix, many products would appear in the total protein instead of in the pure protein, and some may not be even extracted properly from the matrix. As shown in our WB results, among the products of Ara h2, only one band of the cross-linking product could be recognized by IgE. Although processed Ara h 2 and Ara h 6 were recognized, the structure and allergenicity might be different from that of raw samples.^{50,51} Many bands could not be recognized by IgE, in spite of their antigenicity to the Ara h 2 polyclone IgG antibody. New peptide fragments appeared during the roasting,⁴³ which could be found by IgG even after the cross-linking. However, the allergenicity of cross-linked products might be lost. After the roasting, the band profiles of the main allergens changed. The disappearance of these bands also altered the immunoreactivity of proteins.²³ Here, the potential allergenicity of APR Ara h 2 increased, but the amount of Ara h 2 monomers decreased dramatically. A large part of Ara h 2 was found in cross-linked products that could not be recognized in patients' sera. In other words, their allergenicity was eliminated after cross-linking.

In this work, Ara h 2 was found to be dramatically decreased. Many kinds of cross-linked products containing Ara h 2 (or its fragments) appeared. Roasting altered the structure and allergenicity of monomers and derivatives of Ara h 2. The assessment on the allergenicity of Ara h 2 itself cannot reflect the allergenicity change of this protein in the matrix after processing, considering the amount and property of its derivatives.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.jafc.1c06828>.

Information of PA patients (PDF)

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Notes

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