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Comparative Proteomic Analysis and IgE Binding Properties of Peanut Seed and Testa (Skin)

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ABSTRACT: To investigate the protein composition and potential allergenicity of peanut testae or skins, proteome analysis was conducted using nanoLC-MS/MS sequencing. Initial amino acid analysis suggested differences in protein compositions between the blanched seed (skins removed) and skin. Phenolic compounds hindered analysis of proteins in skins when the conventional extraction method was used; therefore, phenol extraction of proteins was necessary. A total of 123 proteins were identified in blanched seed and skins, and 83 of the proteins were common between the two structures. The skins contained all of the known peanut allergens in addition to 38 proteins not identified in the seed. Multiple defense proteins with antifungal activity were identified in the skins. Western blotting using sera from peanut-allergic patients revealed that proteins extracted from both the blanched seed and skin bound significant levels of IgE. However, when phenolic compounds were present in the skin protein extract, no IgE binding was observed. These findings indicate that peanut skins contain potentially allergenic proteins; however, the presence of phenolic compounds may attenuate this effect.

KEYWORDS: *IgE binding, LC-MS/MS, peanut, proteomics, skins, testae*

INTRODUCTION

Peanut (*Arachis hypogaea* L.), a legume, is one of the top five oilseeds produced worldwide, grown extensively as a source of oil and protein. In legumes, within the hull and immediately encasing the cotyledon is the testa, often referred to as the seed coat, but within the peanut industry it is more commonly called the skin. The skin is a distinct plant structure that is critical for seed development and functions to regulate nutrient uptake and defend against various environmental stresses such as fungal invasion, and it is key in regulating imbibition for germination.[1] Accordingly, the physiology of the skin has direct and important consequences to the agronomic performance of peanuts (and similar oilseeds). In much of North America and elsewhere, peanuts are primarily consumed roasted, as part of peanut butter or confections or as whole seed snack nuts. In the preparation of these products, the skins are typically removed from the seed after blanching (mild dry heat) or dry roasting. Therefore, skins, which compose approximately 3% of the seed weight, are a major byproduct of the peanut-processing industry with hundreds of thousands of tons being produced annually.[2]

Tannins are generally defined as secondary plant metabolites that have molecular weights of at least 500 Da, contain multiple phenolic moieties, and have the capacity to precipitate proteins.[3,4] For many years, peanut skins have been established as a rich source of tannins,[5] and in recent years the specific polyphenolic makeup of peanut skins has been elucidated with the identification of various procyanidins.[6−8] Whereas peanut skin composition is expected to vary with cultivar and growing conditions, a typical proximate composition is 19.7% fat, 18.6% protein, 2.2% ash, 18.1% fiber, and 41.4% other components.[8] Despite being an appreciable source of protein on a proximate basis, peanut skins have limited application as a feed ingredient as this procyanidin-rich material negatively affects feed performance by binding protein and reducing nitrogen availability in the gastrointestinal tracts of livestock such as pigs and cattle.[9] More recently, a multitude of health benefits have been associated with procyanidin sources, including peanut skins. Examples include antioxidant properties and defense against inflammation, cardiovascular disease, and cancer.[10−12] As such, there is intense interest in identifying value-added applications for this unique and readily available biomaterial as highlighted in recent research.[8,13,14]

Peanuts contain proteins that are major food allergens; therefore, the allergenicity of peanut skins must be addressed for any potential food application involving this byproduct. The International Union of Immunological Societies (IUIS) currently recognizes 13 known allergenic peanut proteins, and they are termed Ara h 1 through Ara h 13. Many of the allergens, including Ara h 1−3, 6, and 7 are seed storage proteins, which exist as several different isoforms. Each of these allergens is expressed in peanuts and can differ in relative abundance depending on cultivar.[15] Currently, no information exists on the expression of these or other proteins in peanut skins. Binding of proteins by phenolic compounds, particularly procyanidins, can alter the structural and functional characteristics of the protein and often renders them insoluble.[16]

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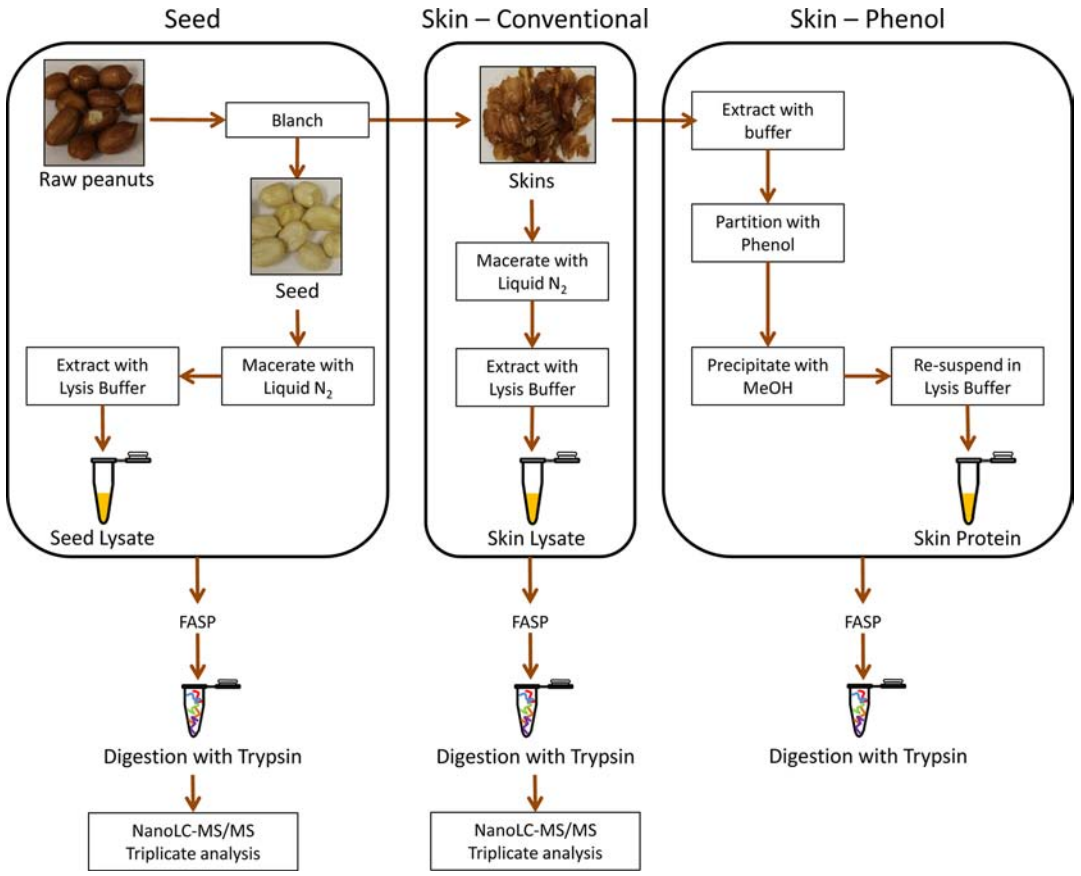

Figure 1. Experimental workflow demonstrating extraction of protein from peanut seed and skins for proteomic analysis.

Evidence also exists suggesting that phenolic compounds can alter IgE binding by the protein.[17]

Proteomics has proven to be a valuable tool in plant research over recent years. For peanuts, this information is helping breeders identify strategies to improve drought tolerance and disease resistance, in addition to improving many other aspects of plant quality.[18] Proteomics-based approaches have proven effective for detection of low levels of peanut allergens in foods.[19,20] More recently, researchers have used proteomics to better understand protein changes during roasting, which have important implications for allergen detection.[21] Most proteomic research involving legumes focuses primarily on the edible portion of the seed or other plant components such as the leaf, but the skins have largely been ignored. Proteomic analysis of the seed coat of *Medicago truncatula*, a model for legume biology, during development has been conducted.[22]

The primary objective of this study was to analyze the proteome of peanut skin in comparison to corresponding blanched peanut seed (skin removed) using nanoLC-MS/MS. To our knowledge, this is the first documentation of the peanut skin proteome. Two procedures for extracting protein from peanut skins were explored, as this procyanidin-rich matrix inherently limits traditional extraction procedures. Furthermore, proteins obtained from the blanched seed and skin were compared for their capacity to bind peanut-specific IgE in human sera obtained from peanut-allergic patients. This study has important implications for peanut breeding and peanut agronomics by aiding in the understanding of protein expression in peanut skin, a distinct component of the seed critical in development, defense, and germination, but ultimately poorly understood on a biochemical level. Additionally, this study contributes to research involving utilization of peanut skins as a byproduct by exploring the potential allergenicity of this unique material.

MATERIALS AND METHODS

Plant Material. Raw-runner-type peanuts from the 2011 growing season were provided by Olam Edible Nuts (Blakely, GA) after being subjected to proprietary blanching temperatures to loosen the skins. The peanuts were stored at 4 °C until use, at which time the skins were removed by hand, ensuring that the skins and seed were from the same small lot.

Compositional Analysis. Proximate analyses of blanched peanut seed and skins were performed by Barrow-Agee Laboratories, LLC (Memphis, TN, USA). Amino acid compositions were determined by first hydrolyzing the protein completely with 6 N HCl containing 1% phenol using a CEM Explorer microwave digestion system at 165 °C for 15 min. Following digestion, amino acids were analyzed using a Hitachi L-8900 Amino Acid Analyzer (Hitachi High Technologies America, Inc.). In this method, glutamine and glutamic acid are expressed as one value as are asparagine and aspartic acid.

Sample Preparation and Protein Isolation. The experimental workflow for sample preparation and protein isolation is shown in Figure 1. The skins from approximately 60 peanut seed were carefully removed, and both skins and blanched seed were coarsely ground using a commercial coffee grinder. Approximately 200 mg each of seed and skins was frozen separately in liquid nitrogen, finely ground, and added to 1.5 mL microcentrifuge tubes with 200 mg of 0.5 mm glass disruptor beads and 1 mL of cold lysis buffer (50 mM Tris, 8 M urea, 2 M thiourea, 10 mM EDTA, 10 mM DTT, 0.001% sodium azide, pH 7.78). Tubes were placed in a Vortex Disruptor Genie (Scientific Industries, Inc., Bohemia, NY, USA) for 1 min and stored on ice for 5 min. Disruption and subsequent cooling were performed two more times. Samples were then centrifuged for 30 min at 14000*g* to remove

insoluble components, and the supernatants were transferred to new tubes. Samples were stored at −80 °C until further analysis.

Phenol Extraction of Peanut Skin Proteins. An additional extraction procedure was performed for peanut skins according to the method of Faurobert et al.[23] with slight modifications for the extraction of proteins from recalcitrant plant tissues. Peanut skins were frozen in liquid nitrogen and finely ground using a mortar and pestle. Samples (0.5 g) were suspended in 5 mL of extraction buffer (500 mM Tris-HCl, 50 mM EDTA, 700 mM sucrose, 100 mM KCl, pH 8.0) and allowed to shake on ice for 10 min. Then, 5 mL of Tris-buffered phenol was added, and tubes were shaken for 10 min at room temperature. Tubes were then centrifuged for at 5500*g* for 10 min at 4 °C. The phenolic phase (top layer) was recovered into a new tube. Extraction buffer (3 mL) was added to the recovered phenolic phase, and tubes were shaken for 3 min at room temperature. Tubes were then centrifuged at 5500*g* for 10 min at 4 °C. The phenolic phase was recovered again and placed into a new falcon tube. Twelve milliliters of precipitation solution (0.1 ammonium acetate in cold MeOH) was added, and protein was precipitated at −20 °C overnight. Following precipitation, samples were centrifuged at 5500*g* for 10 min at 4 °C. The pellet was rinsed three times with cold precipitation solution and one time with cold acetone. The pellet was frozen in liquid nitrogen, ground with a mortar and pestle, resuspended in lysis buffer, and stored at −80 °C until further analysis.

SDS-PAGE. Approximately 50 μg of protein from each sample was diluted 1:1 with Laemmli buffer containing 5% mercaptoethanol and loaded into the wells of a 10−20% Tris-HCl 1D gel (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Proteins were separated on the gel at 200 V for 55 min using Tris/glycine/SDS running buffer, and bands were stained using Bio-Rad Bio-Safe Coomassie stain.

Western Blot. Following electrophoresis, proteins from gels were transferred onto a polyvinyl difluoride (PVDF) membrane using an iBlot Gel Transfer Device (Life Technologies, Carlsbad, CA, USA). Membranes were stained with Ponceau S for protein visualization and then blocked with phosphate-buffered saline/Tween (PBST) containing 2% BSA for 1−2 h and then transferred to diluted (1:10) human sera pooled from six confirmed peanut-allergic patients (average IgE content of 336.6 kU/L) overnight with gentle shaking. Human sera were obtained from the Department of Pediatrics at the University of North Carolina. The blots were then incubated with biotinylated goat IgG−anti-human IgE diluted 1:4000 with PBST for 1 h followed by NeutrAvidin horseradish peroxidase conjugate diluted 1:10000 in PBST with 2% BSA for 30 min. The membrane was then submerged in SuperSignal West Pico Chemiluminescent Substrate for 5 min and imaged on a ChemiDoc Imaging system. Between each incubation step, the membrane was washed thoroughly in PBST.

Filter-Aided Sample Preparation (FASP). FASP was performed according to a previously published procedure with some modifications.[24,25] Briefly, approximately 200 μg of seed or skin protein from the above extraction protocols was reduced using 5 mM DTT at 56 °C for 30 min. Reduced samples were added to 200 μL of 8 M urea in 0.1 M Tris-HCl, pH 8.5, and placed into individual Vivacon 500 30 kDa MWCO filters (Fisher Scientific, Hampton, NH, USA). Samples were concentrated (14000*g* for 15 min) and alkylated with 100 μL of 0.05 M iodoacetamine in 8 M urea in 0.1 M Tris-HCl, pH 8.5. Samples were concentrated again (14000*g* for 15 min) and exchanged three times with 8 M urea in 0.1 M Tris-HCl, pH 8.5, and three times with 0.05 M ammonium bicarbonate (pH 8.0). Digestion was performed by adding trypsin (1:100 enzyme to protein ratio) in 40 μL of 0.05 M ammonium bicarbonate and incubating in a wet chamber at 37 °C for 18 h. Peptides were eluted from the filter unit by centrifugation (14000*g* for 10 min), and peptide concentration was determined by UV−vis (λ = 280 nm) using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

NanoLC-MS/MS. Each sample was reconstituted in mobile phase A to a concentration of 0.12 μg/μL according to NanoDrop readings. A 5 μL (600 ng) sample was aspirated into a 5 μL loop and loaded onto the trap column. NanoLC-MS/MS was performed on a cHIPLC-Nanoflex system (Eksigent, Dublin, CA, USA) at 26 °C. A trap column, 200 μm × 0.5 mm in-line with a 75 μm × 15 cm analytical column, both packed with Chrom XP C18-CL 3 μm 120 Á, and 350 nL/min flow rates were utilized for separation. A 5−40% B gradient was run for 120 min, and three technical replicates of each sample were recorded. Mobile phase A was composed of 98% H_2O, 2% acetonitrile, and 0.2% formic acid, and mobile phase B was composed of 98% acetonitrile, 2% H_2O, and 0.2% formic acid.

MS analysis was performed on a hybrid LTQ-Orbitrap XL MS (Thermo Fisher Scientific, Bremen, Germany). Optimized instrument parameters recently published by Andrews et al.[26] were utilized with one minor change, such that MS/MS data were collected in centroid mode.

Data Analysis. Raw files were searched against a concatenated target-reverse peanut (*A. hypogaea*) Uniprot database with 877 protein entries in MASCOT. Distiller option Orbitrap_low_res_MS2_2.opt was used as peak picking algorithm, and search parameters were 5 ppm MS tolerance, 0.6 Da MS/MS tolerance, and two allowed missed cleavages. Carbamidomethylation of cysteines was set to be a fixed modification, and variable modifications were oxidation of methionines and deamidation of glutamines and asparagines. ProteoIQ version 2.3.02 (BioInquire, Athens, GA, USA) was used to apply a 1% protein false discovery rate (FDR) for confident protein identifications.

RESULTS AND DISCUSSION

Compositional Analysis. The proximate composition of the blanched peanut seed and peanut skins is presented in Table 1. The blanched seed were low mositure (5.1%) and composed primarily of fat (47.0%) and protein (28.3%) with lower amounts of fiber (6.6%), ash (1.9%) and "other" components (11.1%), which include sugars and other nonfiber carbohydrates. The proximate composition of the skins differed from the blanched seed as they were higher in moisture (11.7%) and fiber (17.0%) and lower in fat (15.7%) and protein (14.9%). The skins also contained very high levels of other components (39.3%), which, in this case, encompassed not only sugars and nonfiber carbohydrates but also polyphenolic compounds, which have been shown to be present at levels up to 18% in peanut skins.[27]

Table 1. Proximate Composition (Percent) of Blanched Peanut Seed and Skins

	blanched peanut seed	peanut skins
moisture	5.1	11.7
protein	28.3	14.9
fat	47.0	15.7
fiber	6.6	17.0
ash	1.9	1.5
other	11.1	39.3

The relative amino acid compositions of the blanched seeds and skins are given in Table 2. The level of each amino acid is significantly ($p < 0.05$) different between the blanched seed and the skin. The most notable differences are in glutamine/glutamic acid, glycine, and arginine. The blanched seed contained 21.0% glutamine/glutamic acid, whereas the skin contained only 7.6%. Additionally, the skins contain 38.0% glycine, whereas the seeds contain only 6.3%. The blanched seed contained 12.5% arginine, whereas the skins contained only 3.2%. Peanuts are known for their high levels of arginine.[28] L-Arginine is a precursor of nitric oxide (NO), and dietary arginine has been shown to enhance NO synthesis, resulting in cardioprotective effects.[29] The difference in amino acid contents between the blanched seed and skin are similar to those previously reported[8] and suggests that differences exist in the proteins expressed in the two plant structures.

Table 2. Amino Acid Composition (Percent)[a] of Blanched Peanut Seed and Skins

amino acid	blanched peanut seed	peanut skins
Asp	13.6 ± 0.4[b]	9.6 ± 0.1
Thr	2.5 ± 0.2	2.1 ± 0.1
Ser	4.7 ± 0.1	10.7 ± 0.3
Glu	21.0 ± 0.1	7.6 ± 0.1
Gly	6.3 ± 0.1	38.0 ± 1.3
Ala	3.8 ± 0.2	1.9 ± 0.1
Val	4.3 ± 0.0	3.2 ± 0.3
Met	0.9 ± 0.0	BDL[c]
Ile	3.2 ± 0.0	2.1 ± 0.3
Leu	7.5 ± 0.1	4.9 ± 0.0
Tyr	4.3 ± 0.1	3.7 ± 0.2
Phe	5.6 ± 0.0	BDL
Lys	2.7 ± 0.0	6.1 ± 0.1
His	2.4 ± 0.0	4.2 ± 0.2
Arg	12.5 ± 0.2	3.2 ± 0.1
Pro	4.7 ± 0.1	2.7 ± 0.4

[a]Values for each amino acid represent a percent of the total amino acids identified. [b]Values within each row are significantly different ($p < 0.05$). [c]Below detection limit.

Protein Extraction and Gel Electrophoresis. Extraction of proteins from plant materials for proteomic analysis is often difficult due to the presence of interfering compounds such as polysaccharides, lipids, and phenolic compounds; therefore, alternative methods for protein isolation such as phenol extraction are often employed.[23] Because peanut skins are a rich source of phenolic compounds, we employed two extraction methods for comparison, a traditional extraction with liquid nitrogen and lysis buffer (same procedure as performed on seed) and a phenol extraction for recalcitrant plant tissues. Likewise, a modified extraction procedure was needed to extract mRNA from the seed coat of *Medicago truncatula* due to interference from phenolic compounds.[22]

Clear differences were observed in the distribution of proteins extracted using the two methods by SDS-PAGE after Coomassie staining (Figure 2a). Using the traditional extraction method, the proteins appeared as one smear down the entire length of the gel, with some banding visible. The smearing was likely due to the presence of polyphenolic compounds, which can bind to proteins and result in aggregation and interference with SDS-PAGE separation. In contrast, the proteins that were extracted by the phenol method appeared as distinct bands, with many corresponding to bands observed in the blanched peanut seed. Although similar amounts of protein were loaded onto the filter for trypsin digestion, proteins from the traditional extraction procedure were not easily digested by trypsin as evidenced by low peptide recoveries after digestion compared to other samples (50.1 μg for seed, 57.2 μg for phenol extracted skin, and 8.2 μg for traditionally extracted skin). This is likely also due to the presence of phenolic compounds, particularly procyanidins, which are known to interfere with enzymatic digestion.[30] The amount of protein extracted from the skins using the phenol method was determined by nitrogen analysis to be 16.6 ± 0.6 g/100 g skins. This agrees well with the protein content of the skins determined by proximate analysis (14.9%) and indicates that the phenol extraction method was effective in extracting protein from the skins. It was determined that this modified extraction method was necessary for protein visualization by SDS-PAGE, trypsin digestion, and subsequent nanoLC-MS/MS analysis and should be employed in future proteomic studies involving peanut skins.

IgE Binding. Proteins extracted from the blanched peanut seed and peanut skin (using both extraction methods) were evaluated for IgE binding from serum obtained from peanut-allergic patients (Figure 2b). Protein extracted from the blanched seed displayed significant IgE binding, and the major allergens Ara h 1, Ara h 2, and Ara h 3 were prominent. The IgE binding pattern of the skin proteins extracted using the phenol method was similar to that of the corresponding blanched peanut seed with the exception of a missing high molecular weight band (~100 kDa) and lower intensity binding in some of the lower molecular weight bands including Ara h 2 (17−20 kDa) in the peanut skin extract. No IgE binding was observed in the peanut skin protein extracted using the conventional method, even though equivalent protein concentrations were analyzed and proteins were visualized when the PVDF membrane was stained with Ponceau S (not shown). This is likely due to the presence of polyphenolic compounds that were extracted into the lysis buffer along with the proteins. Polyphenolic compounds, particularly procyanidins, which are abundant in peanut skins, are known to bind proteins and alter both structure and functionality.[3,16] Therefore, such binding might have an effect on the allergenicity of the proteins. In fact, simple phenolic compounds such as caffeic, chlorogenic, ferulic,

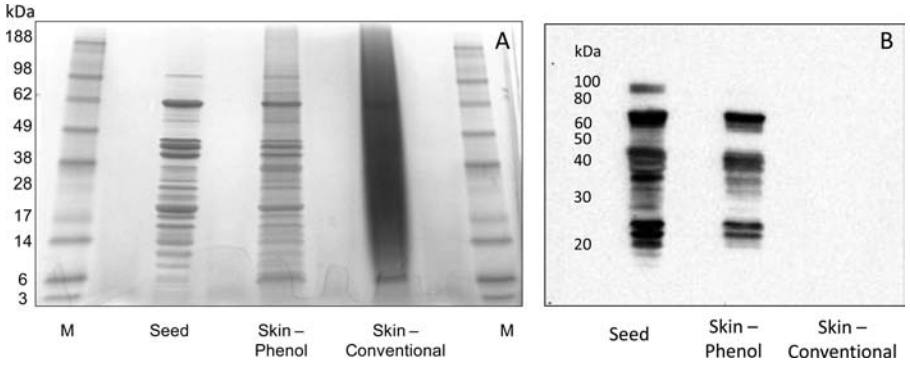

Figure 2. (a) SDS-PAGE of blanched peanut seed and peanut skin samples. (b) Western blot of blanched peanut seed and peanut skin samples exposed to pooled sera from six peanut allergic patients with a total concentration of peanut-specific IgE of 336.6 kU/L. Protein from "Skin−Phenol" was extracted using the phenol extraction method, and "Skin−Conventional" was extracted using liquid N_2 maceration and lysis buffer. Approximately 50 μg of protein was loaded per lane.

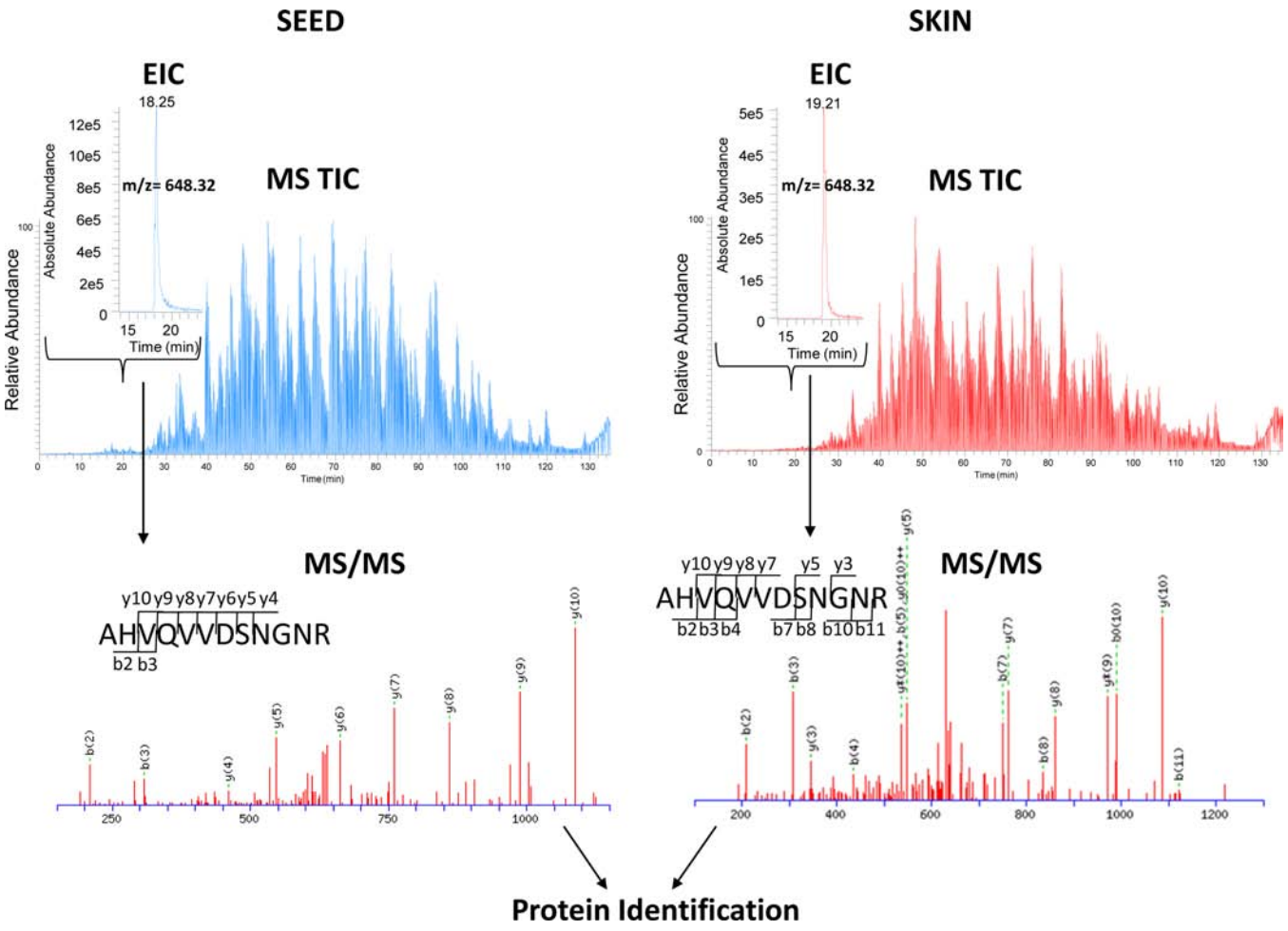

Figure 3. Total ion chromatograms (TIC) from blanched seed (left) and skin (right). An extracted ion chromatogram (EIC) was performed for the m/z 648.32 peak, shown in the expansion of the MS total ion chromatogram. Below is the resulting MS/MS spectrum identifying the fragment peaks and sequence of the m/z 648.32 obtained from MASCOT. This is a conserved sequence that maps to multiple proteins.

and tannic acids have been demonstrated to reduce IgE binding in peanut extracts and liquid peanut butter by forming insoluble complexes with the allergenic peanut proteins.[17,31] Although Western blotting using sera from peanut-allergic patients measures only IgE binding and is not a true indicator for effector activity or in vivo allergenicity, evidence suggests that procyanidins from peanut skins and other procyanidin-rich foods such as apples, tea, and grape seed may actually provide some protection against allergenic responses.[32−34] Related, an A-type dimer from peanut skin was recently shown to inhibit degranulation of rat basophilic leukemia cells that were stimulated by an antigen, which suggests that peanut skin polyphenolics may attenuate allergic responses.[35]

Protein Identification by NanoLC-MS/MS and Bioinformatics. Proteins in the blanched seed and skin were identified by nanoLC-MS/MS and subsequent MASCOT searching against the *A. hypogaea* database. Total ion chromatograms from the blanched seed and skin and extracted ion chromatograms for the m/z 648.32 peak are shown in Figure 3. Also shown is an example MS/MS spectrum showing the fragment peaks identifying the sequence of the m/z 648.32 peak obtained from MASCOT. This example peptide is a conserved sequence that maps to several proteins. Sequencing of the peanut genome is an ongoing process and, thus, protein database entries specific to the peanut are limited. Therefore, many of the peptides (>88%) present in the digest were not matched to proteins in the database.

A total of 123 unique proteins were identifed between the samples with 83 of the proteins common to both the blanched seed and the skin. Two proteins identified in the blanched seed were not found in the skin, whereas 38 proteins were identified in the skin but not in the blanched seed. Lists of the proteins identified in the blanched seed and skin along with their corresponding biological process and molecular function (if known) are provided in Tables 3 and 4, respectively. Proteins within each table are sorted by their normalized spectral abundance factor (NSAF), which relates to relative abundance in each sample.[36] NSAF is a method of data analysis that accounts for both protein size and variability between runs. It is based on spectral counting, which has been demonstrated to be effective for quantitative proteomic studies including those involving peanuts.[37] The number of spectral counts for each protein is divided by the mass or protein length (i.e, number of amino acids) to determine the spectral abundance factor (SAF). Individual SAFs are normalized to 1 to account for run-to-run variation by dividing by the sum of all SAFs.[38]

Many of the known allergenic proteins were identified in both the blanched seed and the skin (Tables 3 and 4). The most abundant proteins identified in both were allergenic seed storage proteins (Ara h 1−3, 6, 7). Interestingly, the single most abundant protein in the seed was Ara h 3, whereas in the

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Table 3. Proteins Identified in Blanched Peanut Seeds[a]

sequence ID[b]	protein name	biological process[c]	molecular function[c]	isoallergen	NSAF value	sequence coverage
A1DZF0	arachin 6		seed storage	Ara h 3	0.0625	72.779
Q9FZ11	Gly1		seed storage	Ara h 3	0.0584	63.327
Q5I6T2	arachin Ahy-4		seed storage	Ara h 3	0.0583	69.115
B5TYU1	arachin Arah3 isoform		seed storage	Ara h 3	0.0571	72.264
Q647H3	arachin Ahy-2		seed storage	Ara h 3	0.0565	67.598
Q647H4	arachin Ahy-1		seed storage	Ara h 3	0.0556	70.336
P43238	allergen Ara h 1, clone P41B		seed storage	Ara h 1	0.0555	64.696
Q647G9	conglutin		seed storage	Ara h 6	0.0530	80.000
P43237	allergen Ara h 1, clone P17		seed storage	Ara h 1	0.0523	65.798
Q8LKN1	allergen Arah3/Arah4		seed storage	Ara h 3	0.0521	68.773
Q6T2T4	storage protein		seed storage	Ara h 3	0.0510	66.791
Q6PSU2	conglutin-7		seed storage	Ara h 2	0.0435	61.047
O82580	glycinin (fragment)		seed storage	Ara h 3	0.0379	48.915
E5G076	Ara h 1 allergen		seed storage	Ara h 1	0.0374	48.627
Q0GM57	iso-Ara h3		seed storage	Ara h 3	0.0283	52.148
A1DZF1	arachin 7 (fragment)		seed storage	Ara h 3	0.0196	28.019
E5FHY1	late embryogenesis abundant protein group 1 protein				0.0106	64.894
P00760	cationic trypsin	digestion	protease		0.0106	45.935
E5FHY0	late embryogenesis abundant protein group 1 protein				0.0096	71.875
Q647G8	2S protein 2		seed storage	Ara h 7	0.0093	50.633
B0YIU5	Ara h 8 allergen isoform	plant defense	pathogenesis-related protein	Ara h 8	0.0087	77.778
Q647G5	oleosin 1	lipid storage			0.0083	41.420
E5FHY2	late embryogenesis abundant protein group 1 protein				0.0080	66.327
E9LFE8	11S arachin (fragment)		seed storage	Ara h 3	0.0072	51.538
Q4U4M1	LEA protein (fragment)				0.0072	68.421
B4XID4	Ara h 7 allergen		seed storage	Ara h 7	0.0067	42.683
E5FHY4	late embryogenesis abundant protein group 3 protein				0.0064	70.13
B6CG41	nonspecific lipid-transfer protein (fragment)	lipid transport	lipid binding	Ara h 9	0.0063	65.217
E5FHY8	late embryogenesis abundant protein group 3 protein				0.0052	53.03
F6KLJ6	annexin		calcium ion binding		0.0048	41.587
Q45W87	oleosin 1	lipid storage		Ara h 11	0.0047	24.088
Q647H1	conarachin		seed storage	Ara h 1	0.0044	37.160
A1E2B0	11S seed storage globulin B1		seed storage	Ara h 3	0.0044	39.344
Q0Q0Q9	type 4 metallothionein		zinc ion binding		0.0042	65.854
B4UW70	fiber annexin (fragment)		calcium ion binding		0.0040	47.826
E5FHZ0	late embryogenesis abundant protein group 4 protein				0.0039	21.084
P01066	Bowman−Birk type proteinase inhibitor A-II		serine-type endopeptidase inhibitor		0.0039	78.571
O20356	ribulose 1,5-bisphosphate carboxylase-oxygenase large subunit (fragment)	photosynthesis	ribulose-bisphosphate carboxylase		0.0037	44.946
Q4JME7	lipoxygenase	fatty acid biosynthesis	iron ion binding/ lipoxygenase		0.0036	47.972
E5FHY9	late embryogenesis abundant protein group 4 protein				0.0036	19.186
B6CEX8	nonspecific lipid-transfer protein	lipid transport	lipid binding	Ara h 9	0.0035	34.483
A7LB60	steroleosin A	oxidation reduction	nucleotide binding/ oxidoreductase		0.0034	35.244
E9LFE9	11S arachin		seed storage	Ara h 3	0.0032	21.875
Q45W86	oleosin 2	lipid storage		Ara h 11	0.0030	24.088
Q647H2	arachin Ahy-3		seed storage	Ara h 3	0.0028	37.603
Q06H19	UDP-glucose pyrophosphorylase (fragment)		nucleotide transfer		0.0027	42
E5FHY3	late embryogenesis abundant protein group 2 protein dehydrin				0.0026	28.986
E5FHZ1	late embryogenesis abundant protein group 5 protein				0.0026	53.636
P02872	galactose-binding lectin		carbohydrate binding		0.0026	40.293
B4UW88	heat shock protein 1 (fragment)	stress response			0.0025	30.597

Table 3. continued

sequence ID[b]	protein name	biological process[c]	molecular function[c]	isoallergen	NSAF value	sequence coverage
Q45W80	nucleoside diphosphate kinase		ATP binding		0.0025	32.215
A7LB59	steroleosin B	oxidation reduction	nucleotide binding/ oxidoreductase		0.0025	24.079
B4UWC0	lipoxygenase 2 (fragment)	oxidation reduction	metal ion binding/ oxidoreductase		0.0025	34.078
E5FHY7	late embryogenesis abundant protein group 3 protein				0.0024	19.5
Q647G3	oleosin	lipid storage		Ara h 10	0.0023	34.337
Q0Q0Q8	type 4 metallothionein		zinc ion binding		0.0021	54.878
A1E2B1	11S seed storage globulin B2		seed storage		0.0021	22.022
Q6PWX0	calmodulin		calcium ion binding		0.0021	22.297
E5FHY5	late embryogenesis abundant protein group 3 protein				0.0020	30.986
Q6J1J8	oleosin	lipid storage			0.0019	20.455
E5FHY6	late embryogenesis abundant protein group 3 protein (fragment)				0.0019	28.834
Q9AXI0	oleosin variant B	lipid storage			0.0017	20.455
G9HPX8	peptidyl-prolyl *cis*−*trans* isomerase	protein folding	peptidyl-prolyl *cis*−*trans* isomerase activity		0.0014	24.419
Q06H32	thioredoxin fold	oxidation reduction	oxidoreductase		0.0013	24.691
E5FHZ7	late embryogenesis abundant protein group 9 protein				0.0011	9.392
Q1HDS7	superoxide dismutase [Cu−Zn]	superoxide metabolic process	netal ion binding		0.0011	13.158
B4UWA1	putative uncharacterized protein (fragment)		lactoylglutathione lyase activity		0.0010	17.857
Q2PXN2	17.5 kDa class I HSP (fragment)				0.0010	17.606
Q06H21	ubiquitin/ribosomal protein S27a	translation	structural constituent of ribosome		0.0009	10.968
Q6VT83	Ara h 8 allergen	plant defense	pathogenesis-related protein	Ara h 8	0.0009	17.197
B1PYZ4	Ara h 8 allergen isoform 3	plant defense	pathogenesis-related protein	Ara h 8	0.0009	17.197
B2ZGS2	pathogenesis-related class 10 protein	plant defense	pathogenesis-related protein	Ara h 8	0.0009	17.197
D3K177	profilin	actin cytoskeleton organization	actin binding	Ara h 5	0.0008	9.924
E5FHZ2	**late embryogenesis abundant protein group 5 protein**				**0.0008**	**19.245**
E5FHZ5	**late embryogenesis abundant protein group 7 protein**	**response to desiccation**			**0.0006**	**22.581**
D8KXZ8	enoyl-ACP reductase 1-		nucleotide binding		0.0006	10.026
B4UW81	glutathione *S*-transferase 2	aromatic amino acid family metabolic process	transferase activity		0.0006	7.263
H6U596	alcohol dehydrogenase (fragment)	oxidation reduction	nucleotide binding/zinc ion binding		0.0006	5.946
B4UWE2	perchloric acid soluble translation inhibitor protein				0.0005	10.053
A6XN43	actin (fragment)				0.0005	8.594
B4UWB2	Kunitz trypsin inhibitor 4	negative regulation of endopeptidase activity	endopeptidase inhibitor activity		0.0005	3.941
B4UW74	fructokinase (fragment)	phosphorylation	kinase activity		0.0004	7.853
G9HPX7	ADP-ribosylation factor	small GTPase mediated signal transduction	GTP binding		0.0002	4.42
E6Y9A5	*β*-hydroxyacyl-ACP dehydratase	fatty acid biosynthesis	hydro-lyase activity		0.0002	3.704
F8UX79	glyceraldehyde-3-phosphate dehydrogenase	glucose metabolism	oxidoreductase		0.0001	1.975

[a]Highlighted (in bold) proteins indicate those that were not identified in the skins. [b]Sequence IDs are according to the *Arachis hypogaea* Uniprot database. [c]Biological process and molecular function were inferred from the Uniprot database.

skin it was Ara h 6. This suggests that even though the seed storage proteins are expressed in both the seed and the skin, the extent to which they are expressed varies between the two plant structures. Ara h 1, Ara h 2, and Ara h 3 are traditionally considered to be major allergens because they are recognized by the IgE of >50% of peanut-allergic patients.[39−41] Ara h 1 belongs to the 7S group of seed storage proteins known as vicilins.[39] Ara h 2 is a 17−19 kDa protein with two isoforms that belongs to the conglutin family of seed storage proteins.[42,40] Ara h 3 belongs to the 11S group of seed storage proteins known as glycinins.[43]

Other allergens, Ara h 5, 8, and 9 were identified in both the blanched seed and the skin (Tables 3 and 4). Ara h 5, a profilin, is a minor peanut allergen, but it is the major allergen in pollen and is considered to be a pan-allergen because it is important in cross-reactions between pollen and peanuts.[44] Ara h 8 is a pathogenesis-related protein involved in plant defense and is also considered to be a pan-allergen because of its sequence

Table 4. Proteins Identified in Peanut Skins[a]

sequence ID[b]	protein name	biological process[c]	molecular function[c]	isoallergen	NSAF value	sequence coverage
Q647G9	conglutin		seed storage	Ara h 6	0.0520	80
P43237	allergen Ara h 1, clone P17		seed storage	Ara h 1	0.0514	69.381
P43238	allergen Ara h 1, clone P41B		seed storage	Ara h 1	0.0505	66.454
A1DZF0	arachin 6		seed storage	Ara h 3	0.0488	73.913
Q647H4	arachin Ahy-1		seed storage	Ara h 3	0.0442	69.963
Q9FZ11	Gly1		seed storage	Ara h 3	0.0430	60.681
B5TYU1	arachin Arah3 isoform		seed storage	Ara h 3	0.0426	73.396
Q647H3	arachin Ahy-2		seed storage	Ara h 3	0.0426	64.804
Q5I6T2	arachin Ahy-4		seed storage	Ara h 3	0.0425	66.29
Q6T2T4	storage protein		seed storage	Ara h 3	0.0419	66.604
Q8LKN1	allergen Arah3/Arah4		seed storage	Ara h 3	0.0396	68.401
E5G076	Ara h 1 allergen		seed storage	Ara h 1	0.0339	49.919
O82580	glycinin (fragment)		seed storage	Ara h 3	0.0271	48.718
Q6PSU2	conglutin-7		seed storage	Ara h 2	0.0226	60.465
Q0GM57	iso-Ara h3		seed storage	Ara h 3	0.0189	69.531
A1DZF1	arachin 7 (fragment)		seed storage	Ara h 3	0.0166	28.019
E5FHY1	late embryogenesis abundant protein group 1 protein				0.0163	67.021
B6CG41	nonspecific lipid-transfer protein (fragment)	lipid transport	lipid binding	Ara h 9	0.0148	86.957
E5FHY0	late embryogenesis abundant protein group 1 protein				0.0147	73.958
E5FHY2	late embryogenesis abundant protein group 1 protein				0.0144	72.449
B0YIU5	Ara h 8 allergen isoform	plant defense	pathogenesis-related protein	Ara h 8	0.0116	89.542
E5FHY4	late embryogenesis abundant protein group 3 protein				0.0116	77.273
Q647G8	2S protein 2		seed storage	Ara h 7	0.0107	54.43
P00760	cationic trypsin	digestion	protease		0.0106	45.935
Q4U4M1	LEA protein (fragment)				0.0105	65.263
Q647G5	oleosin 1	lipid storage			0.0095	41.42
B6CEX8	nonspecific lipid-transfer protein	lipid transport	lipid binding	Ara h 9	0.0093	46.552
B4XID4	Ara h 7 allergen		seed storage	Ara h 7	0.0091	53.049
Q06H32	thioredoxin fold	oxidation reduction	oxidoreductase		0.0084	93.827
B2ZGS	pathogenesis-related class 10 protein	plant defense	pathogenesis-related protein	Ara h 8	0.0082	59.873
E5FHZ0	late embryogenesis abundant protein group 4 protein				0.0080	33.133
Q06013	**endochitinase 1B (fragment)**	**plant defense/chitin degradation**	**glycosidase**		**0.0072**	**63.043**
B4UW70	fiber annexin (fragment)		calcium ion binding		0.0072	55.901
E5FHY9	late embryogenesis abundant protein group 4 protein				0.0070	30.233
Q6VT83	Ara h 8 allergen	plant defense	pathogenesis-related protein	Ara h 8	0.0066	40.764
B1PYZ4	Ara h 8 allergen isoform 3	plant defense	pathogenesis-related protein	Ara h 8	0.0066	43.949
B4UW78	**glutamine synthetase GS56 (fragment)**	**glutamine biosynthetic process**	**glutamate-ammonia ligase activity**		**0.0061**	**70.701**
Q6J1J8	oleosin	lipid storage			0.0059	26.705
Q9AXI0	oleosin variant B	lipid storage			0.0059	26.705
Q45W80	nucleoside diphosphate kinase		ATP binding		0.0058	42.282
E5FHY6	late embryogenesis abundant protein group 3 protein (fragment)				0.0058	54.601
A7LB60	steroleosin A	oxidation reduction	nucleotide binding/ oxidoreductase		0.0058	39.828
Q42515	**chitinase (class II)**	**cell wall macromolecule catabolic process**	**chitnase**		**0.0058**	**60.606**
E5FHY7	late embryogenesis abundant protein group 3 protein				0.0052	26.5
A1Z1T1	**cytosolic ascorbate peroxidase**	**response to oxidative stress**	**heme binding/ peroxidase activity**		**0.0051**	**58.4**
G9HPX8	peptidyl-prolyl *cis*−*trans* isomerase	protein folding	peptidyl-prolyl *cis*−*trans* isomerase activity		0.0051	56.977
E9LFE8	11S arachin (fragment)		seed storage	Ara h 3	0.0045	42.692
Q06H19	UDP-glucose pyrophosphorylase (fragment)		nucleotide transfer		0.0044	53.333
P02872	galactose-binding lectin		carbohydrate binding		0.0044	37.363
B4UWD5	**proteasome subunit alpha type (fragment)**	**ubiquitin-dependent protein catabolic process**	**threonine-type endopeptidase activity**		**0.0042**	**41.606**
Q4JME7	lipoxygenase	fatty acid biosynthesis	iron ion binding/ lipoxygenase		0.0042	46.002

Table 4. continued

sequence ID[b]	protein name	biological process[c]	molecular function[c]	isoallergen	NSAF value	sequence coverage
P01066	Bowman−Birk type proteinase inhibitor A-II		serine-type endopeptidase inhibitor		0.0041	78.571
Q6PWX0	calmodulin		calcium ion binding		0.0039	43.919
F6KLJ6	annexin		calcium ion binding		0.0038	37.143
Q647H2	arachin Ahy-3		seed storage	Ara h 3	0.0034	36.364
Q1HDS7	superoxide dismutase [Cu−Zn]	superoxide metabolic process	metal ion binding		0.0033	36.842
Q06H21	ubiquitin/ribosomal protein S27a	translation	structural constituent of ribosome		0.0032	21.935
E5FHY8	late embryogenesis abundant protein group 3 protein				0.0031	40.909
Q0Q0Q9	type 4 metallothionein		zinc ion binding		0.0030	54.878
B5TKB7	**actin (fragment)**				**0.0030**	**42.029**
E5FHY3	late embryogenesis abundant protein group 2 protein dehydrin				0.0026	28.986
B4UWC0	lipoxygenase 2 (fragment)	oxidation reduction	metal ion binding/ oxidoreductase		0.0025	22.346
G9HPX7	ADP-ribosylation factor	small GTPase mediated signal transduction	GTP binding		0.0025	23.757
E5FHZ1	late embryogenesis abundant protein group 5 protein OS = *Arachis hypogaea* GN = LEA5-1 PE = 2 SV = 1				0.0024	63.182
A1E2B0	11S seed storage globulin B1		seed storage	Ara h 3	0.0024	17.705
Q647H1	conarachin		seed storage	Ara h 1	0.0024	23.716
Q2PK12	**actin depolymerizing factor-like protein**		**actin binding**		**0.0021**	**16.547**
B4UWD3	**putative mitochondrial ATP synthase (fragment)**				**0.0019**	**18.75**
B4UWA1	putative uncharacterized protein (fragment)		lactoylglutathione lyase activity		0.0019	22.449
A7LIS6	germin-like protein subfamily 2 member 1		manganese ion binding		0.0019	18.721
B4UWD9	threonine endopeptidase (fragment)	proteolysis involved in cellular protein catabolic process	threonine-type endopeptidase activity		0.0018	29.814
H6U596	alcohol dehydrogenase (fragment)	oxidation reduction	nucleotide binding/zinc ion binding		0.0018	19.459
O20356	ribulose 1,5-bisphosphate carboxylase-oxygenase large subunit (fragment)	photosynthesis	ribulose-bisphosphate carboxylase		0.0018	24.946
Q2PXN2	17.5 kDa class I HSP (fragment)				0.0018	23.239
B4UW7	fructokinase (fragment)	phosphorylation	kinase activity		0.0017	20.419
Q2HWT8	**phospholipase D**	**lipid catabolic process**	**NAPE-specific phospholipase D activity**		**0.0016**	**21.561**
B4UW88	heat shock protein 1 (fragment)	stress response			0.0015	18.657
E5FHZ7	late embryogenesis abundant protein group 9 protein				0.0014	9.392
Q1PCR5	**proteasome subunit beta type (fragment)**	**proteolysis involved in cellular protein catabolic process**	**threonine-type endopeptidase activity**		**0.0013**	**24.215**
E9LFE9	11S arachin		seed storage	Ara h 3	0.0013	7.422
B4UW51	**class II small heat shock protein Le-HSP17.6 (fragment)**	**response to stress**			**0.0013**	**20.155**
E0WN93	**cystein proteinase inhibitor**	**negative regulation of peptidase activity**	**thiol protease inhibitor**		**0.0013**	**16.327**
D3K177	profilin	actin cytoskeleton organization	actin binding	Ara h 5	0.0013	9.924
Q647G3	oleosin	lipid storage			0.0012	27.108
Q45W87	oleosin 1	lipid storage			0.0012	11.679
E5FHY5	late embryogenesis abundant protein group 3 protein				0.0012	15.493
B4UW77	**gibberellin-regulated protein**				**0.0012**	**11.215**
B4UW73	**universal stress protein**	**response to stress**			**0.0011**	**8.287**
A7LB59	steroleosin B	oxidation reduction	nucleotide binding/ oxidoreductase		0.0011	14.448
Q06H37	**syringolide-induced protein 19-1-5 (fragment)**	**carbohydrate metabolic process**	**hydrolase activity**		**0.0010**	**19.34**
E6Y9A5	*β*-hydroxyacyl-ACP dehydratase	fatty acid biosynthesis	hydro-lyase activity		0.0010	15.741
B4UW79	**glutathione peroxidase (fragment)**	**response to oxidative stress**	**glutathione peroxidase activity**		**0.0010**	**15.278**
C0L2 V3	**putative phosphoglycerate dehydrogenase (fragment)**	**L-serine biosynthetic process**	**amino acid binding**		**0.0009**	**16.592**
B4UWA3	**putative uncharacterized protein**				**0.0009**	**16.022**
Q45W86	oleosin 2	lipid storage		Ara h 11	0.0009	11.679
D8KXY1	**acyl carrier protein**	**fatty acid biosynthesis**	**phosphopante-theine binding**		**0.0009**	**10.714**
B4UWE2	perchloric acid soluble translation inhibitor protein				0.0009	17.989
B4UW54	**GroES-like protein (fragment)**	**protein folding**	**ATP binding**		**0.0008**	**17.327**
F8UX79	glyceraldehyde-3-phosphate dehydrogenase	glucose metabolism	oxidoreductase		0.0008	1.975

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Table 4. continued

sequence ID[b]	protein name	biological process[c]	molecular function[c]	isoallergen	NSAF value	sequence coverage
B4UWB2	Kunitz trypsin inhibitor 4	negative regulation of endopeptidase activity	endopeptidase inhibitor activity		0.0008	9.852
Q45W77	**ubiquitin-conjugating enzyme 1**	**UbI conjugation pathway**	**ATP binding**		**0.0008**	**12.418**
G4WJT1	**sucrose synthase (fragment)**	**sucrose metabolic process**	**sucrose synthase activity**		**0.0008**	**4.49**
C9W977	**phosphoenolpyruvate carboxylase**	**carbon fixation**	**phosphoenolpyruvate carboxylase activity**		**0.0007**	**11.594**
B4UW81	glutathione *S*-transferase 2	aromatic amino acid family metabolic process	transferase activity		0.0007	7.263
B7UCQ3	**cysteine protease-like protein**	**proteolysis**	**cysteine-type peptidase activity**		**0.0007**	**6.319**
A6XN43	actin (fragment)				0.0006	8.594
C9W980	**phosphoenolpyruvate carboxylase**	**carbon fixation**	**phosphoenolpyruvate carboxylase activity**		**0.0006**	**8.256**
D8KXZ8	enoyl-ACP reductase 1-		nucleotide binding		0.0006	7.969
B4UWA9	**putative inorganic pyrophosphatase (fragment)**	**phosphate-contatining compound metabolic process**	**inorganic diphosphatase activity**		**0.0006**	**12.782**
C9W979	**phosphoenolpyruvate carboxylase**	**carbon fixation**	**phosphoenolpyruvate carboxylase activity**		**0.0006**	**10.766**
Q1PCR4	**putative IN2-1 protein (fragment)**				**0.0006**	**22.302**
Q84TU2	**subtilisin-like seed-specific protein (fragment)**	**proteolysis**	**serine-type endopeptidase**		**0.0005**	**9.836**
Q0Q0Q8	type 4 metallothionein		zinc ion binding		0.0005	17.073
A1E2B1	11S seed storage globulin B2		seed storage		0.0004	8.664
Q5XXY3	**PR protein 4A (fragment)**	**defense response**			**0.0004**	**21.277**
D8KXY8	***β*-ketoacyl-ACP synthase I-1**	**fatty acid biosynthesis**	**transferase activity**		**0.0004**	**16.596**
B0LXE5	**phosphoenolpyruvate carboxylase**	**carbon fixation**	**phosphoenolp-yruvate carboxylase activity**		**0.0004**	**5.062**
E6Y9A4	**biotin carboxylase**		**ATP binding**		**0.0003**	**7.407**
Q06H26	**tumor-related protein-like (fragment)**				**0.0002**	**7.306**
Q43375	**galactose-binding lectin (fragment)**		**carbohydrate binding**		**0.0002**	**4.839**

[a]Highlighted (in bold) proteins indicate those that were not identified in the seed. [b]Sequence IDs are according to the *Arachis hypogaea* Uniprot database. [c]Biological process and molecular function were inferred from the Uniprot database.

identity and cross-reactivity with Bet v 1, a known birch pollen allergen.[45] Ara h 9, a nonspecific lipid transfer protein, was also identified in both the blanched seed and the skin. Ara h 9 has been identified as a major peanut allergen in the Mediterranean population and to a lesser extent in non-Mediterranean populations.[46] Several oleosins were identified in both the blanched seed and the skin. Oleosins are proteins associated with the membranes of oil storage bodies in vascular plants and act as emulsifiers. Two oleosins, Ara h 10 (16 kDa) and Ara h 11 (14 kDa), have been recognized as potential peanut allergens and may be involved in allergic cross-reactions between peanuts and soybeans.[47] Ara h 11 was identified in both the peanut seed and peanut skin, whereas Ara h 10 was identified only in the seed. Recently, the IUIS has recognized as allergens Ara h 12 and 13, both of which are defensins that function to protect against fungi and bacteria. These proteins were not included in the analysis because they are not in the Uniprot database.

Many of the proteins that were identified in the skin but not in the blanched seed are related to defense and stress response. For example, chitinases are an important class of defense proteins that hydrolyze chitin, a major structural polysaccharide present in fungi and the exoskeletons of insects.[48] Two chitinases (endochitinase 1B and class II chitinase) were identified in the peanut skin but not in the blanched seed. Because chitinases are important in defense against fungal infections, this suggests that the peanut skin is key in providing protection from fungal contamination. Although not identified in the present study, chitinase has previously been isolated from peanut seed.[49] The expression of chitinases in plants depends upon the presence of environmental stresses such as microbial invasion and, therefore, varies from plant to plant. Identification of ways to up-regulate expression of chitinases in the peanut may lead to plants with increased resistance to *Aspergillus*, the fungal species responsible for aflatoxin contamination, and other microorganisms, which would have a positive economic impact on the peanut industry. Two lipoxygenase enzymes were also identified in both the blanched seed and skin. Lipoxygenase enzymes are important to the plant's response to fungal infections as they break down fatty acids into hydroperoxides, which have been shown to inhibit *Aspergillus* spore germination.[50] Multiple lipoxygenase isozymes have been purified from peanuts.[51] *Aspergillus* infection has been shown to induce expression of lipoxygenase enzymes in peanut seed.[52]

Seed coats of legumes, including peanuts, consist of specialized cells that provide protection to the seed, function in both dormancy and germination, and enhance seed dispersal.[53] A map of the peanut skin proteome is key to understanding how the skin interacts with the enclosed seed and how the plant responds to environmental stresses. Proteomic analysis of peanut skin, as demonstrated in this study, could be used to determine the differential expression of various proteins during development or in response to certain stresses such as fungal infection. Comparative proteomic studies of peanut skins could help plant breeders identify proteins in the skins that are important to seed development and plant defense. Proteomic analysis of *M. truncatula* during seed development revealed preferential expression of certain proteins in the seed coat that are necessary for seed growth including proteolysis enzymes that provide amino acids to the

embryo for protein synthesis.[22] A similar study in peanut is warranted to determine specific functionality of the peanut skin during seed development. Additionally, an understanding of proteome modifications in response to fungal contamination could lead to identification of defense proteins, which may help breeders to develop new resistant species.

In conclusion, peanut skins contain many of the same proteins as blanched peanut seed, including all of the major seed storage proteins and other allergens. The phenolic compounds in the skins hinder protein digestion by trypsin when extracted using conventional methods; therefore, phenol extraction of proteins was necessary to remove interfering compounds. Proteins extracted from peanut skins did not bind peanut-specific IgE when phenolic compounds were present, whereas they did bind IgE in the absence of phenolic compounds. This suggests that phenolic compounds may bind to the proteins and prevent them from binding IgE or, conversely, bind to IgE, thus hindering IgE binding to the proteins. Further investigation into this mechanism is warranted. To our knowledge, this is the first report of the peanut skin proteome. This research contributes to research regarding utilization of peanut skins by highlighting the presence of allergenic proteins in the skins. Additionally, the methods used to analyze the peanut skin proteome could be applied in other studies to determine how the skin is involved in seed growth and defense.

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Notes

The authors declare no competing financial interest.

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