JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Transglutaminase Polymerization of Peanut Proteins

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Transglutaminase promotes protein cross-linking reactions through an acyl transferase mechanism involving protein-bound glutaminyl residues and primary amines including the ϵ-amino group of lysine residues in soy, myosin, gluten, oat globulin, casein, and whey. Herein, we present a first report of exogenous transglutaminase catalysis of several peanut protein fractions, including purified Ara h 1. In most cases, SDS-PAGE banding patterns revealed the formation of high molecular weight polymers while catalysis of Ara h 1 resulted in distinct dimer formation. Cross-linking effects were accomplished in the presence and absence of the reducing reagent, dithiothreitol. Ortho-phthaldialdehyde assays, used to quantify the degree of polymerization, indicated ~21% and ~30% coupling over a similar time interval, using either cold hexane extracted peanut protein fractions or lightly roasted flour dispersions, respectively. Rheological measurements established that transglutaminase-modified peanut extracts exhibited lowered viscosity readings compared to nontreated dispersions. Peanut protein polymers and glycoprotein conjugates, created by covalent linkage between protein substrates and monosaccharide amino sugars, exhibited similar IgE binding activity, compared to control solutions. These results suggested that potential allergic responses were not enhanced after enzymatic modification. Ultimately, these approaches may provide novel peanut-based food ingredients with unique functional characteristics for expanded applications within the world marketplace.

KEYWORDS: Transglutaminase; peanuts (*Arachis hypogaea Linn*); protein functionality; food allergies

INTRODUCTION

The production of peanuts on a worldwide scale is estimated to be approximately 20.4 million metric tons. In the United States alone, 1.8−2.0 million metric tons are currently utilized for food applications (*1*). Presently, this commodity is added to numerous edible foods including peanut-buttered snacks, ice cream, candies, beverages, and confections (*2*). However, on a global basis, peanuts are primarily grown to extract oil for cooking purposes, and during this process, residual protein-rich "pucks" are generated that may be used to manufacture partially defatted peanut meals for nutritive usages. Oftentimes, these materials can be prepared for human consumption in the form of commercial flours, peanut protein concentrates and isolates, flakes, or grits if suitable handling and food-processing conditions are maintained (*3*).

Peanut-based food ingredients can be modified using both chemical means and physical manipulations including acidification, heating, proteolysis, and enzymatic cross-linking, all in the presence and absence of different salts. Previous work had already demonstrated that transglutaminase [protein-glutamine: amine γ-glutamyl-transferase, EC 2.3.2.13] promoted cross-linking reactions of soy, myosin, gluten, oat globulin, casein, and whey proteins (*4*−*8*); therefore, to test the potential reactivity of the enzyme with peanut protein substrates, several experimental samples were prepared under a variety of conditions. These fractions included raw peanuts, cold and hot hexane extracted fractions, preheated protein solutions (60 °C), dried peanut flours, and purified Ara h 1. To our knowledge, this report represents a first documentation of peanut protein catalysis by an exogenous transglutaminase.

In this work, we have characterized protein−protein interactions that occurred on the basis of the nature of initial starting materials. In addition, experiments were designed to measure the effects of polymerization on peanut protein solubility, to illustrate the SDS-PAGE protein-banding patterns for all conjugates, and to quantify the degree of cross-linking. Rheological changes were assessed upon monitoring the changes in apparent viscosity after treatment of peanut protein dispersions with the enzyme. IgE binding parameters, a measure of immunogenic activity, were calculated for polymeric protein and glycoprotein conjugates, generated by covalent linkages between peanut protein substrates with monosaccharide amino sugars.

Allergenicity remains a major hurdle with respect to ensuring the safe ingestion of peanut-based food products for some consumers. With these studies, we have demonstrated that transglutaminase modification of dried flour materials did not lower IgE binding to peanut protein epitopes; however, it may be possible to create alternative enzyme-modified protein

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10.1021/jf062309t CCC: $37.00
Published on Web 12/29/2006

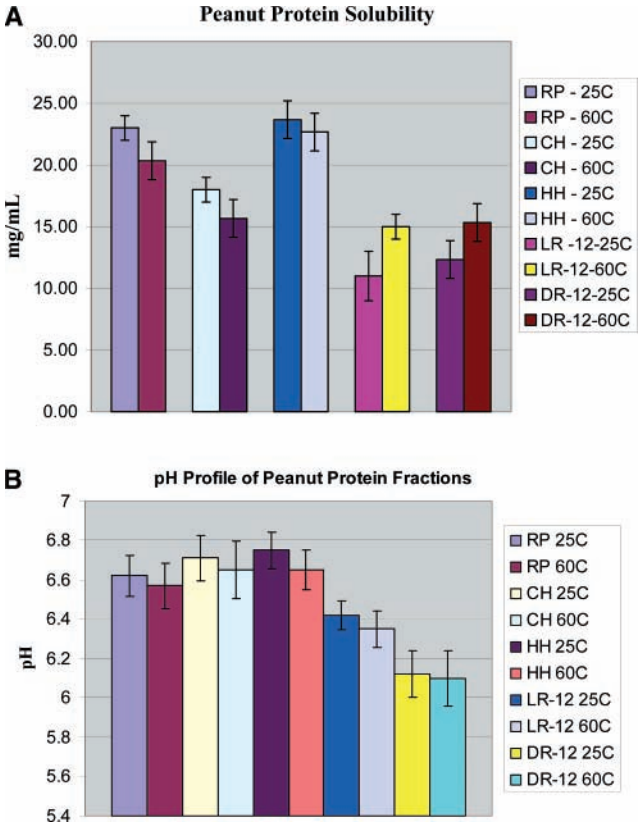

Figure 1. (**A**) Solubility study. Raw (RP), cold (CH) and hot (HH) hexane extracted peanut protein fractions, and two peanut flours, light roast, 12% fat (LR-12), and dark roast, 12% fat (DR-12), were prepared at 10% solids (w/v) in deionized water at 25 °C and 60 °C as described in Materials and Methods. (**B**) pH study. The pH of the solution was measured directly.

fractions that elicit diminished immunogenic responses using different starting materials or variant experimental reaction conditions. Since we established that transglutaminase reacted with peanut proteins under a wide range of solution parameters, ongoing research will be directed toward optimization of enzyme-treated protein fractions, exhibiting superior performance traits, for utilization as a new food ingredient.

With the relative abundance of underutilized peanut protein resources, there is a critical need for the development of innovative strategies that promote new usages within the food industry. Ultimately, we anticipate that these approaches will afford increased utility for peanut proteins by expanding its functional effectiveness within a given food product as previously demonstrated in other protein-based nutritional systems (*3*, *9*−*12*).

MATERIALS AND METHODS

Materials. *Peanut Protein Resources.* Raw peanuts (both runner and Virginia types) were obtained for these studies. Light and dark roasted, partially defatted (12% fat content), peanut flours were provided as a gift from Golden Peanut Company (Alpharetta, GA). Amidated pectin, GENU Type LM-20AS, was contributed by CP Kelco (Wilmington, DE). *N*-Carbobenzoxy (CBZ)-glutaminyl-glycine, catalog #6154, was obtained from Sigma-Aldrich (St. Louis, MO) as were all other reagent-grade chemicals.

Transglutaminase. Purified microbial transglutaminase was donated by Ajinomoto Food Ingredients LLC (Japan) and was stored at −20 °C prior to use. The enzyme was supplied as a solid powder, mixed with maltodextrin. The specific activity was measured as 800 units per gram/powder using *N*-carbobenzoxy (CBZ)-glutaminyl-glycine and hydroxylamine upon assay according to the method of Folk and Chung

Table 1. Summary of Peanut Protein Fractions Polymerized by Transglutaminase

peanut protein dispersions[a]	heat treatment	reducing solution conditions, 10 mM DTT	pH
I. raw peanuts:	25 °C	(+) DTT	6.62
Runner and Virginia lines	60 °C		
II. cold hexane extracted:	25 °C	(±) DTT	6.71
Runner and Virginia, ∼4% fat	60 °C		
III. hot hexane extracted:	25 °C	(±) DTT	6.74
Runner and Virginia, ∼4% fat	60 °C		
IV. peanut flours:			
light roast, 12% fat	25 °C	(±) DTT	6.41 and 8.0
	60 °C		
dark roast, 12% fat	25 °C	(±) DTT	6.12 and 8.0
	60 °C		
V. purified Ara h 1	25 °C	(±) DTT	7.0

[a] Peanut protein dispersions (10% solids, w/v) were prepared at 25 °C and 60 °C as detailed in Materials and Methods. Most samples were supplemented with 10 mM dithiothreitol (+). Reaction assay mixtures lacking 10 mM DTT are denoted by a minus sign (–). Ara h 1 was purified as described (*16*).

(*4*) with slight modification by Truong et al. (*13*). More specifically, the final reagent concentrations in the assay mixture were as follows: 100 mM hydroxylamine, 15 mM CBZ-glutaminyl-glycine, 5 mM calcium chloride, 10 mM dithiothreitol in 200 mM Tris acetate buffer, pH 6.0. Under these experimental conditions, one unit of enzyme activity was defined as an absorbance change of 0.29/min at 525 nm at pH 6.0, forming 1 μmol of hydroxamate/min, at 37 °C. The purity was >90% according to electrophoretic data (*14*).

Methods. *Hydration of Peanut Flour*. Peanut flour, containing 12% fat content, was dispersed in deionized water to either 10% or 20% (w/v). In some cases, the pH was adjusted to 8.0 with 2 N NaOH and stirred continuously for 2 h, at which time the pH was readjusted to 8.0 if required. All peanut protein suspensions were then stored overnight at 4 °C before analysis.

Cold Hexane Extraction (Defatting Method). Runner and Virginia type peanuts were ground using a standard homogenizer (Braun, Boston, MA), were placed into a large centrifuge tube at one-third filled capacity, and hexane (Fisher Scientific) was added to the top of the tube. The contents were then shaken for 30 min and centrifuged at 2600*g* for 15 min to separate soluble fractions from insoluble particulate matter. This process was repeated three times (3×). The defatted peanut meal was then air-dried under a chemical fume hood for 16 h (*15*).

Hot Hexane Extraction (Defatting Method). Runner and Virginia type peanuts were ground using a standard homogenizer (Braun, Boston, MA), and the homogenate was placed into a cellulose thimble, 25 mm × 80 mm (Whatman, Maidstone, England), filled to approximately three-fourths capacity (45.0 g). Soxhlet hexane extraction of lipid materials was then conducted at ∼50 °C for 6 h. Peanut meals, with reduced fat content, were air-dried under a chemical fume hood for 16 h (*15*).

Purification of Ara h 1. Five grams of raw, defatted, peanut material was added to ∼40 mL of 0.1 M phosphate buffer, pH 8.0, and the samples were incubated at 37 °C/3.5 h while shaking. Subsequently, Ara h 1 was purified according to the method of Basha and Cherry (*16*).

Solubility and pH Studies. Raw peanuts, cold and hot hexane extracted, and two peanut flours [light roast, 12% fat (LR-12), and dark roast, 12% fat (DR-12)] were dissolved in deionized water at 10% solids (w/v) at 25 °C and 60 °C. Heated samples were maintained at 60 °C for ∼3 h and were held overnight at 4 °C prior to use. Afterward, all samples were centrifuged at 11950*g* for 30 min at 4 °C, and the protein concentration was measured according to bicinchoninic acid (BCA) methodologies developed by Pierce Inc. (Rockford, IL). The pH of all test solutions was measured directly using an Orion pH meter, model 405 (Fisher Scientific). Solubility and pH measurements were made in triplicte.

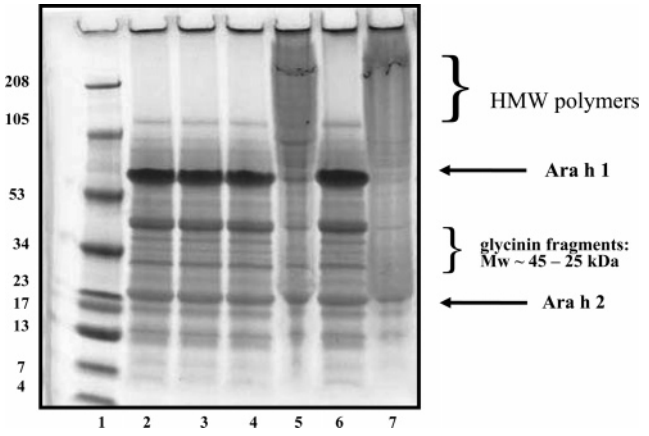

Figure 2. SDS-PAGE banding pattern of transglutaminase-treated cold hexane extracted peanut protein (CH–PP) fractions. CH–PP samples were incubated with transglutaminase (TG) at 37 °C for the times indicated. Lane 1: Marker (kDa); lane 2: CH–PP/0 h; lane 3: CH–PP + TG/0 h; lane 4: CH–PP/1 h; lane 5: CH–PP + TG/1 h; lane 6: CH–PP/7 h; lane 7: CH–PP + TG/7 h. HMW: high molecular weight.

Ortho-Phthaldialdehyde (OPA) Analysis. Experimental samples were appropriately diluted with deionized water and microcentrifuged for 5 min. Twenty microliters of the resultant supernatant was then added to 2.0 mL of the OPA reagent, and the sample was incubated for 2 min at 25 °C. Then the absorbance was read at A_{340} nm using a Gilford 2600 spectrophotometer. All final readings fell within the range of [0.1−1.0]. This procedure was adapted from the method of Church et al. (*17*). Test samples were analyzed in triplicate.

Enzymatic Polymerization of Peanut Protein Substrates. Peanut protein dispersions were typically prepared at 10% or 20% solids content (w/v), pH 8.0, as previously described. Microbial transglutaminase, added at a ratio of ~5 units/gram of dried peanut flour, was slowly mixed with the protein substrate in a rotating incubator at 37 °C. Aliquots were removed at timed intervals for SDS-PAGE and OPA analyses. In most cases, soluble supernatants, removed after centrifugation at 11950*g*, were tested. Also, BCA methods were used to measure residual protein content in transglutaminase-treated samples maintained at 37 °C for 24 h. Control solutions, devoid of enzyme, were evaluated as well.

SDS-PAGE Electrophoresis. All samples were initially assayed for protein content (BCA method) to ensure equivalent loading amounts into each lane. The fractions were appropriately diluted and mixed (1:1, v/v) with sample buffer [8% SDS, 0.9 M Tris buffer containing 5.0% β-mercaptoethanol (InVitrogen Inc., Carlsbad, CA)]. Each sample was then heated at 100 °C for 10 min, a process which also stops the enzymatic reaction, prior to loading onto 10−20% Tris-Tricine gradient polyacrylamide gels (InVitrogen, Inc). After electrophoresis, the samples were stained directly for visualization of proteins using a colloidal Coomassie Blue staining reagent (InVitrogen, Inc.).

Apparent Viscosity. A 20% (w/v) dispersion of LR-12 was prepared in deionized water at pH 8.0. Dispersions containing microbial transglutaminase, and nontreated control samples, were then incubated at 37 °C with agitation for 6 h prior to rheological analysis using a stress-controlled rheometer (StressTech, Reologica Instruments AB, Lund, Sweden). The apparent viscosity was monitored as the test fraction was heated from 40° to 80 °C at 1 °C/min at a constant shear rate of 50 1/s.

ELISA Methodology. LR-12 dried flours were prepared at 20% solids content (w/v) in deionized water, the pH was adjusted to 8.0, and the sample was centrifuged at 11950*g* for 30 min prior to testing. Supernatant fractions included (1) transglutaminase-treated LR-12 peanut protein samples, (2) glycoprotein conjugates formed by enzymatic catalysis between 20% LR-12 dispersions, pH 8, mixed with either 5% glucosamine or galactosamine (w/v) at 37 °C for 24 h, and (3) 20% LR-12 suspensions containing 1%-amidated pectin (w/v) that were heated for 3 h at 60 °C.

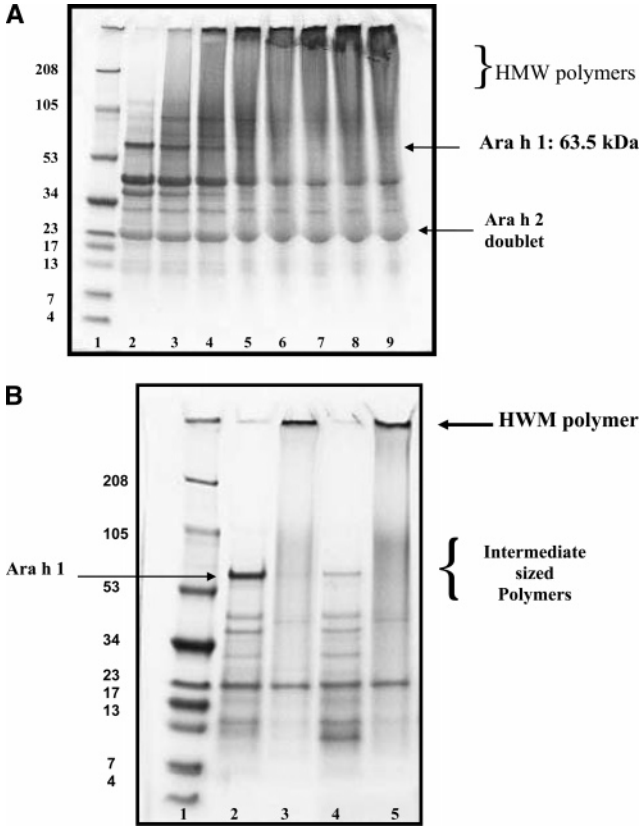

Figure 3. (**A**) SDS-PAGE banding pattern of transglutaminase-treated LR-12 peanut flour dispersions. LR-12 dispersions, prepared in deionized water containing 10 mM dithiothreitol, pH 8.0, were incubated with transglutaminase at 37 °C for the times indicated. Lane 1: Marker (kDa); lane 2: 0 h; lane 3: 0.5 h; lane 4: 1 h; lane 5: 2 h; lane 6: 4 h; lane 7: 6 h; lane 8: 9 h; lane 9: 24 h. (**B**) Effect of preheating on LR-12 peanut protein polymer formation. LR-12 flour dispersions were prepared in deionized water, pH 8.0, at 25 °C and 60 °C (maintained for 3 h) as described in Materials and Methods. Test fractions were treated with transglutaminase (TG) for 3 h at 37 °C. Lane 1: Marker (kDa); lane 2: LR-12, 25 °C; lane 3: LR-12, 25 °C (+) TG; lane 4: LR-12, 60 °C; lane 5: LR-12, 60 °C (+) TG.

Table 2. Degree of Protein Polymerization

peanut protein sample[a]	time point	absorbance 340 nm	standard deviation
CH 60 °C	0 h	0.443	±0.05
(+) TG	0.5 h	0.398	±0.02
(+) TG	1 h	0.374	±0.01
(+) TG	3 h	0.35	±0.01
(+) TG	6 h	0.343	±0.02
LR-12, 25 °C	0 h	0.84	±0.04
(+) TG	2 h	0.68	±0.05
(+) TG	4 h	0.58	±0.03

[a] Peanut protein dispersions, CH–PP (10% solids, w/v), and LR-12 (20% solids, w/v), pH 8.0, were prepared at 60 °C and 25 °C as detailed in Materials and Methods. Soluble supernatant fractions were used directly for enzymatic cross-linking at 37 °C. LR-12 dispersions contained 10 mM dithiothreitol. The degree of polymerization was determined by OPA analysis.

All test samples were diluted to an equivalent protein concentration on the basis of BCA protein data. Glucosamine/galactosamine glycoprotein conjugates were adjusted to comparable values on the basis of an A_{280} nm reading of ~0.02 since reducing sugar components interfered with BCA assay protocols. ELISA methodologies were then performed according to the instructions provided in the Veratox Peanut Allergen

Table 3. Loss of Soluble Protein After Treatment of LR-12 and DR-12 with Transglutaminase

peanut protein sample[a]	time point	protein mg/mL	standard deviation
LR-12, 37 °C	0 h	11.97	1.41
(+) TG	24 h	8.63	1.13
DR-12, 37 °C	0 h	11.46	1.40
(+) TG	24 h	5.83	0.85

[a] Peanut protein dispersions, LR-12 (10% solids, w/v) and DR-12 (10% solids, w/v), pH 8.0, were prepared at 25 °C. After treatment of each solution with transglutaminase for 24 h at 37 °C, soluble supernatant fractions were measured for residual protein concentrations using BCA methods (Materials and Methods).

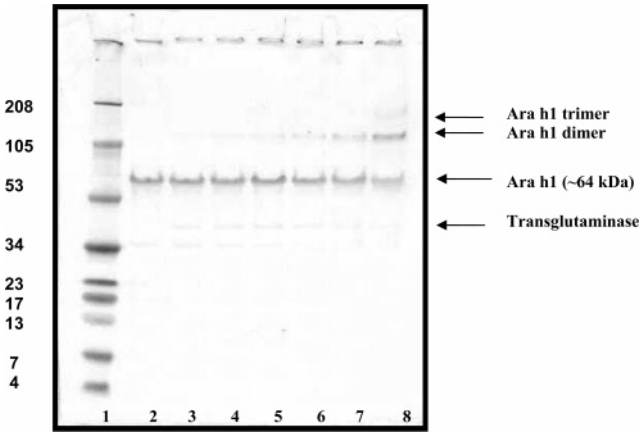

Figure 4. SDS-PAGE banding pattern of transglutaminase-treated Ara h 1. Ara h1 was incubated with transglutaminase (TG), and cross-linking reactions were performed at 37 °C in the presence of 10 mM dithiothreitol for the times indicated. Lane 1: Marker (kDa); lane 2: 0 h; lane 3: 0.5 h; lane 4: 1 h; lane 5: 2 h; lane 6: 3 h; lane 7: 5 h; lane 8: 20 h.

test kit, specifically designed for quantitative analysis of IgE binding activity (Neogen Corporation, Lansing, MI).

RESULTS AND DISCUSSION

Characterization of Peanut Protein Substrate Fractions. To address the potential reactivity of transglutaminase polymerization of various peanut protein fractions, we first assessed general solubility parameters in an aqueous medium using raw peanuts, cold and hot hexane extracted samples, and two peanut flour dispersions prepared at either 25 °C or 60 °C. We hypothesized that heated solutions may slightly denature the protein substrate, providing increased reactive sites for enzyme catalysis. Also, under these experimental conditions, additional glycoconjugates might have formed as a result of Maillard reactivity given that native carbohydrate was present in some of these test samples.

As shown in **Figure 1A**, raw and hexane-extracted peanut preparations contained approximately 1.5−2.0 times more soluble protein than the flours at equivalent total solids content (10%, w/v). Furthermore, native pH values varied slightly, especially between differentially roasted flour samples where longer heating treatments, used in preparing dark roasted flours, caused increased acidity (**Figure 1B**).

Enzymatic Catalysis of Minimally Processed Peanut Protein Substrates. Initially, only soluble peanut protein substrates were tested for transglutaminase catalysis under a variety of experimental conditions (**Table 1**). In fact, polymerization was observed in raw peanut fractions, cold and hot hexane extracted protein solutions, and peanut flour suspensions

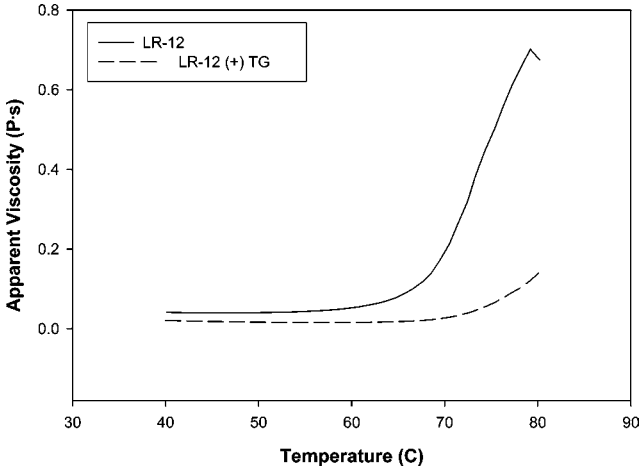

Figure 5. Apparent viscosity of LR-12 peanut flour dispersions after polymerization with transglutaminase. LR-12 protein dispersions, pH 8.0, were incubated with transglutaminase at 37 °C with agitation for 6 h prior to rheological analysis.

prepared over a pH range of 6−8. Furthermore, catalysis was accomplished in the presence and absence of dithiothreitol (DTT), a reducing denaturant. In one example, cold hexane extracted fractions, preheated at 60 °C for 3 h, were extensively cross-linked after a 60-min incubation period even in the absence of DTT (**Figure 2**). Concomitantly, high molecular weight polymers were observed at the top of the gel, near the interface.

Transglutaminase Reactivity with Roasted Peanut Flours. After determining that minimally processed peanut protein samples, such as raw peanuts and cold/hot hexane extracted materials were polymerized by the enzyme, solution parameters were further refined to study the effects of enzymatic catalysis on peanut flours, a readily available commercial source of protein starting material. Peanut flour extracts, per se, were devoid of endogenous transglutaminase activity using CBZ-glutaminyl-glycine and hydroxylamine substrates under the reaction conditions described. In first experiments, dithiothreitol was added to the lightly roasted flour dispersions, an effect previously reported to increase transglutaminase activity by maintaining the redox status of the cysteine residue residing at the active site (*14*).

Under these solution conditions, SDS-PAGE results of soluble LR-12 flour dispersions revealed considerable formation of high molecular weight polymers, demonstrated by amplified smearing at the top of the gel (**Figure 3A**). Furthermore, these reaction end products ranged in their size distribution in contrast to major reaction end products generated by transglutaminase catalysis of dark roasted dispersions (DR-12), containing dithiothreitol, that almost exclusively produced highly cross-linked protein polymers at the gel interface (data not shown). This latter result might be attributed to larger-sized protein substrates already present in DR-12 flour materials that were generated under higher thermal processing parameters (e.g., increased temperatures, longer heating times) as a result of the Maillard reaction.

As evidenced in **Figure 3B**, electrophoresis data illustrated that heating alone diminished the amount of Ara h 1 that was visible in the peanut flour test sample prepared at 60 °C (lane 4) versus the dispersion dissolved at room temperature, 25 °C (lane 2). Furthermore, in the absence of dithiothreitol, the rate of the transglutaminase-catalyzed reaction was slower, resulting in the formation and visualization of intermediately sized polymers using substrates that were thermally denatured (60 °C, 3 h) prior to exposure to the enzyme (lane 5). This

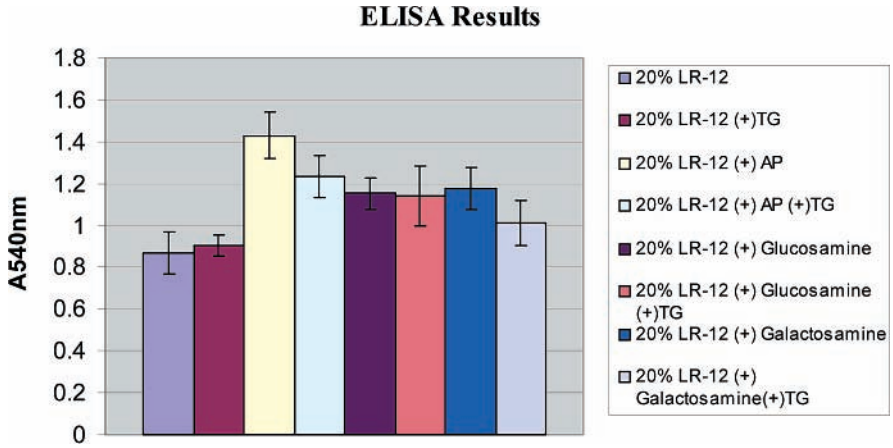

Figure 6. IgE binding activity of transglutaminase-treated peanut protein fractions. Transglutaminase treated peanut protein test suspensions included (1) LR-12, (2) glycoprotein conjugates, and (3) LR-12 suspensions containing 1% amidated pectin (w/v). All protein samples were diluted to an equivalent protein concentration. ELISA protocols were performed according to the instructions provided in the Veratox Peanut Allergen test kit.

phenomenon was also noted in enzyme-modified fractions prepared at 25 °C (lane 3), although to a lesser degree. These findings supported the observations of other investigators who recently reported that transglutaminase reactivity was enhanced upon utilization of preheated protein substrates (*18*). Prolonged incubation of the enzyme with all flour dispersions (e.g., 24 h at 37 °C) resulted in significant precipitation, a finding which suggested decreased solubility of extensively cross-linked protein materials. This hypothesis was confirmed by quantitative protein analyses of supernatant fractions obtained after removal of an insoluble pellet (**Table 3**). Taken together, these data demonstrated that the specific form of the polymeric end product differed considerably on the basis of the variability between preprocessed protein-starting materials.

Interestingly, a couple of these banding areas were not extensively cross-linked under these reaction conditions. For example, the subunits ranging from ~28 to 45 kDa, likely representing glycinin fragments (*19*), were not polymerized, at least to the same degree, as Ara h 1 at equivalent time intervals (**Figure 3A**). Furthermore, Ara h 2, another major peanut allergen, reportedly migrates as a doublet (*20*) [22 and 20 kDa] and appeared to remain essentially intact even after a 2−3 h treatment with the enzyme (**Figure 3A**). Since both of these peanut protein fractions contain glutaminyl and lysyl residues, as discerned from a search of the protein sequence data base provided by the National Center for Biotechnology Information (NCBI, www.ncbi.nlm.nih.gov/), it may be that they are folded in such a way so as to prevent their ready accessibility to the enzymatic catalytic site. Alternatively, enzyme/substrate binding affinities or concentration effects may limit polymerization under these reaction conditions. In contrast, the disappearance of Ara h 1 subunits in these fractions was essentially complete after a 4-h incubation period (**Figure 3A**).

Transglutaminase Polymerization of Ara h 1. In earlier work, Ara h 1, a major protein found in peanut extracts, was reported to be among the most reactive antigens (*21*), and the results of these investigations demonstrated that transglutaminase catalysis of Ara h 1 resulted in distinct dimer formation (**Figure 4**). Concurrently, the density of the Ara h 1 subunit, itself, was diminished. Notably, there was only slight evidence of trimer formation (192 000 Da), while tetramer assembly (256 000 Da) and so forth was not observed during this time frame. Perhaps, in this case, the accessibility of polymeric substrates, such as Ara h 1 dimers, to the enzymatic catalytic site was more limited.

OPA Assay Results. The degree of covalent cross-linking was quantitatively analyzed using OPA assay methods, a test that measures the disappearance of free amino groups. As summarized in **Table 2**, a decrease of ~21% was noted in the enzyme-treated cold hexane extracted peanut protein suspension (10% solids, w/v) that was preheated to 60 °C for 3 h prior to exposure to the enzyme. Similarly, a LR-12 dispersion (20% solids, w/v), pH 8.0, was more highly cross-linked, ~30%, during a similar incubation period. In this latter instance, however, the experimental sample contained supplemental dithiothreitol, known to improve the catalytic parameters of transglutaminase (*14*).

Viscosity Measurements of Transglutaminase-Modified Peanut Proteins. Since transglutaminase is currently used in numerous functional food applications (*6*−*8*, *10*−*12*), experiments were designed to investigate the potential effect of enzymatic cross-linking reactions on the apparent viscosity of peanut protein solutions. Typically, the flow characteristics of a particular protein are influenced by the molecular shape, charge, size, and water-holding capacity; therefore, experimental parameters such as pH, temperature, ionic strength, concentration, and preprocessing treatments, including enzyme modification, can affect the "thickness" of any given solution (*22*).

LR-12 dispersions, pH 8.0, were incubated with transglutaminase for approximately 6 h at 37 °C, and the results indicated that the apparent viscosity of enzyme-treated samples was lower than that of the controls over a broad temperature range (~60−80 °C). For example, at ~74 °C, the magnitude of this difference was ~5 fold. Also, a significant increase in the flow characteristics of the untreated sample was observed at ~67 °C as compared to a less dramatic rise at ~72 °C, for enzyme-modified LR-12 dispersions, suggesting delayed network formation (**Figure 5**). Taken together, these data may reflect relevant differences in diffusion parameters between high molecular weight protein polymers as compared to non-cross-linked proteins that exhibit a wider range in their size distribution. Recently, Eissa and Khan reported that transglutaminase-treated whey protein isolate (WPI) dispersions also exhibited a lowered viscosity that was on the order of several magnitudes (*23*). Furthermore, they proposed that enzymatic cross-linking minimized hydrophobic interactions, causing formation of compact, structured, protein polymers as a result of inter- and intramolecular bonding. Alternatively, these rheological findings may simply reflect a lowered "soluble" protein content since long term incubation of these samples with transglutaminase eventually caused significant precipitation of the treated material. Typically, viscosity readings are correlated with the total amount of protein in any given solution.

Allergenicity. Peanut proteins remain one of the most important food allergens, and the heat-stable glycoproteins, Ara h 1 (63.5 kDa) and Ara h 2 (20, 22 kDa), are considered to be the major immunogenic epitopes (*21*, *24*, *25*). Potentially, cross-linking reactions may alter in vivo allergic responses, as noted in a previous study. Chung et al. examined the effects of peroxidase treatment on IgE binding to peanut protein epitopes and reported that immunogenic responses of roasted fractions, especially Ara h 1 and Ara h 2, were diminished upon treatment with this enzyme (*26*).

Herein, protein polymerization was accomplished using microbial transglutaminase, and the antigenic characteristics of several conjugates were evaluated. Test samples included (1) lightly roasted protein polymers created through inter- and intramolecular bond formation, (2) transglutaminase-generated protein−carbohydrate complexes, formed as a result of covalent linkages between peanut proteins with either glucosamine or galactosamine, and (3) peanut flour dispersions, mixed with amidated pectin, under Maillard reaction conditions (60 °C, 3 h, pH 8.0).

ELISA test results indicated that IgE binding was higher, to varying degrees, in most experimental fractions supplemented with carbohydrate, as compared to the control, lightly roasted flour dispersions (**Figure 6**). Furthermore, IgE readings were essentially equivalent in transglutaminase cross-linked protein samples as compared to nontreated fractions. Peanut flour dispersions, prepared with amidated pectin, exhibited the highest IgE response of all. Glycoproteins, generated by conjugation of peanut proteins with either glucosamine or galactosamine, exhibited elevated IgE binding capacities compared to peanut flour dispersions per se, although this data was not significantly different from control samples containing equivalent amounts of the monosaccharide amino sugar.

In other systems, however, transglutaminase modification was shown to have a diminished effect on immunogenic responses. Previously, Watanabe et al. concluded that treatment of either soft or hard wheat flour with the enzyme decreased allergenicity using both salt-soluble and salt-insoluble fractions, an observation attributed to a masking of IgE antibody binding sites as a result of cross-linking (*27*). Also, Babiker and co-workers examined the effect of enzymatic processing on soy protein immunogenic responses and observed that acid-precipitated soy proteins were less immunogenic after polymerization with transglutaminase than control samples (*28*, *29*); however, both were considerably less effective at lowering IgE responses than soy−galactomannan conjugates prepared via the Maillard reaction.

To our knowledge, this study provides the first report of peanut protein catalysis by an exogenous transglutaminase. Moreover, enzymatic cross-linking of these substrates was achieved in the presence and absence of DTT although the rate of the reaction varied. Since initial rheological data suggested that enzymatic modification impacted functional characteristics, such as the final viscosity of a given solution, future studies will be directed toward developing peanut protein-based food ingredients that deliver superior performance characteristics.

Allergenicity remains one of the key limitations with regard to the utilization of peanut materials in the food industry, and these results established that transglutaminase catalysis of peanut flour protein solutions did not diminish IgE binding responses. It remains to be seen, however, whether or not these approaches might be used to decrease/eliminate potential allergic responses by creating alternative peanut protein derivatives. In this event, the expanded use of peanut protein resources in the world marketplace can be safely achieved.

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Received for review August 10, 2006. Revised manuscript received October 28, 2006. Accepted November 16, 2006. Paper 07-07 of the Journal Series of the Department of Food Science, North Carolina State University, Raleigh, NC. The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service of products named or criticism of similar ones not mentioned.

JF062309T