

## Effects of Twin-Screw Extrusion of Peanut Flour on In Vitro Digestion of Potentially Allergenic Peanut Proteins

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### ABSTRACT

Partially defatted peanut flour was processed in a twin-screw extruder. Resulting extrudates were dried, ground, and incubated with simulated gastric fluid for various time periods. Soluble protein content of the resulting digesta was measured after 10% trichloroacetic acid treatment to evaluate the digestibility. In vitro digestion using pepsin increased the solubility of peanut protein in 10% trichloroacetic acid solution from 2 to 6% to 65 to 75%. Four strong IgE-binding subunits (65, 22, 17, and 14 kDa) were found with immunoblotting in peanut proteins extracted from unextruded peanut flour; no IgE-binding bands were observed in extrudates. The 65-kDa (putative Ara h 1) subunit was insolubilized during extrusion, and its IgE-binding property was susceptible to in vitro digestion. Following extrusion cooking, no IgE-binding bands were detected by immunoblotting, including the strongly IgE-binding 14-kDa fraction, a strong IgE-binding band from native peanut protein that is stable in pepsin. The 22- and 17-kDa (putative Ara h 2) subunits retained a small amount of IgE-binding potential and became susceptible to pepsin hydrolysis after extrusion.

Peanut (*Arachis hypogea*) is one the most important causes of food allergy reactions in the United States, affecting 1.1% of Americans (20). Peanut allergy is becoming increasingly important because of the pervasive use of peanuts in food manufacturing, making them difficult to avoid. Peanut allergy is distinguished by its potential severity and its tendency to be life-long (19). The threshold dose of peanut protein ranges from milligrams to grams, depending on the individual (22). Peanut allergy is among the most severe food allergies and can result in anaphylactic shock and even death (23).

Peanut allergens are proteins or glycoproteins. Multiple peanut allergens have been reported in the past two decades (2, 10, 11); Ara h 1 (63.5 kDa) (6) and Ara h 2 (17 kDa) (5) have been most widely studied.

Peanut allergens are stable to heat processing over a pH range of 2.8 to 10 (2). Roasted peanut has an even higher level of IgE-binding capacity than does raw peanut (15, 18). Food allergens tend to be stable to pepsin digestion (1). However, proteolytic treatment, i.e., pepsin hydrolysis followed by hydrolysis with trypsin and chymotrypsin to mimic human digestion, is more efficient in reducing peanut IgE-binding ability (100-fold) than is thermal treatment (7). In a study of purified peanut allergens, Astwood et al. (1) found that Ara h 1 lost immunoreactivity within 15 s in simulated gastric fluid (SGF) and Ara h 2 was completely stable after 1 h of digestion.

Extrusion cooking reduces allergenicity of soybean and buckwheat proteins (12). Proteins are susceptible to both conformational changes and chemical reactions during extrusion. Our previous research (9) has indicated that extru-

sion with a single-screw extruder reduces IgE-binding potential of phosphate-buffered saline (PBS)-soluble peanut protein by 36 to 99%, as measured by radioallergosorbent inhibition. The effect of high pH on peanut allergens was critical; 99 to 100% of IgE-binding capability of peanut proteins was destroyed when they were extruded at pH 9. However, intensive alkaline treatment induces chemical reactions that deprive the final products of the desired sensory properties as food. In another study (9), we found that twin-screw extrusion at the natural pH of peanut meal reduced IgE-binding potential by 53 to 95%. Hydrophobic interaction and formation of disulfide bonds play an important role in insolubilizing peanut allergens such as Ara h 1 and Ara h 2 in PBS buffer. Although insoluble proteins are unlikely to enter the immune system and trigger allergenic responses, the protein hydrolytic enzymes in the human digestive system may solubilize these proteins, recovering their allergenicity. The objectives of this research were to investigate (i) the allergenicity of PBS-insoluble peanut proteins, (ii) the fate of peanut allergens subject to in vitro digestion, and (iii) the effects of extrusion on in vitro pepsin digestion of allergenic peanut proteins.

### MATERIALS AND METHODS

**Raw materials.** Partially defatted peanut flour was obtained from Seabrook Enterprises (Sylvester, Ga.). The peanut flour contained 6% fat, 5 to 6% moisture, and 60% protein.

**Extrusion.** Extrusion was carried out on a corotating twin-screw extruder (model MPF1700-30, APV Baker Ltd., Peterborough, UK) with a slit die measuring 1 by 20 mm. Barrel and die temperature was set at 115 or 135°C, but the first temperature-controlled zone close to the feeder was set at 100°C. Screw speed was set at 300 or 500 rpm. A screw configuration recommended by the manufacturer was used to impart sufficient mechanical en-

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ergy to feed materials containing significant fat. Peanut meal was fed into the extruder inlet port with a volumetric feeder (model K2VT20, K-Tron, Pitman, N.J.). The feed rate was set at 10 kg/h (dry basis). Moisture content of the raw material was adjusted to the lower level (20%) before extrusion. Peanut meal was hydrated to the desired moisture content by slowly adding water in a mixer (model A-200, Hobart Mfg. Co., Troy, Ohio) at the lowest speed. The material was mixed for 15 min to make it homogeneous. The higher moisture level (40%) was achieved by pumping the calculated amount of water into the barrel during extrusion through a proportioning pump (type N-P31, Bran and Lube, Buffalo Grove, Ill.). Material was collected for several minutes after steady state at each condition had been achieved.

**In vitro digestion with pepsin.** Extrudates were dehydrated at 60°C overnight in a laboratory oven (Jouan, Inc., Winchester, Va.). The resulting dry products (moisture, <5%) were ground in a coffee mill for 1 min. The fine powder obtained was defatted by extraction overnight with petroleum ether using a Goldfish extractor (model 3500, Laboratory Construction Co., Kansas City, Mo.). SGF contained 0.32% (wt/vol) pepsin (Sigma, St. Louis, Mo.) and 0.03 M NaCl at pH 1.2 (3). In vitro digestion was performed as follows. Defatted samples (0.050 g) were suspended in 2.0 ml of SGF and incubated at 37°C in a shaking water bath for 1 h. The effect of digestion time on protein solubility was studied with an extrudate sample (extruded at 135°C, with 20% moisture content and screw speed of 500 rpm) using various incubation times: 0, 15, and 30 s and 1, 2, 4, 8, 15, 30, and 60 min. Digestion was quenched by neutralization with 0.635 ml of 0.24 M Na<sub>2</sub>CO<sub>3</sub> to obtain a final pH of 8. The quenched digests contained 138 mM Na<sup>+</sup>, which provided the same ion strength as did the saline solution. Part of the digesta was centrifuged at 20,000 × *g* for 20 min at 25°C in a refrigerated centrifuge (model J2-21M, Beckman Instruments, Palo Alto, Calif.). The supernatant was subjected to protein assay for evaluation of protein solubility in saline, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), and immunoblotting. The remaining digesta was treated with 10% (wt/vol) trichloroacetic acid (TCA) to precipitate large undigested protein molecules (16) and evaluate the digestion. The suspension was centrifuged at 20,000 × *g* for 20 min at 25°C in the refrigerated centrifuge. The supernatant was collected for protein content determination.

**SDS and 2-mercaptoethanol extraction.** To extract the insoluble protein after in vitro digestion, SDS and 2-mercaptoethanol were added to the quenched digests to obtain final concentrations of 1% and 0.1 M, respectively. After 2 h of incubation at room temperature with gentle agitation, the extracts were centrifuged at 20,000 × *g* for 20 min at 25°C. The resulting supernatant was subject to protein content assay, SDS-PAGE, and immunoblotting.

**Protein content determination.** Nitrogen content of all samples was measured by the Dumas combustion method using an FP-2000 protein/nitrogen analyzer (LECO Corporation, Warrendale, Pa.). The percent nitrogen obtained was multiplied by 5.46 to give protein content (24).

**SDS-PAGE.** SDS-PAGE was performed according to the method of Laemmli (13) using discontinuous gels (12% separating gel and 4% stacking gel).

**Immunoblotting.** Immunoblotting was applied to visualize the peanut-specific IgE-binding proteins in the polyacrylamide gels. Proteins were transferred to a 0.45-μm nitrocellulose membrane (Bio-Rad Laboratories, Hercules, Calif.) with a semidry

blotter (Bio-Rad). The membrane was blocked with 0.01 M PBS (pH 7.4) containing 1% casein and 0.025% Tween 20 for 1 h at room temperature. After blocking, the membrane was incubated with an 11:1 dilution (PBS) of pooled sera from allergic individuals (kindly supplied by the Food Allergy Resource and Research Program, University of Nebraska, Lincoln) overnight at 4°C. The membrane was washed four times with PBS solution containing 0.025% Tween 20 and then incubated with goat anti-human IgE–horseradish peroxidase conjugate (1,000:1) (Bethyl Laboratories, Montgomery, Tex.) overnight at 4°C. The membrane was again washed four times, and chemiluminescence blotting substrate (POD, Boehringer Mannheim GmbH, Mannheim, Germany) was added for 1 min before determination of chemiluminescence with a model 440 image station (Kodak, Rochester, N.Y.). Negative controls were treated in the same manner except that the serum did not contain peanut-specific IgE.

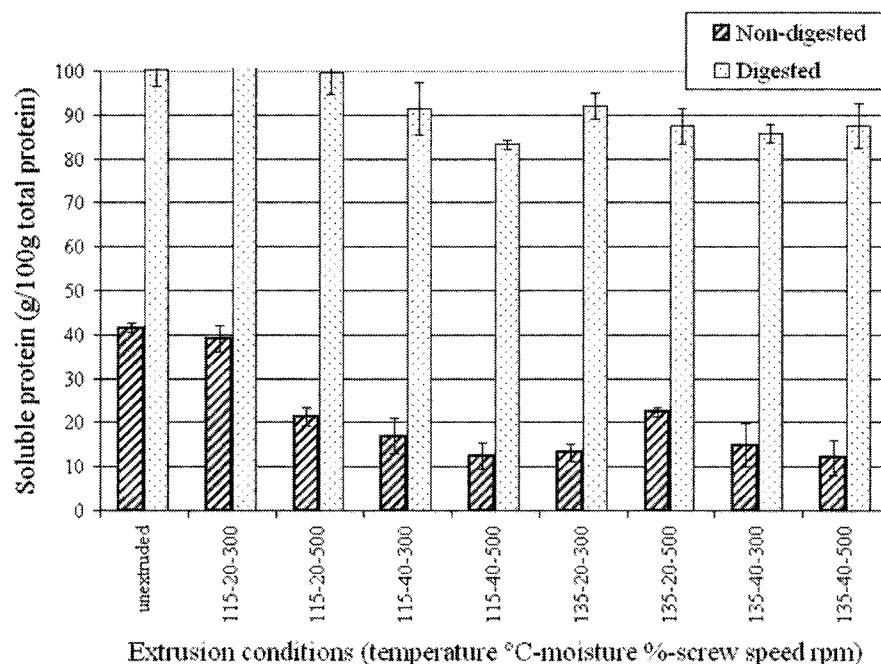
## RESULTS AND DISCUSSION

**Effects of extrusion on in vitro digestion of peanut extrudates.** In vitro digestion was carried out to imitate the fate of proteins in the human digestive system. In a preliminary experiment (data not shown), incubation of defatted extrudates with SGF for 1 h hydrolyzed most of the protein substrate. Therefore, we chose to perform in vitro digestion of extrudates from all extrusion treatments for 1 h.

The solubility profile of the proteins before and after digestion without TCA treatment is shown in Figure 1. Protein solubility was expressed by percentage of soluble protein in the total protein of the samples (grams per 100 g of total protein). In vitro digestion drastically improved the solubility of peanut protein in saline. Extrusion reduced the solubility of peanut protein by 5 to 70%, depending on the extrusion conditions. Unextruded peanut protein and proteins in extrudates from low-temperature and low-moisture conditions became approximately 100% soluble after pepsin treatment. Under other extrusion conditions, there was still a small amount of protein (8 to 17%) that was insoluble after digestion, although solubility increased by four- to sevenfold after digestion. Protein denatured by extrusion has a linear random structure and is more susceptible to enzymatic cleavage (8). However, if the denatured proteins aggregate by hydrophobic interaction and form large aggregates, inner proteins may be isolated from enzymes, accounting for the insoluble protein after pepsin digestion. Chemical reactions induced by extrusion, such as intermolecular cross-links of the lysinoalanine and lanthionine types, Maillard products, and isopeptides (21), may also generate products that are resistant to enzymatic hydrolysis.

TCA is widely used as a precipitant of protein from digesta (4, 14, 24), even though there is little information on its efficiency. TCA treatment resulted in comparable overall values for the free amino acids plus small (<700 Da) peptides (16), which are considered hydrolyzed protein. Extrudates, before and after digestion, were treated with TCA, and the solubility is shown in Figure 2. Less than 6% of TCA-soluble proteins or small peptides existed in extrudates and unextruded peanut flour before digestion. After in vitro digestion, 65 to 75% of protein was TCA soluble. The extrudates had an average of 69% TCA-soluble protein, whereas unextruded peanut had 66% TCA-

FIGURE 1. Protein solubility in saline solution of unextruded peanut flour and peanut extrudates before and after SGF treatment for 1 h at 37°C.



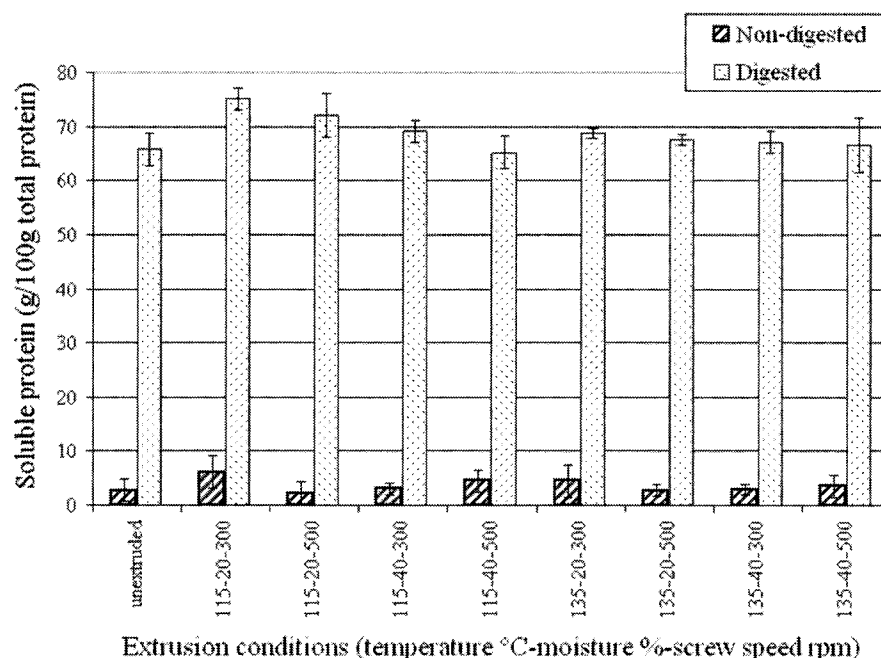
soluble protein. This finding indicates that extrusion may slightly increase the overall digestibility of peanut protein.

The effect of digestion time on protein solubility was also determined. The soluble protein content was determined before and after TCA precipitation (Figs. 3 and 4). Protein solubility increased with the time of pepsin treatment (Fig. 3). Unextruded peanut had 53% soluble protein at the beginning of digestion, but the solubility increased to 100% within 4 min. The extruded sample also exhibited increasing solubility with digestion time. However, it took a longer time (15 to 30 min) for the percentage of soluble protein to reach a consistent high level (around 90%). Figure 4 shows the percentage of hydrolyzed protein (TCA soluble) with digestion time. Both the extrudate and unextruded sample displayed a rapidly increasing rate of hydro-

lysis in the first 4 min. After that, the hydrolysis slowed down because of lack of substrate and the increasing difficulty of access to substrate receptor sites. At the end of 30 to 60 min of digestion, both samples reached the highest point of around 65% hydrolyzed protein (Fig. 2).

The effect of in vitro digestion on the SDS-PAGE profile of unextruded peanut and extrudate is shown in Figures 5 and 6. Most of the bands from unextruded peanut faded within 30 s of digestion, with increased smearing in the low-molecular-weight area. This finding indicates a degradation of high-molecular-weight proteins into numerous smaller species. SDS-PAGE of extrudate produced a similar profile, except that there were no strong bands above 30 kDa. Because the radioallergosorbent inhibition test is always applied to the soluble protein fraction, the incomplete

FIGURE 2. TCA-soluble protein of unextruded peanut flour and peanut extrudates. Proteins were extracted with or without 1 h of incubation with SGF at 37°C.



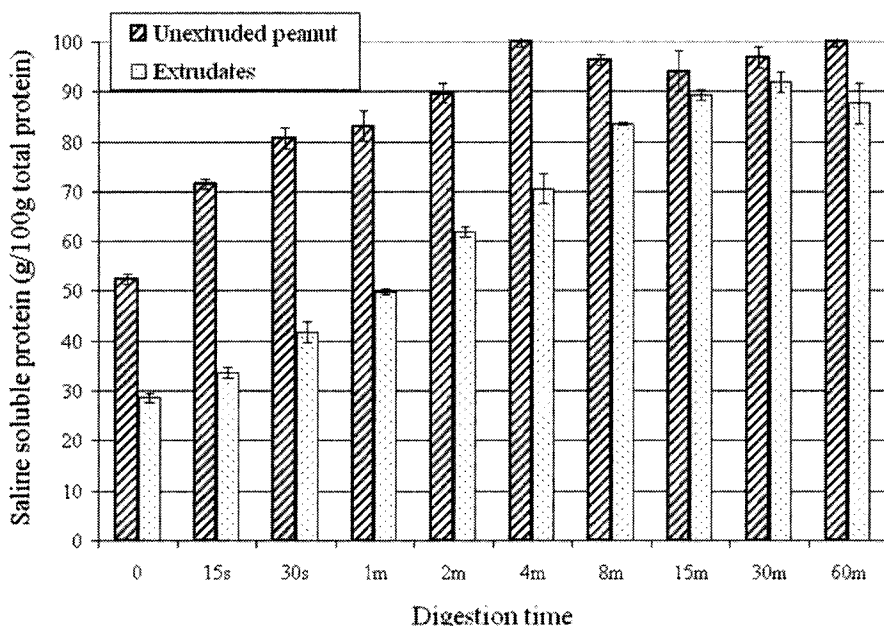


FIGURE 3. Effect of digestion time on protein solubility of unextruded peanut flour and peanut extrudates (extruded at 135°C, with 20% moisture content and screw speed of 500 rpm). Digestion time was set to 0, 15, and 30 s and 1, 2, 4, 8, 15, 30, and 60 min.

recovery of the insoluble proteins in a digestive system may challenge its accuracy as a method of evaluating IgE-binding potential.

**Effect of *in vitro* digestion on peanut allergenic proteins.** To trace the fate of peanut allergens during *in vitro* digestion, the digesta of extrudate and unextruded peanut protein were subject to SDS-PAGE and immunoblotting (Figs. 5 and 6). Negative controls for immunoblotting did not produce any visible bands different from background (data not shown).

Most of the bands in unextruded peanut flour disappeared after 30 s of pepsin treatment (Fig. 5). Large protein molecules were cleaved by pepsin into smaller fragments. Background smear increased with digestion time. Immunoblotting showed the 65-kDa band, putative Ara h 1, was not stable in SGF. This result confirmed what Astwood et al. (1) found, that purified Ara h 1 lost its immunoreactivity

within 15 s in SGF. However, in that study, a monoclonal antibody was used instead of IgE from allergic subjects. They found that this monoclonal antibody failed to detect fragments of Ara h 1. They also used purified Ara h 1 instead of allergens from a food matrix. In our study, putative Ara h 1 lost its IgE-binding properties within 30 s and these properties were not restored with increasing protein solubility due to digestion.

In addition to Ara h 1, three intensive IgE-binding bands of 14, 17, and 22 kDa were seen on the membrane before digestion. These bands were not as strong in the polyacrylamide gel because of the sensitivity differences between Coomassie blue stain and chemiluminescence development. The 14- and 22-kDa allergens were fairly stable in SGF. Although the intensity was reduced with digestion time, their IgE-binding bands were still visible after 4 min of pepsin digestion. The 17-kDa allergen lost its IgE-bind-

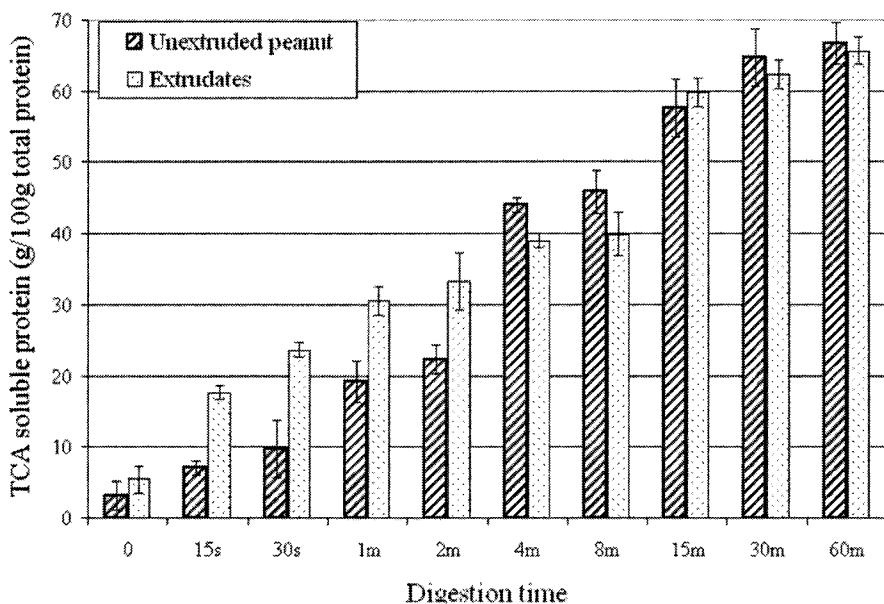
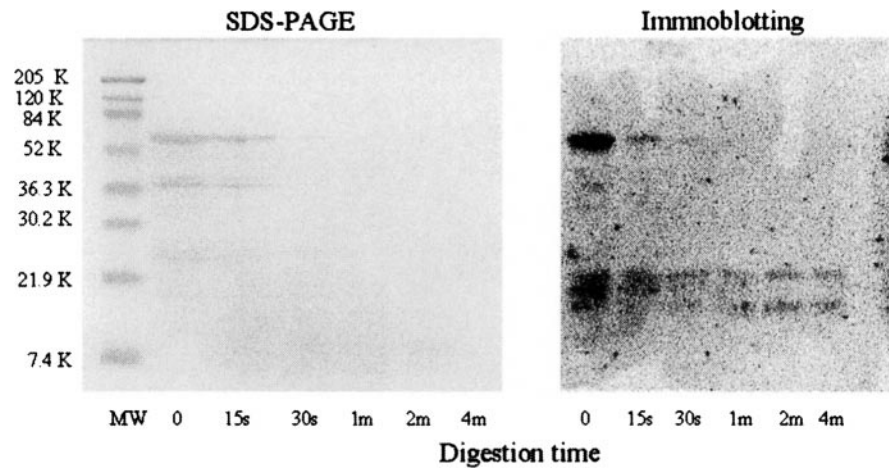


FIGURE 4. TCA-soluble protein of unextruded peanut flour and peanut extrudates (extruded at 135°C, with 20% moisture content and screw speed of 500 rpm). Digestion time was set to 0, 15, and 30 s and 1, 2, 4, 8, 15, 30, and 60 min.

FIGURE 5. Gel electrophoresis (SDS-PAGE) and Western blot of unextruded peanut proteins with IgE from pooled sera of five peanut-allergic patients. Unextruded peanut flour was incubated with SGF for 0, 15, and 30 s and 1, 2, and 4 min. The soluble fraction of resulting digests (30  $\mu$ g of protein) was loaded on the gel. An identical polyacrylamide gel was used for the Western blot.



ing capacity within 1 min. Ara h 2 (17 kDa) falls into the molecular weight range where the three IgE-binding bands were found. Astwood et al. (1) found that the purified Ara h 2 was completely stable in SGF, which remained on the gel after up to 60 min of digestion.

The SDS-PAGE and immunoblotting profiles of extrudate are quite different from those of the unextruded peanut protein (Fig. 6). No strong bands were seen in the polyacrylamide gel at the beginning of digestion. The bands became weaker with digestion time, with an indistinct, hazy background built up in the low-molecular-weight area. Immunoblotting did not produce visible IgE-binding bands that could be distinguished from the background. Thus, there was no IgE-binding potential observed in the digesta.

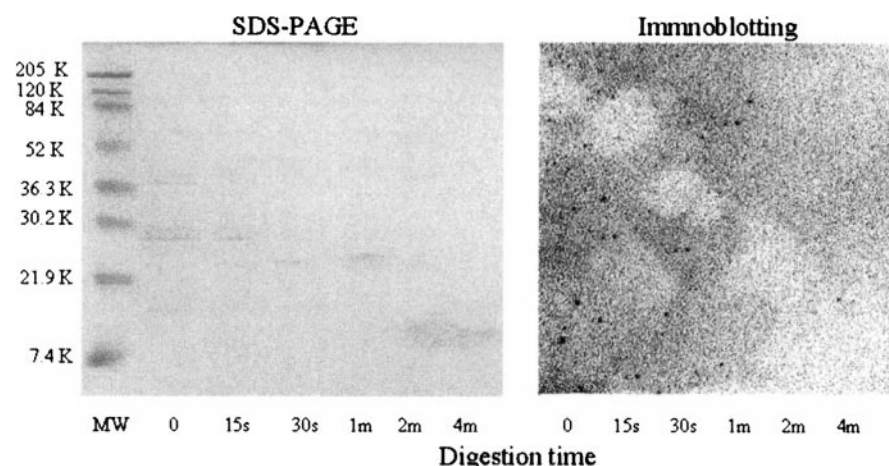
In vitro digestion cleaves the peptide bond and breaks protein molecules into smaller fragments. It affects the allergens in two ways. First, enzymatic hydrolysis increases the solubility of protein. As we found previously, Ara h 1 (65 kDa) is insolubilized by hydrophobic interaction, and the other three intensive IgE-binding protein subunits around 22 kDa (including Ara h 2) are insolubilized by formation of disulfide bonds (9) during extrusion. These allergen aggregates might be dissolved by SGF and some of their IgE-binding property recovered. However, pepsin may also hydrolyze the epitopes and eliminate their IgE-binding potential. In our study, no IgE-binding potential was observed in the digesta by immunoblotting, which in-

dicates that either the epitopes were rapidly destroyed by pepsin hydrolysis when the allergen recovered solubility or some of the allergens were still insoluble after in vitro digestion. Ara h 1, which is not stable to SGF, is likely to experience rapid hydrolysis and lose its IgE-binding capacity early in the digestion process. Meanwhile, pepsin-resistant allergens, such as 14- and 22-kDa allergens, may remain insoluble after digestion or may become less able to bind IgE.

To evaluate the IgE-binding potential of the PBS-soluble and -insoluble protein fractions, SDS and mercaptoethanol were added to digesta to extract as much protein as possible. The resulting extracts were subject to protein content determination, SDS-PAGE, and immunoblotting. Both SDS-mercaptoethanol treatment and in vitro digestion may increase the solubility of peanut proteins; however, the SDS-mercaptoethanol treatment does not affect IgE-binding potential as enzymatic hydrolysis does.

Figure 7 shows the efficiency of SDS-mercaptoethanol extraction. Unextruded peanut protein was highly soluble (>90%) in most digesta. The solubility of extrudate in SDS-mercaptoethanol increased with digestion time. Up to 88% of protein was extracted after incubation with pepsin for 15 min. Compared with the results in Figure 3, SDS-mercaptoethanol treatment helped to extract more proteins from the digesta, especially for the samples with short digestion times.

FIGURE 6. Gel electrophoresis (SDS-PAGE) and Western blot of peanut extrudate protein (extruded at 135°C, with 20% moisture content and screw speed of 500 rpm) with IgE from pooled sera of five peanut-allergic patients. Peanut extrudate was incubated with SGF for 0, 15, and 30 s and 1, 2, and 4 min. The soluble fraction of resulting digests (30  $\mu$ g of protein) was loaded on the gel. An identical polyacrylamide gel was used for the Western blot.



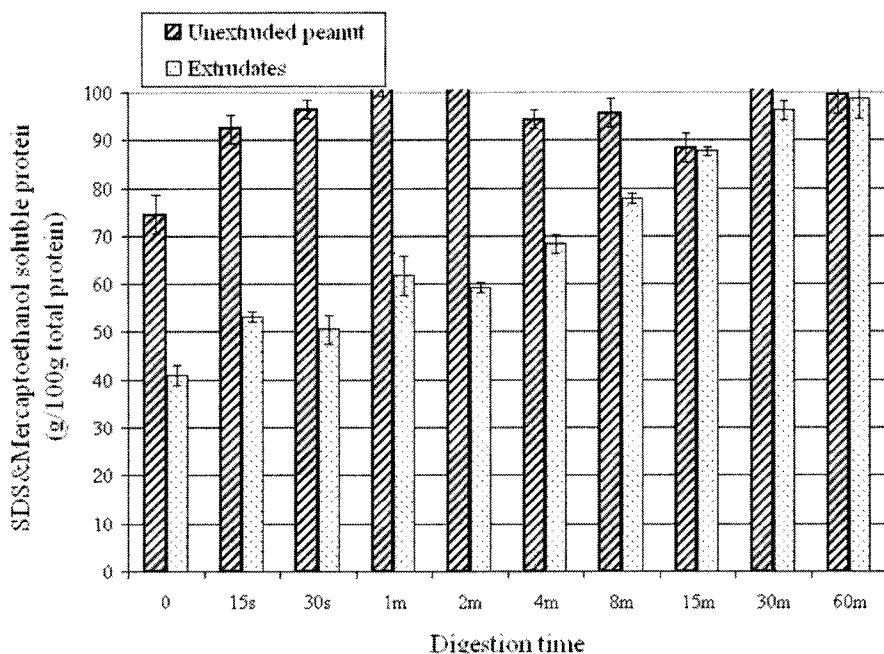


FIGURE 7. Solubility of peanut protein in SDS and 2-mercaptoethanol after *in vitro* pepsin digestion. Unextruded peanut flour and peanut extrudates (extruded at 135°C, with 20% moisture content and screw speed of 500 rpm) were incubated with SGF for 0, 15, and 30 s and 1, 2, 4, 8, 15, 30, and 60 min. After digestion, SDS (1%) and mercaptoethanol (0.1 M) were added to digests. Soluble nitrogen content of the resulting extracts was measured and converted to protein content.

SDS-PAGE and immunoblotting of unextruded protein extracted by SDS-mercaptoethanol treatment are shown in Figure 8. Negative controls for immunoblotting produced a blank background (data not shown). Most of the subunits on the polyacrylamide gel decreased in intensity with digestion time. The 65-kDa band diminished with time and was absent at the end of 4 min of digestion. Immunoblotting revealed an increase of IgE binding at the first 1 min of digestion. After that, binding tailed off with digestion time. However, at the end of 60 min of digestion, there was still a small amount of IgE binding remaining, especially to the smallest subunits. The increase of IgE binding in the first 1 min and the remaining IgE-binding potential was due to increasing protein solubility in SDS and mercaptoethanol, which suggests that some of the allergenic proteins containing Ara h 1 remained insoluble in digesta.

Two strong bands that did not bind to IgE, one at around 40 kDa and another at around 25 kDa, were seen on the polyacrylamide gel at the beginning of pepsin treatment, and all bands became invisible within 4 min.

Three strong IgE-binding bands were found at 14, 17, and 22 kDa, even though they were not intense bands on the Coomassie blue-stained polyacrylamide gel. All these protein subunits exhibited an increase in IgE binding in the first 1 min of digestion, but binding decreased gradually thereafter. The IgE binding of the 22-kDa fraction diminished within 15 min, whereas that of the 14- and 17-kDa subunits was still visible after 60 min of digestion. This finding suggests that the 14- and 17-kDa subunits are more stable in SGF than is the 22-kDa subunit. The increase of IgE binding in the first 1 min of digestion indicates that these subunits are resistant to enzymatic hydrolysis and remain insoluble in digesta during this period of time. SDS-mercaptoethanol treatment and *in vitro* digestion dissolved these subunits and facilitated their increase in IgE-binding capability.

Figure 9 shows the SDS-PAGE and immunoblotting profiles for the SDS-mercaptoethanol-extracted extrudate. Immunoblotting without peanut-specific IgE (negative control) had a clean background (data not shown). The poly-

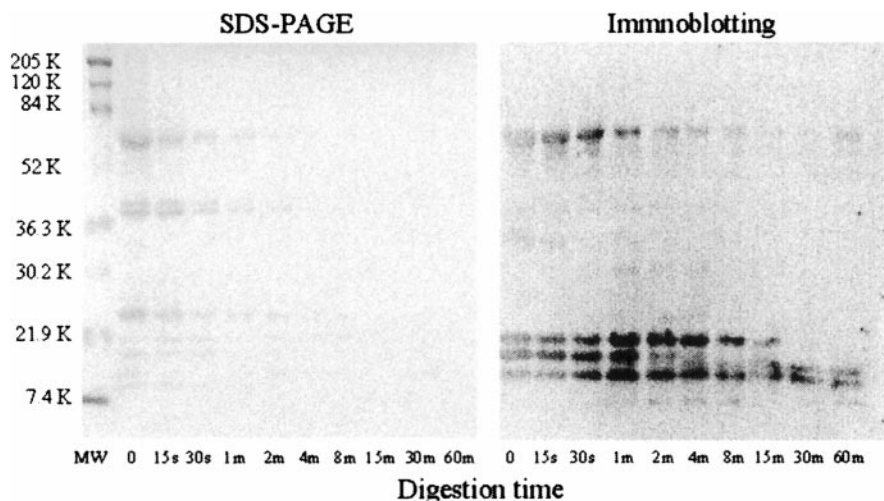
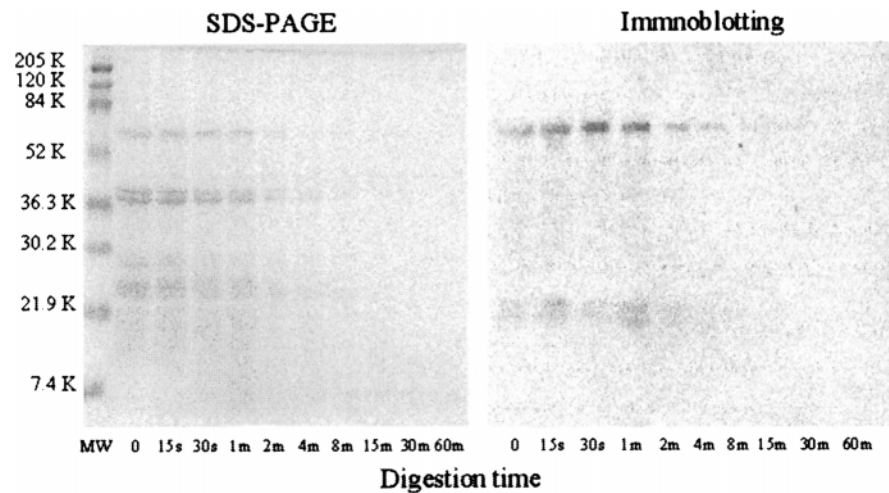


FIGURE 8. Gel electrophoresis (SDS-PAGE) and Western blot of unextruded peanut proteins with IgE from pooled sera of five peanut-allergic patients. Unextruded peanut flour was incubated with SGF for 0, 15, and 30 s and 1, 2, 4, 8, 15, 30, and 60 min. After digestion, SDS (1%) and mercaptoethanol (0.1 M) were added to the digests. The soluble fraction of resulting digests (30 µg of protein) was loaded on the gel. An identical polyacrylamide gel was used for the Western blot.

FIGURE 9. Gel electrophoresis (SDS-PAGE) and Western blot of proteins extracted from peanut extrudates (extruded at 135°C, with 20% moisture content and screw speed of 500 rpm) with IgE from pooled sera of five peanut-allergic patients. Extruded peanut flour was incubated with SGF for 0, 15, and 30 s and 1, 2, 4, 8, 15, 30, and 60 min. After digestion, SDS (1%) and mercaptoethanol (0.1 M) were added to the digests. The soluble fraction of resulting digests (30 µg of protein) was loaded on the gel. An identical polyacrylamide gel was used for the Western blot.



acrylamide gel with protein from the peanut extrudate had a profile similar to that with unextruded peanut. Most of the bands diminished with digestion time and were absent after 4 min. However, the immunoblotting membrane did not exhibit as much as IgE binding as was seen in unextruded peanut. This finding suggests that extrusion reduced the IgE-binding potential of peanut protein by both insolubilizing the allergens and by eliminating the immunoactivity of their epitopes.

Compared with the negative IgE-binding profile of digesta in saline (Fig. 6), immunoblotting of samples dissolved in SDS-mercaptoethanol revealed that the 65-kDa band remained insoluble in the digesta for up to 15 min before it was hydrolyzed and deprived of its IgE-binding property. Two IgE-binding bands (17 and 22 kDa) were barely visible within the first 4 min of digestion, and the 14-kDa band, which was strong in unextruded peanut, was completely absent. These findings indicate that small amounts of the 17- and 22-kDa allergens remained insoluble in digesta after the first 4 min of digestion but were hydrolyzed and destroyed after that. The 14-kDa allergen lost its IgE-binding property during extrusion. Compared with the strong IgE-binding profile of unextruded peanut protein (Fig. 8), extrusion cooking helped to reduce the IgE binding of these three allergenic protein subunits or made them susceptible to enzymatic hydrolysis.

IgE binding is a prerequisite for elicitation of an immune response; however, it does not necessarily predict the cascade of allergic reaction because of the complex relationship between cross-linking of basophil- or mast cell-bound IgE by allergens and the occurrence of allergic signs. Also, the way in which the allergen gets into the immune system is not well known. Therefore, an in vivo test, such as a double-blind placebo-controlled food challenge, should be conducted to evaluate the true allergenicity of the extrudates.

Extrusion cooking reduced overall protein solubility in peanuts; however, it slightly increased protein digestibility. No IgE binding was observed by immunoblotting for extrudates at various stages of in vitro digestion. Four strong IgE-binding protein subunits were found at 14, 17, 22, and 65 kDa. Ara h 1 (65 kDa) was susceptible to enzymatic

hydrolysis. The 14-, 17-, and 22-kDa subunits were fairly resistant to in vitro hydrolysis, but extrusion helped to reduce their IgE-binding capacities and made them more susceptible to enzymatic hydrolysis. These observations suggest that extrusion cooking may substantially reduce the allergenic potential of peanut protein.

## REFERENCES

1. Astwood, J. D., J. N. Leach, and R. L. Fuchs. 1996. Stability of food allergens to digestion in vitro. *Nat. Biotechnol.* 14:1269–1273.
2. Barnett, D., and M. E. H. Howden. 1986. Partial characterization of an allergenic glycoprotein from peanut (*Arachis hypogaea* L.). *Biochim. Biophys. Acta* 882:97.
3. Board of Trustees (ed.). 1995. Simulated gastric fluid, TS, p. 2053. *In* The United States pharmacopoeia 23. The National Formulary 18. U.S. Pharmacopoeia Convention, Inc., Rockville, Md.
4. Braude, R., K. G. Mitchell, M. J. Newport, and J. W. G. Porter. 1970. Artificial rearing of pigs. I. Effect of frequency and level of feeding on performance and digestion of milk proteins. *Br. J. Nutr.* 76:235–241.
5. Burks, A. W., L. W. Williams, and C. Connaughton. 1992. Identification and characterization of a second major peanut allergen, Ara h II, with use of the sera of patients with atopic dermatitis and positive peanut challenges. *J. Allergy Clin. Immunol.* 90:962–969.
6. Burks, A. W., L. W. Williams, R. M. Helm, C. Connaughton, G. Cockrell, and T. O'Brien. 1991. Identification of a major peanut allergen, Ara h I, in patients with atopic dermatitis and positive peanut challenges. *J. Allergy Clin. Immunol.* 88:172–179.
7. Burks, A. W., L. W. Williams, W. Thresher, C. Connaughton, G. Cockrell, and R. M. Helm. 1992. Allergenicity of peanut and soybean extracts altered by chemical and thermal denaturation in patients with atopic dermatitis and positive food challenges. *J. Allergy Clin. Immunol.* 90:889.
8. Camire, M. E. 2001. Extrusion and nutritional quality, p. 108–129. *In* R. Guy (ed.), *Extrusion cooking—technologies and applications*. Woodhead Publishing, Cambridge, UK.
9. Chen, L. 2001. Reduction of peanut allergenicity by extrusion cooking. Ph.D. dissertation. University of Georgia, Athens.
10. DeJong, E. C., M. Van Zilverden, S. Spanhaak, S. J. Koppelman, H. Pellegrom, and A. H. Penninks. 1998. Identification and partial characterization of multiple major allergens in peanut protein. *Clin. Exp. Allergy* 28:743–751.
11. Gleson, P. A., and M. A. Jermyn. 1977. Leguminous seed glycoproteins that interact with concanavalin A. *Aust. J. Plant Physiol.* 4:25.
12. Hayakawa, I., Y. Linko, and P. Linko. 1996. Novel mechanical treatments of biomaterials. *Lebensm.-Wiss. Technol.* 29:395–403.
13. Laemmli, U. K. 1970. Cleavage of the structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680–685.

14. Low, A. G. 1979. Studies on digestion and absorption in the intestines of growing pigs. 5 measurements of the flow of nitrogen. *Br. J. Nutr.* 41:137–146.
15. Maleki, A. J., S.-Y. Chung, E. T. Champagne, and J.-P. Raufman. 2000. The effects of roasting on the allergenic properties of peanut proteins. *J. Allergy Clin. Immunol.* 106:763–768.
16. Moughan, P. J., A. J. Darragh, W. C. Smith, and C. A. Butts. 1990. Perchloric and trichloroacetic acids as precipitants of protein in endogenous ileas digesta from the rat. *J. Sci. Food Agric.* 52:13–21.
17. Munro, H. N., and A. Fleck. 1969. Analysis for nitrogenous constituents, p. 423–525. In H. N. Munro (ed.), *Mammalian protein metabolism III*. Academic Press, New York.
18. Nordlee, J. A., S. L. Taylor, R. T. Jones, and J. W. Yunginger. 1981. Allergenicity of various peanut products as determined by RAST inhibition. *J. Allergy Clin. Immunol.* 68:376–382.
19. Sampson, H. A., and A. W. Burks. 1996. Mechanisms of food allergy. *Annu. Rev. Nutr.* 16:161–177.
20. Sicherer, S. H., A. Munoz-Furlong, A. W. Burks, and H. A. Sampson. 1999. Prevalence of peanut and tree nut allergy in the US determined by a random digit dial telephone survey. *J. Allergy Clin. Immunol.* 103:559–562.
21. Stanley, D. W. 1989. Protein reactions during extrusion processing, p. 321–341. In C. Mercier, P. Linko, and J. M. Harper (ed.), *Extrusion cooking: the theory and practice of food extrusion*. American Association of Cereal Chemists, St. Paul, Minn.
22. Woodroof, J. G. 1983. Composition and nutritive value of peanuts, p. 165–179. In J. G. Woodroof (ed.), *Peanuts: production, processing, products*, 3rd ed. AVI Publishing, Westport, Conn.
23. Yunginger, J. W., K. G. Sweeney, W. Q. Sturmer, L. A. Giannandrea, J. D. Teigland, M. Bray, P. A. Benson, J. A. York, L. Biedrzycki, D. L. Squillace, and R. M. Helm. 1988. Fatal food-induced anaphylaxis. *J. Am. Med. Assoc.* 260:1450.
24. Zebrowska, T., A. G. Low, and H. Zebrowska. 1983. Study on gastric digestion of protein carbohydrate, gastric secretion and exocrine pancreatic secretion in the growing pig. *Br. J. Nutr.* 49:401–410.