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Allergenic Proteins in Soybean: Processing and Reduction of P34 Allergenicity

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Soybean ranks among the "big 8" of the most allergenic foods, and with increasing consumption of soybean products, the incidence of soy-caused allergies is expected to escalate. Soybean and its derivatives have become ubiquitous in vegetarian and many meat-based food products, and as a result, dietary avoidance has become difficult. However, soybeans can be manipulated in a variety of ways to alter their allergenicity. Several studies have focused on reducing the allergenicity of soybeans by changing the structure of the immunodominant allergen P34 using food processing, agronomic, or genetic manipulation techniques. A review of the literature pertaining to these studies is presented here.

Key words: allergenicity, soybean, soy products, Gly m Bd 30K, β -conglycinin, glycinin

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Overview of Food Allergy

A food allergy is an immune system reaction to a specific food.¹ Proteins in foods can be allergenic by acting as antigenic molecules that cause an immune reaction. The allergen induces an initial IgE antibody response, followed by a secondary IgE antibody response, which signals an allergic reaction.² Antibodies are found on the surface of mast cells and basophils. Upon binding of the allergen to the antibody during the second exposure, mediators are released. These mediators, such as histamine and cytokines, induce the inflammatory response indicative of an allergic reaction.³

About 5% to 7.5% of children and 1% to 2% of adults are affected by food allergies. Children are more susceptible to food allergies, but usually develop resistance as they grow older.⁴

Any food containing protein may induce an allergic reaction. The "big 8" are those foods that account for 90% of all IgE-mediated food allergies, and include cow's milk, eggs, fish, crustaceans, peanuts, soybeans, tree nuts, and wheat.^{3,5} Although rare, allergic reactions may also occur due to cross-reactivity between similar allergens. For example, Wensing et al.⁶ reported several anaphylaxis cases indicating cross-reactivity between peas and peanut because IgE antibodies to pea vicilin reacted with peanut vicilin. This is due to homology in the amino acid sequences found among various allergic proteins. This is also the case with P34, the major allergenic soy protein that shares approximately 70% sequence homology with peanut's main allergen (Ara h 1) and 50% to 70% with the immunodominant cow's milk allergen (2-S1-casein).^{6,7} Due to this homology and close botanical relationship, peanuts and soybeans contain common allergenic components, and for this reason IgE antibodies to peanut proteins can also react with soybean proteins.⁸ This may explain a study in Sweden that reported three anaphylactic deaths in patients ages 9 to 17 after consumption of meat products fortified with 2.2% to 7% soy protein; these patients had a previously known allergy to peanuts but not to soybeans.

Peanuts, tree nuts, fish, and shellfish are likely to cause childhood allergies that persist as an individual matures. Other food allergies in children, such as those from milk, soy, egg, and wheat, are likely transient.⁴

Soybeans

The soybean (*Glycine max*) is a member of the legume family.^{9,10} Its protein is being used in an increasing number of products, in part because of a plethora of claimed health benefits. Soy protein consists of 136 phytochemicals,¹¹ and there is evidence that individuals who consume soybean-rich diets exhibit a lower prevalence

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lence of high plasma cholesterol, cancer (including bowel and kidney), diabetes mellitus, and obesity.^{12,13} Clinical trials have also shown reductions in triglycerides and total and low-density-lipoprotein (LDL) cholesterol when soybean protein is substituted for animal protein.¹⁴ Hypercholesterolemic patients show a greater response to the soybean protein diet than those who are normocholesterolemic.¹¹ Isoflavones from soybeans may lower the risk of coronary heart disease and may protect women against breast cancer.^{15,16} This protective effect could also be due to other factors such as the Bowman-Birk inhibitor or phospholipids present in soybeans. The Bowman-Birk inhibitor is a chymotrypsin and trypsin inhibitor found in soybean seeds. It is also thought to possess anticarcinogenic and radioprotective activity; it has been shown to suppress free radical production and to kill human cancer cells.^{12,17}

When used as a meat substitute in vegetarian diets, soy products provide an alternative source of protein and may reduce the risk of cardiovascular disease compared with a traditional meat-based diet.¹⁸ This may be due to the ability of soy protein to modulate LDL receptor levels in the liver.¹⁹

Overview of Soybean Allergy

Soybean allergies affect about 1% to 6% of infants.⁴ In adults, the incidence is increasing because more products are being produced with soy.²⁰ According to the US Department of Agriculture, the revenue generated from soybean production in North America alone totaled over 1 trillion dollars in 2003.²¹

There are three main types of soy allergenic reactions. The first type consists of IgE-mediated reactions that can produce respiratory, cutaneous, and gastrointestinal symptoms. The second type are non-IgE-mediated reactions, and include soy-induced enterocolitis, which can often be outgrown; symptoms of these reactions usually include fever, vomiting, and diarrhea.³ The third and least common reaction is anaphylaxis, which in the United States affects an average of 10.8/100,000 persons per year. Anaphylaxis is the most severe allergic reaction. Food anaphylaxis is characterized by a sudden onset of symptoms typical of IgE-mediated hypersensitivity after the ingestion of a food. The reaction results from the release of potent bioactive mediators from mast cells and basophils that have effects on typically two or more target organs. Food anaphylaxis can induce respiratory, cutaneous, cardiovascular, and gastrointestinal symptoms, and even death.²²

There are several allergenic proteins in soybeans. However, allergic reactions to soybean proteins are mostly transient and non-life-threatening, and are usually outgrown by the age of 3 years.³ Other individuals seem to become tolerant within 3 to 5 years after the initial

diagnosis.¹⁰ Although this allergy may be transient and is usually outgrown, its severity and frequency have increased, particularly in adults. Therefore, there is a need to remove allergenic proteins from soy products whenever possible.⁷

When an allergic reaction occurs, there are few suggested treatments. Epinephrine injections, antihistamines, systemic steroids, and respiratory treatments are commonly administered to reduce the symptoms.²³ Peptide immunotherapy, DNA immunization, and humanized anti-IgE monoclonal antibody treatments are three of the newest therapeutic options being studied.^{24,25} However, these new treatments have not yet been implemented, and the best prevention to date is dietary vigilance. Sensitive individuals need to avoid products that contain soybean protein or its derivatives, but determining which products contain these ingredients is increasingly difficult.⁴ If a product label says it "may contain" a certain allergenic ingredient, or if the product was made close to or using the same equipment used with an allergenic ingredient, it is wise to avoid that product. The threshold for allergens is usually very low, and a very small amount may be enough to trigger a reaction. For instance, a statistical model projects that 0.3 g of soy flour will elicit an allergic response in 1 out of every 100 soy-sensitive people.²⁶ Sicherer et al.²⁷ reported that 28% (n = 53) of soy-sensitive children with atopic dermatitis exhibited an allergic response after ingesting less than 0.5 g of soy flour. This corresponds to approximately 41 mg of soy protein.

Identification of Soy Proteins with Allergenic Activity

There are at least 21 allergenic proteins in soybean that have been identified and present IgE binding.²⁸ Table 1 lists several of the allergenic proteins in soybean, including P34. A number of these soybean proteins have been found to cause asthma-related allergic reactions. Among these are the newly discovered proteins Gly m 1A, 1B, and 2, which are contained in soybean hulls. Heating enhances the allergenicity of these hull proteins. Another allergenic protein is rGly m 3, which is a 12- to 15-kD allergenic soybean profilin that warrants further study.^{29,30}

Plants store proteins in their developing seeds to serve as a source of nitrogen, sulfur, and carbon.³¹ Seed proteins in soybean comprise two major fractions that account for 70% to 80% of total protein composition: 11S and 7S globulins.¹⁰ The 11S globulin fraction contains the hexameric pure protein glycinin, and each of its subunits contains an acidic and a basic polypeptide linked by a disulfide bond.³² All subunits of glycinin, in oligomeric form, generate an antibody response in mice when fed soy-protein-containing diets. This indicates

Table 1. Summary of Soybean Allergens

Molecular Weight of IgE-Binding Soy Protein (kD)	Name of Protein or Protein Fraction	Reference
7.0	Gly m 1a; hull protein	Rodrigo et al. ⁸¹
7.5	Gly m 1b; soybean hydrophobic protein; hull protein	Rodrigo et al. ⁸¹
8.0	Gly m 2; hull protein	Gonzalez et al. ⁸²
12–15	rGly m 3; profilin	Rihs et al. ²⁹
17	2S-globulin fraction	Ogawa et al. ⁸³
20	Kunitz trypsin inhibitor; 2s globulin	Ogawa et al. ⁸³
18–21	Whey fraction	Ogawa et al. ⁸³
22	Glycinin G2; basic chain of glycinin 11S-globulin	Helm et al. ⁸⁴
28	Gly m Bd 28K; 7s globulin	Tsuji et al. ⁸⁵
30–34	Gly m Bd 30K, P34; immunodominant allergen	Ogawa et al. ⁸³
29–31	Whey fraction	Ogawa et al. ⁸³
32	Soy lectin; soybean agglutinin	Metcalf et al. ⁶⁷ ; Bals and Welsch ⁸⁶
33–35	7S-Globulin	Ogawa et al. ⁸³
35–38	7S-Globulin	Ogawa et al. ⁸³
35–40	Glycinin G1; acidic chain of glycinin; 11S-globulin	Beardslee et al. ⁸⁷
40–41	7S-Globulin	Ogawa et al. ⁸³
42	β subunit of beta conglycinin	Thanh and Shibasaki ⁸⁸ ; Ogawa et al. ⁸³
47–50	7S-Globulin	Ogawa et al. ⁸³
52–55	7S-Globulin	Ogawa et al. ⁸³
63–67	α subunit of beta conglycinin; Gly m Bd 60K	Rihs ²⁹ ; Ogawa et al. ³⁵
71	α' subunit of beta conglycinin	Rihs et al. ²⁹

that glycinin is allergenic and resistant to processing.³³ The 7S globulin fraction is composed primarily of β -conglycinin, which includes three subunits: α (~67 kD), α' (~71 kD), and β (~50 kD).²⁹ The antibody against the α -subunit is found in 25% of soybean-allergic patients' sera.^{34,35} The Gly m Bd 28K protein is also contained within the 7S globulin fraction and shares sequence homology with proteins in pumpkin and carrot.²⁹ They are vicilin-like glycoproteins that also have approximately a 25% prevalence in allergic reactions of soybean-sensitive individuals. This allergen is not present in many soybean accessions; in fact, testing showed that 80% of Japanese soybean varieties did not contain Gly m Bd 28K.³⁶ For this reason, the majority of soybean allergen research has concentrated on the immunodominant allergen P34, considered the major and most studied allergenic protein in soybean.

The Immunodominant Soybean Allergen: Gly m Bd 30K/P34

Of the numerous allergens present in soybean, Gly m Bd 30K, also known as P34 or Gly m 1, has been classified as the immunodominant allergen.^{9,10,28,32,35–41} Sixty-

five percent of soy-sensitive patients with atopic dermatitis exhibit an allergenic response to this protein.^{9,10,37,38,41,42} The N-terminal amino acid sequence and the amino acid composition of Gly m Bd 30K and P34 are identical and therefore considered similar proteins with interchangeable denomination.⁴³ P34 also shares 30% sequence homology to Der p 1, a dust mite allergen.⁴¹

P34 is a monomeric, insoluble glycoprotein consisting of 257 amino acid residues attached by disulfide linkages in the 7S globulin protein fraction, and may play a role in protein folding.^{36,39,44} P34 is present predominantly in the seed cotyledon, which becomes the leaves of the plant embryo and associates with the oil body after cell lysis.^{45,46} Oil bodies are small organelles that hold the reserve oils of seeds and consist mainly of triglycerides, phospholipids, and a few polypeptides.

P34 has been previously characterized as an outlying member of the papain superfamily of cysteine proteases. It is post-translationally derived from a 46- to 47-kD precursor protein by the partial removal of 122 N-terminal amino acid residues from the carboxyl side of an asparagine residue—probably by a thiol protease in

the developing seeds—and its tertiary conformation is consistent with the papain family.^{9,35,43,44} P34 exhibits unique properties in that it possesses a glycine substitution at the position 38 cysteine amino acid in the active site, unlike all of the other proteases of this family. While other proteins of this family exhibit enzymatic activity, the absent catalytic action of cysteine suggests that its allergenicity may be structural in nature rather than induced by enzymatic activity.^{38,44}

In terms of biological function, P34 may act as a defense protein against *Pseudomonas*, a gram-negative bacteria affecting many plants, by binding syringolides secreted by the bacteria that normally trigger defense responses in the affected host. It has been reported that soybean cultivars with higher levels of P34 are resistant to this bacteria and may serve as a receptor to mediate syringolide signaling.⁴⁵

Previous epitope mapping using recombinant technology has elucidated a minimum of 12 distinct linear epitopes on the P34 protein. Five of these have been synthesized and characterized: amino acids 3-12, 100-110, 229-238, 299-308, and 331-340.^{9,47} Approximately 30% of the amino acids in the peptides are aspartic and glutamic acids, histidine, lysine, and arginine.¹⁰ Kalinski et al.⁴⁴ published a deduced amino acid sequence using cloned DNA similar to P34¹⁷ (Figure 1). Although there is a large amount of diversity in the immunodominant IgE-binding epitopes, it appears that substituting alanine at a single site may reduce or eliminate binding to some patients' sera.³⁷ Amino acid sequence analysis showed that its sugar chain (mannose, N-acetylglucosamine, fucose, and xylose at a molar ratio of 3:2:1:1) binds to the asparagine residue, and suggests that the fucosyl group may serve as an additional epitope.^{36,39} α -1,3-Fucosylated glycoconjugates can elicit histamine release from mast cells, which suggests that glycans can contribute to an allergic reaction.^{48,49} Wilson et al.⁵⁰ conducted an exhaustive survey of N-glycans in foods, and found that soy showed extensive binding to anti-horseradish peroxidase using the core α -1,3-linked fucose-specific monoclonal antibody. In addition, asparagine-linked oligosaccharides are a probable source of carbohydrate-mediated cross-reactions between foods.⁵¹

Degradation of P34

Table 2 summarizes the various processing methods that have been utilized in the reduction of soy protein allergenicity. P34 protein remains allergenic even under adverse chemical treatments such as treatment with 2-mercaptoethanol and 4 M urea.³⁹ Only about 62% of P34 was separated into the precipitate of soy milk containing NaCl by ultracentrifugation in the presence of 100 mM 2-mercaptoethanol. These results further substantiate the claim that P34 is associated with β -conglycinin (found in

the supernatant) through disulfide bonds, which can be broken by a high concentration of 2-mercaptoethanol.⁵²

In 1994, Samoto et al.⁵³ developed a procedure for the simple and efficient removal of P34 by salting out the protein with 1 M Na₂SO₄ in an acidic environment (pH = 4.5) with centrifugation. This process resulted in soy milk with a 90% reduction in P34 and no significant loss of ingredient functionality when used in the production of common soybean protein products, such as tofu. This study did not test for any other allergens, nor did it determine the nutritional and sensory profile, such as total protein and isoflavone content, digestibility, viscosity, taste, and appearance, of the subsequent product.

There are few soybean cultivars naturally lacking P34; this mandates that food scientists engineer novel processes to eliminate this immunodominant allergen. Current studies have focused on manipulating the protein structure as a potential method to reduce its allergenicity. Studies have indicated that not only is P34 highly resistant to vigorous treatments and important for the immunity of soybeans, but also that it may be coded by a single gene representing merely 2% to 3% of the total protein content.^{10,39,45} Consequently, if removed, the nutritional value of the soy protein may not be compromised.

In summary, soybeans are processed in a number of ways, some of which affect the allergenic potential of P34. Overall, the processing techniques that have been shown to reduce allergenicity do so by manipulating the protein structure in a particular way; altering P34's structure appears to make it less available to antibody receptors.

Heat Treatment

Heat treatment, which is used widely in food production, causes protein denaturation and is responsible for the reduction in inhibitory capacity of soybean protease inhibitors such as the Kunitz trypsin inhibitor and the chymotrypsin and trypsin Bowman-Birk inhibitor. However, because of the complex structure of the number of epitopes present in P34, it is unlikely that heat alone will denature the protein sufficiently to reduce allergenicity. In fact, the IgE-binding activity of P34 has been reported to be enhanced by autoclave treatment that involves superheated steam.⁵⁴ Therefore, coupling heat treatment with a structure-modifying element, such as chemical modifications, may be more beneficial in the reduction of the antinutritional properties of soybean.¹²

Sulphydryl-disulfide interchange using a heat treatment in the presence of cysteine has been used to modify classic inhibitors found in soybean (Kunitz trypsin inhibitor, Bowman-Birk inhibitor) by impairing their ability to complex with trypsin and proteolytic enzymes, thereby minimizing the undesirable inhibitory effects. The resulting soybean protein chains show an increase in cysteine content as well as a reduction in glycinin and

P34 deduced amino acid sequence	M G F L V L L L F S L L	
Amino acid number	120	
GGT CTC TCT TCT AGT TCC AGC ATA TCA ACT CAT CGT TCC ATA TTG GAC CTT GAC CTA ACC		98
G L S S S S S I S T H R S I L D L D L T		
110	100	
AAG TTT ACC ACA CAG AAA CAG GTG TCT TCA CTG TTC CAA CTA TGG AAG AGT GAG CAT GGA		158
K F T T O K O V S S L F Q L W K S E H G		
90	80	
CGT GTC TAC CAT AAC CAC GAA GAA GAG GCA AAG AGA CTT GAG ATT TTC AAG AAT AAC TCG		218
R V Y H N H E E E A K R L E I F K N N S		
70	60	
AAC TAT ATC AGG GAC ATG AAT GCA AAC AGA AAA TCA CCC CAT TCT CAT CGT TTA GGA TTG		278
N Y I R D M N A N R K S P H S H R L G L		
50	40	
AAC AAG TTT GCT GAC ATC ACT CCT CAA GAG TTC AGC AAA AAG TAC TTG CAA GCT CCC AAG		338
N K F A D I T P Q E F S K K Y L Q A P K		
30	20	
GAT GTG TCG CAG CAA ATC AAA ATG GCC AAC AAG AAA ATG AAG AAG GAA CAA TAT TCT TGT		398
D V S Q Q I K M A N #K K M K K E Q Y S C		
10	1	
GAC CAT CCA CCT GCA TCA TGG GAT TGG AGG AAA AAA GGT GTC ATC ACC CAA GTA AAG TAC		458
*D H P P A S W D W R K K G V I T Q V K Y		
20		
CAA GGG GGC TGT GGA AGG GGT TGG GCG TTT TCT GCC ACG GGA GCC ATA GAA GCA GCA CAT		518
Q G G C G R G W A F S A T G A I E A A H		
40		
GCA ATA GCA ACA GGA GAC CTT GTT AGC CTT TCT GAA CAA GAA CTC GTA GAC TGT GTG GAA		578
A I A T G D L V S L S E Q E L V D C V E		
60		
GAA AGC GAA GGT TCT TAC AAT GGA TGG CAG TAT CAA TCG TTC GAA TGG GTT TTA GAA CAT		638
E S E G S Y N G W Q Y Q S F E W V L E H		
80		
GGT GGC ATT GCC ACT GAT GAT GAT TAT CCT TAC AGA GCT AAA GAG GGT AGA TGC AAA GCC		698
G G I A T D D D Y P Y R A K E G R C K A		
100		
AAT AAG ATA CAA GAC AAG GTT ACA ATT GAC GGA TAT GAA ACT GTA ATA ATG TCA GAT GAG		758
N K I Q D K V T I D G Y F T L I M S D E		
120		
AGT ACA GAA TCA GAG ACA GAG CAA GCG TTC TTA AGC GCC ATC CTT GAG CAA CCA ATT AGT		818
S T L S E T E Q A F L S A I L E Q P I S		
140		
GTC TCA ATT GAT GCA AAA GAT TTT CAT TTA TAC ACC GGG GGA ATT TAT GAT GGA GAA AAC		878
V S I D A K D F H L Y T G G I Y D G E N		
160		
TGT ACA AGT CCG TAT GGG ATT AAT CAC TTT GTT TTA CTT GTG GGT TAT GGT TCA GCG GAT		938
C T S P Y G I N H I V L L V G Y G S A D		
180		
GGT GTA GAT TAC TGG ATA GCG AAA AAT TCA TGG GGA GAA GAT TGG GGA GAA GAT GGT TAC		998
G V D Y W I A K N S W G E D W G E D G Y		
200		
ATT TGG ATC CAA AGA AAC ACG GGT AAT TTA TTA GGA GTG TGT GGG ATG AAT TAT TTC GCT		1058
I W I Q R N T G N L L G V C G M N Y F A		
220		
TCA TAC CCA ACC AAA GAG GAA TCA GAA ACA CTG GTG TCT GCT CGC GTT AAA GGT CAT CGA		1118
S Y P T K E F S I T L V S A R V K G H R		
240		
AGA GTT GAT CAC TCT CCT CTT TGA AGCGTAAAA GGTTT AATAC AACGAGTGCT TGTTCCTTA		1181
R V D H S P L stop		
257		
GGACAAGCATGTACTTATGTATGATTCTGTGTAACCATGTCTCCACGTTGTACTAATGTGAAGG		1249
CAAAAATAAAA CACACAACAAGTTCGTTTTCTCAAT poly A tail of 63 As		1350

Figure 1: Complete cDNA sequence and predicted amino acid sequence of P34. The underlined protein sequence indicates the position and sequence of the amino terminus as directly determined. The number sign indicates the position of the P34 amino terminus. The asterisk indicates the position of the P32 amino terminus resulting from the developmentally regulated processing of P34 during seedling growth. The italic sequence indicates the position of the consensus polyadenylation sequence. Figure adapted from Kalinski et al.⁴⁴

Table 2. Reduction in the Allergenicity of Soy Proteins

Product	Process	Reduced Allergenicity	Remaining Allergenicity (other than P34)	Reduced P34	Functionality	Nutritional Profile	Reference
Soy milk	1 M Na ₂ SO ₄ at pH 4.5	Yes	NT	>80%	NT	NT	Samoto et al. ⁵¹
Soy flour	Disulfide bond modification	Yes (glycinin)	NT	NT	NT	Improved	Friedman et al. ¹²
Mold-hydrolyzed soy sauce, tempeh, miso	Fermentation	Yes	Yes (RAST inhibition <50%)	NT	Yes	NT	Herian et al. ⁵³
Soybean-koji miso	Koji fermentation	Yes	NT	100% after 3 months mixing	Yes	NT	Tsuji et al. ⁵⁵
Natto	<i>Bacillus natto</i> fermentation	Yes	NT	100% after 24 hrs.	Yes	NT	Yamanashi et al. ⁵²
“Boiled beans”	Enzymatic hydrolysis Proleather (250 units) and Protease N (5000 units)	Yes	NT	Yes	Negative	NT	Yamanashi et al. ⁴¹
Soy protein isolate	Enzymatic hydrolysis Proleather FG-F (20 units/g isolate)	Yes (β-conglycinin)	Yes (glycinin)	NT	Yes	Yes	Tsumara et al. ⁵⁷
Acid-hydrolyzed soy sauce	Galactomannan conjugate	Yes	NT	ELISA value < 0.05	Improved	NT	Babiker et al. ²⁷
Soy milk	Tohoku 124 + 1M Na ₂ SO ₄ at pH 4.5	Yes (α-subunit of β-conglycinin 28 kD)	Yes (α' subunit of β-conglycinin)	99.8%	NT	NT	Samoto et al. ⁵⁹
Soy protein	Silenced P34 gene	Yes	NT	100%	NT	Yes	Herman et al. ³⁷
Soybean seeds	Mutant line QF2	Yes (glycinin, β-conglycinin)	Yes (β subunit of β-conglycinin)	Increased	NT	NT	Takahashi et al. ⁶⁰
	Nitrogen fixation	Yes (β-subunit of β-conglycinin, glycinin)	NT	NT	NT	NT	Paek et al. ⁶⁶
	Glutathione application	Yes (β-subunit of β-conglycinin)	Yes (glycinin)	NT	NT	NT	Awazuhara et al. ⁶⁷
Texturized soy protein	Extrusion at high temperature	NT	NT	17–21 mg P34/g N	Yes	NT	Tsuji et al. ⁷⁰
		Yes	Yes (glycinin)	Yes	Yes	NT	Franck et al. ⁶⁰

NT = Not tested.

increased digestibility. In this study, tests for P34 allergenicity were not conducted. This technique may be useful only to denature inhibitors with disulfide bonds. Because P34 is not a protease inhibitor and possesses a glycine substitution for a cysteine amino acid, it is possible that this procedure would not effectively denature this immunodominant allergen.¹² Further specific experimentation with thermally induced disulfide interchange and P34 needs to be conducted.

Fermentation

Fermentation has been investigated with regard to its effect on the allergic potential of soybean proteins. Fermentation hydrolyzes proteins into smaller peptides that may cause less allergenicity. Proteases from bacteria used in the process appear to be responsible for the reduction. This suggests that the type of bacteria used in the fermentation process can play an integral role in determining which hydrolyzed proteins are present and whether they retain the necessary conformation to be recognized by antibodies.

The radioallergosorbent test, or RAST, is the standardized method to detect IgE responses to allergens. Herian et al.⁵⁵ used RAST to compare the allergenicity of five traditional soy-based foods, including soybean sprouts, soy sauce (both acid-hydrolyzed and mold-hydrolyzed), tempeh, and miso. If specific IgEs to the allergen of interest are found in a patient's sera, they will bind to the solid phase. In this case, a raw soybean solid phase was prepared by coupling raw soybean extract to cyanogen bromide-activated microcrystalline cellulose particles, combining with serum from soy-allergic subjects, and incubating overnight. Next, labeled rabbit anti-human IgE was added to the solid phase and the radioactivity was determined in a gamma scintillation counter. Results were expressed as percent binding. All products were found to be comparable to raw soybean in their ability to bind with the IgE of allergic patients' sera in the RAST test. This indicates that all products possessed, to some extent, allergenic soy proteins or fragments, in some cases after extensive processing.

It is interesting that mold-hydrolyzed soy sauce exhibited a much lower inhibitory percentage than its acid-hydrolyzed counterpart, which suggests that the mold component of hydrolysis plays an important role in reducing the allergenic protein content. The fermented soy products (tempeh, miso, and mold-hydrolyzed soy sauce) presented significantly less inhibition than their non-fermented counterparts.⁵⁵ This study validates the claim that fermented soy products retain less allergenicity than non-fermented soy products. The allergens responsible for the allergic response were not identified; the information gleaned from this study may implicate highly resistant soybean allergens, including P34.

Several more recent studies also substantiate the

claimed hypoallergenicity of fermented soy products.^{33,38,59} Tsuji et al.⁶⁰ found that soybean-koji miso (fermented soy paste) showed no P34 immunoreactivity in the sera of soybean-sensitive patients. Yamanashi et al.⁵⁷ simulated natto (traditional Japanese fermented soybeans) fermentation with autoclaved soybeans soaked in water overnight and inoculated with *Bacillus natto* (0.8 mg/g soybean). After 24 hours of fermentation, the product obtained did not bind with the monoclonal antibody against P34 or with soy-allergic patients' sera. All proteins were hydrolyzed (molecular weight < 10 kD), and the subsequent peptides showed no immunoreactivity. This study suggests that proteases particular to *B. natto* digested the soy proteins into peptides that could not be recognized by antibodies.⁵⁴

Fermentation has the potential to be utilized in creating a hypoallergenic soy protein, particularly with respect to P34. However, careful consideration must be given to the type of bacterial protease used and the conditions under which the fermentation is performed. The ability of this process to hydrolyze soy protein into smaller peptides may also reduce the adverse nutritional effect of indigestibility.⁵⁸ Although a hypoallergenic soy product may be produced through fermentation, the characteristic flavor of fermented soybeans is not universally well accepted. Further studies regarding hypoallergenic soybeans may also need to focus on ways to manipulate the flavor of these products in order to make them not only toxicologically safe, but also more palatable.

Enzymatic Hydrolysis

Enzymatic hydrolysis is an effective procedure to inactivate P34 allergenicity. Compared with other proteases, Proleather, and, to a lesser extent, Protease N (both proteases produced by *Bacillus subtilis*), were found to markedly decompose P34. It could not be detected by immunoblotting analysis with monoclonal antibody when using more than 250 units of Proleather or 5000 units of Protease N per gram of autoclaved soybean.⁴² However, these proteases are not specific to P34 and, as a result, all proteins will be hydrolyzed in the process of destroying this major allergen. The subsequent products did not retain any gel-forming ability for processing, and no information was given concerning the amino acid profile, total protein, or isoflavone content.⁵⁹

Tsumura et al.⁵⁹ performed selective enzymatic digestion using Proleather FG-F, an alkaline protease from *B. subtilis*, to hydrolyze P34, Gly m Bd 28K, and β -conglycinin to produce tofu with adequate gelation properties and no reactivity with patients' sera containing P34-specific IgE antibodies. The P34 level was effectively reduced by 99.2%. The most effective environment for Proleather FG-F (20 units/g soy protein isolate) degradation of β -conglycinin and P34 (as evidenced by SDS-PAGE against monoclonal antibodies)

was found to be 70°C at pH 7.0 using native soy protein isolate as substrate. However, glycinin could not be removed due to a different denaturation temperature at neutral pH. Glycinin is thought to aid in the gelation of soy protein, and its inclusion seemed to preserve the functionality of the product. No data were provided on the reactivity of patients' sera to the glycinin, Gly m Bd 28K, or β -conglycinin allergenic components.⁵⁹

Carbohydrate Conjugation

Galactomannan, prepared from guar gum, was found to mask the antibody-recognizing structure of P34, thus eliminating its allergenic potential. Acid-precipitated soy protein was conjugated with galactomannan through a Maillard reaction between amino groups in acid-precipitated soy protein and the reducing end carbonyl group in galactomannan. When this conjugate was subjected to SDS-PAGE and immunoblotting with both monoclonal antibodies and human sera of allergic patients, no bands were found to indicate cross-reactivity. The functional properties were not affected by the treatment; in fact, the subsequent product had improved solubility, heat stability, and emulsifying properties.²⁸ However, no data were presented regarding protein quality, isoflavone content, or allergic potential due to soybean allergens other than P34. Further studies are needed to investigate the presence of other allergens and examine the product's behavior after in vitro or in vivo digestion.

Genetic Modification

A P34 protein of soybean has been expressed in *E. coli* using a pET expression system providing a model system for the production of this allergenic substance by bacterial fermentation. This may facilitate the implementation and evaluation of new methods to eliminate or counteract the allergenic effect of P34.³⁹

Tohoku 124, a mutant line of soybean, has been induced by irradiation and chemical breeding to lack Gly m Bd 28K, the α and α' subunits of β -conglycinin, and to reduce the β subunit of β -conglycinin.⁶⁰ Defatted soy milk from this mutant soybean can be manipulated with a physicochemical treatment involving a reducing agent (Na_2SO_4) and optimal pH to remove 99.8% of the P34.⁶¹ A preliminary trial found that 80% of soybean-sensitive patients could ingest these products without adverse reactions. However, these proteins retain no gel-forming ability for use in traditional products and no data were available as to the subsequent nutritional quality of the soybean.³⁵

A genetically engineered soybean silenced for the P34 gene has been developed. Such a product lacks any other manipulation except for the elimination of this allergen.³⁸ Takahashi et al.⁶² have generated a soybean mutant line, QF2, whose seeds lack both glycinin and β -conglycinin, both of which have been implicated in

IgE-mediated soybean allergenicity. However, the subsequent product exhibits an abundance of other proteins and free amino acids to compensate for the nitrogen stored in the protein components that were removed. These overabundant proteins include P34, the immunodominant allergen in soybean.⁶² This study elucidates novel information about the storage of nitrogen in soybean; however, this mutant line presents obvious issues with regard to allergenicity.

Genetic modification has been subjected to considerable scrutiny, despite the fact that GM crops have been a part of the American diet for many years.⁶³ GM glycophosphate-tolerant soybean seeds are equivalent to their non-GM counterparts.⁶⁴ Genetic modification may result in the introduction of new proteins. As the majority of allergens are proteins, clearly these novel proteins have the potential to be allergenic. This is especially true if the transgenic material originates from a known allergenic source. For example, methionine-rich albumin from Brazil nuts was introduced into soybeans to compensate for a deficiency in this essential amino acid. Immunoblotting of sera from subjects allergic to Brazil nuts revealed that serum IgE recognized the allergen in transgenic soybeans.⁶⁵ As a result, this product was not commercialized.⁶³ In 1996, the International Food Biotechnology Council, in collaboration with the International Life Sciences Institute, introduced a systematic hierarchical approach to assess the potential allergenicity of transgenic products.^{66,67} This approach has been implemented by genetic engineers in order to prevent the introduction of previously described or novel allergens into transgenic crops.

Agronomic Nutrition

The nutritional supplementation of soybeans during cultivation in greenhouses has been studied with regard to its effect on protein composition. Nitrogen fixation decreases the sulfur-poor β -subunit of β -conglycinin and, to a lesser degree, glycinin, which improves the protein seed composition.⁶⁸ Another example is the application of both reduced and oxidized glutathione, which decreases the accumulation of the sulfur-deficient β -subunit of β -conglycinin and increases the glycinin component.⁶⁹ The β -subunit of β -conglycinin showed enhanced accumulation when soybean plants were supplemented with nitrogen.⁷⁰ The information gleaned from these studies could be used to develop a crop with less of this sulfur-deficient, and often allergenic, protein in soybeans. Experimentation with respect to allergenicity has yet to be conducted.

Extrusion

Extrusion is a common food processing method that has recently been investigated with regard to its impact on allergenicity, namely in texturized soy protein, which

can be used as an inexpensive meat extender, constituting up to 12% of products such as meatballs and Salisbury steak. Tsuji et al.⁵⁶ found that meatballs and beef croquettes supplemented with soy derivatives contained 17 and 21 mg P34 per gram of nitrogen, respectively. Texturized soy protein is produced by extruders that manipulate soy ingredients using pressure and temperature to make them flow (allowing the protein molecules to align), expand, and then collapse to produce a meat-like texture.⁷¹ A study comparing various soy-protein-containing products suggested that the processes used to texturize soy protein could also eliminate P34. Soybean-sensitive patients' sera was found to react with the 38- and 50-kD proteins in texturized soy protein, which may correspond to the acidic and basic chains of G1 glycinin, respectively. Although a 31- to 34-kD band was observed in the texturized product, it did not bind IgE, suggesting that P34's binding ability was eliminated during the extrusion process.⁷²

Hydrolysates

Hydrolysates are predicted to show no reactivity with allergen-specific IgE. Results from ELISA tests show that protein hydrolysates have substantially lower immunogenicity than their parent proteins.⁷³ Soy protein hydrolysates are made by heat and enzymatic hydrolysis of soybeans and converted into a mixture of amino acids. The nutritional value does not seem to be affected by this processing; however, the safety of these hydrolysates is dependent upon the degree of hydrolysis and the allergenic fragments subsequently present.^{5,74} In fact, small amounts of native proteins from which the product is derived may be present in hydrolyzed formulas, indicating that these surviving proteins or protein fragments could potentially cause an allergic response, even triggering an anaphylactic reaction.^{2,75,76} Soy formulas are often indicated when an infant is allergic to cow's milk; however, there is evidence that 8% to 14% of infants with cow's milk allergy are also reactive to soy formulas.^{77,78} Ahn et al.⁷⁹ studied the prevalence of soy protein hypersensitivity in children with cow's milk allergy; 18.3% of 224 children were found to have positive soy-specific IgE. Hydrolyzed formulas may be potentially useful for preventing allergic symptoms, but further studies are needed to evaluate this possibility.⁷⁷

Most studies that have been conducted on soy protein hydrolysate formulas focus on cow's milk allergy, not soy protein allergy. The subsequent soy protein hydrolysate formulas are less allergenic than cow's milk but still remain somewhat allergenic to those with cow's milk allergy; however, allergenicity varies with the degree of hydrolysis and the particular allergy from which the patient suffers. Rice-hydrolyzed proteins may be beneficial for those with allergies to both cow's milk and soy.⁸⁰ Further clinical trials conducted with soy protein

hydrolysates should focus on the reactions they produce in those suffering from soy protein allergy specifically, and should investigate the role of P34 in those allergic reactions.

Conclusion

Soybean processing can result in the reduction of soybean allergenicity, particularly of P34, and can be achieved by denaturation, hydrolysis, or conjugation. Nevertheless, there is no single procedure (other than gene knockout) that completely eliminates P34 allergenicity. For example, heat alone is not effective but becomes more so when combined with chemical treatment, and the effect of fermentation depends upon the degree of hydrolysis and the type of microorganism employed. It is possible that combined treatments can yield better results than a single approach. To date, no comprehensive information exists about the relationship between the secondary structure of P34, its manipulation by processing, and allergenicity. However, the integrity of the structure seems to play an important role. Further research is needed to thoroughly evaluate the effect of processing P34 and other soybean allergens on remaining allergenicity and on its nutritional implications.

There are over 21 identified allergenic proteins in soybean, and this presents a challenge to food scientists and geneticists, who need to develop a process to eradicate immunodominant allergens while maintaining functionality, nutritional value, and efficacy in the subsequent soybean product. The Food Allergen Labeling and Consumer Protection Act of 2004 will require FDA-regulated food ingredient statements to identify a major food allergen, such as soybean, in any food product, ingredient, flavoring, coloring, or incidental additive. The food allergen labeling requirements are scheduled to take effect on January 1, 2006.

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