

This article was downloaded by: [Advanced Institute of Sanita]
[Advanced Institute of Sanita]

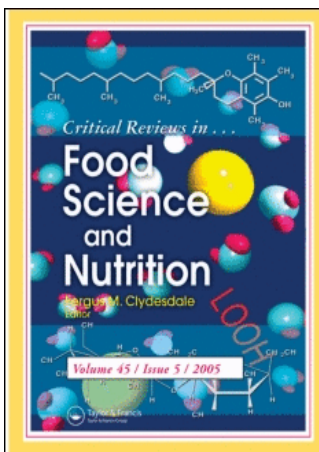
On: 21 February 2007

Access Details: [subscription number 769486267]

Publisher: Taylor & Francis

Informa Ltd Registered in England and Wales Registered Number: 1072954

Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Critical Reviews in Food Science and Nutrition

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title-content=t713606380>

Allergenicity of Soybean: New Developments in Identification of Allergenic Proteins, Cross-Reactivities and Hypoallergenization Technologies

To link to this article: DOI: 10.1080/10408390600626487

URL: <http://dx.doi.org/10.1080/10408390600626487>

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article maybe used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

© Taylor and Francis 2007

Allergenicity of Soybean: New Developments in Identification of Allergenic Proteins, Cross-Reactivities and Hypoallergenization Technologies

LAMIA L'HOCINE and JOYCE I. BOYE

Food Research and Development Centre, Agriculture and Agri-Food Canada, St-Hyacinthe, Quebec, Canada

Soybean is considered one of the “big eight” foods that are believed to be responsible for 90% of all allergic reactions. Soy allergy is of particular importance, because soybeans are widely used in processed foods and, therefore, represent a particularly insidious source of hidden allergens. Although significant advances have been made in the identification and characterization of soybean allergens, scientists are not completely certain about which proteins in soy cause allergic reactions. At least 16 allergens have been identified. Most of them, as with other plant food allergens, have a metabolic, storage, or protective function. These allergens belong to protein families which have conserved structural features in relation with their biological activity, which explains the wide immunochemical cross-recognition observed among members of the legume family. Detailed analysis of the structure-allergenicity relationships has been hampered by the complexity and heterogeneity of soybean proteins. A variety of technological approaches have been attempted to decrease soybean allergenicity. This paper provides a comprehensive review of the current body of knowledge on the identification and characterization of soybean allergens, as well as an update on current hypoallergenization techniques.

Keywords soybean, allergens, allergenicity, allergenic proteins, cross-reactivity, hypoallergenization

INTRODUCTION

The soybean (*Glycine max*), often called the miracle crop, is today the world's foremost provider of protein and oil. Probably native to China, it provided food and oil for Asian countries for thousands of years before its introduction to the rest of the world in the twentieth century. In western countries the history of soybeans for human consumption dates back only several decades; yet today, more soybeans are grown in the United States than anywhere else in the world. World soybean production, in 2004, reached 217 million metric tons, and soybean meal represented 67% of the world total protein meal consumption.¹

Since the 1960s, new soy protein products have been developed, such as soy flour, flakes, grits, soy protein concentrate, and soy protein isolate. Due to their excellent nutritional and

functional properties, such as water and fat absorption, emulsification, foaming, gelation, and binding, these protein products are now widely used (Fig. 1) as ingredients in formulated foods, meat and poultry products, bakery and pastry products, dairy products, and edible spreads, etc.² Soybeans are also being used today in a variety of cosmeceutical, pharmaceutical, and industrial applications (Fig. 2). One of the difficult problems limiting the expanded use of soy proteins products is the presence of allergenic proteins; soybean is included in the “big eight” foods that are believed to be responsible for 90% of all food allergies.^{3,4}

The terminology surrounding food allergy (hypersensitivity) as opposed to food intolerance has been very confusing.^{5–7} A generally accepted definition of food allergy, in North America, is that it is an abnormal immunological (usually an immunoglobulin E (IgE)-related) reaction resulting from the exposure (ingestion, skin contact, or inhalation) to exogenous food macromolecules (generally proteins) known as allergens or antigens.^{4,6} This reaction results in a marked increase in reactivity to that

Address correspondence to Joyce I. Boye, Food Research and Development Centre, Agriculture and Agri-Food Canada, 3600 Casavant Blvd. West, St-Hyacinthe, J2S 8E3, Quebec, Canada. E-mail: boyej@agr.gc.ca

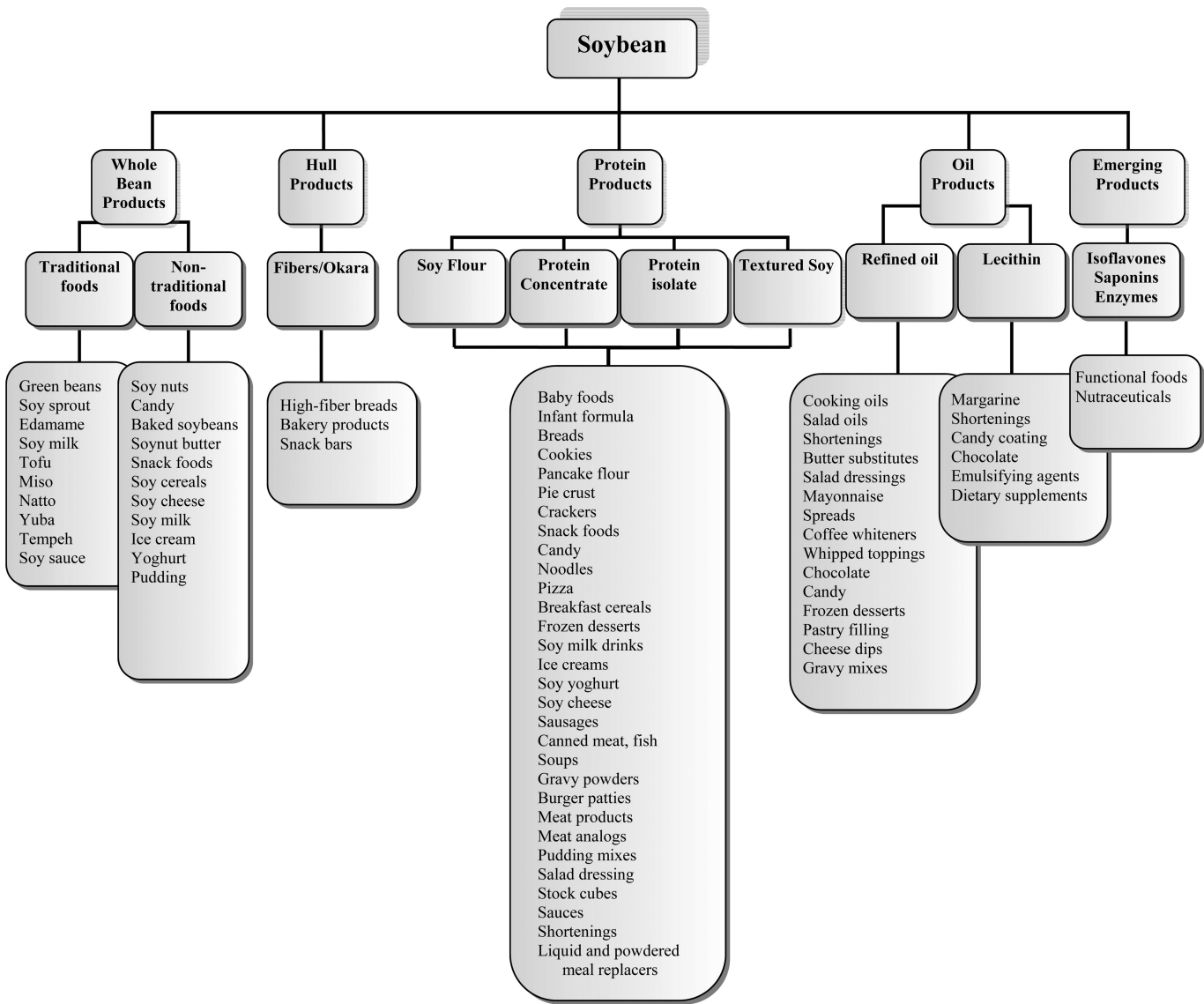


Figure 1 Soybean foods and ingredients.

allergen on subsequent exposures, resulting in adverse effects. Food intolerance, on the other hand, is defined as a reproducible non-immune-mediated reaction to an ingested food or food component. It can include idiosyncratic, metabolic, or toxic responses to food.⁴ The European Academy of Allergy and Clinical Immunology (EAACI) uses the term intolerance as an umbrella term for allergic and non-allergic reactions, as is common practice in UK.⁵ In this review, we adopt the North American definition and use the term allergy to refer to immune-mediated hypersensitivity.

The exact prevalence of soy allergy in the general population is unknown and is likely to be dependent on local feeding habits and exposure; however, a prevalence rate of 0.3–0.4% in the general population has been quoted.⁸ Soy allergy appears to affect 6% of atopic children and 14% of children with cow's milk allergy.⁹ Estimates of the threshold levels for triggering adverse reaction in soy allergic individuals vary widely and have been

reported to vary from 0.0013 mg to 500 mg.⁸ Clinical manifestations of soy allergy are similar to other food allergies, ranging from severe enterocolitis, to atopic eczema, and immediate IgE-mediated systemic multi-system reactions. Although severe reactions to soy are rare compared to reactions to peanuts, tree nuts, fish, and shellfish, "fatal events" after eating soy have been reported.¹⁰ Anaphylactic reactions to bread, pizzas, or sausage extended with soy protein date back at least to 1961.¹¹ Subsequent studies have confirmed that the risk may be rare but very real.^{12–16}

Soy allergy is of particular importance, because soybeans are widely used in processed foods and, therefore, represent a particularly insidious source of hidden allergens. Their avoidance in the diet can be very challenging; especially, when the regulation for labelling of allergens, as in many countries, covers only allergens present in ingredients but not hidden allergens in compound ingredients.¹⁷ For example, if margarine is added to a food

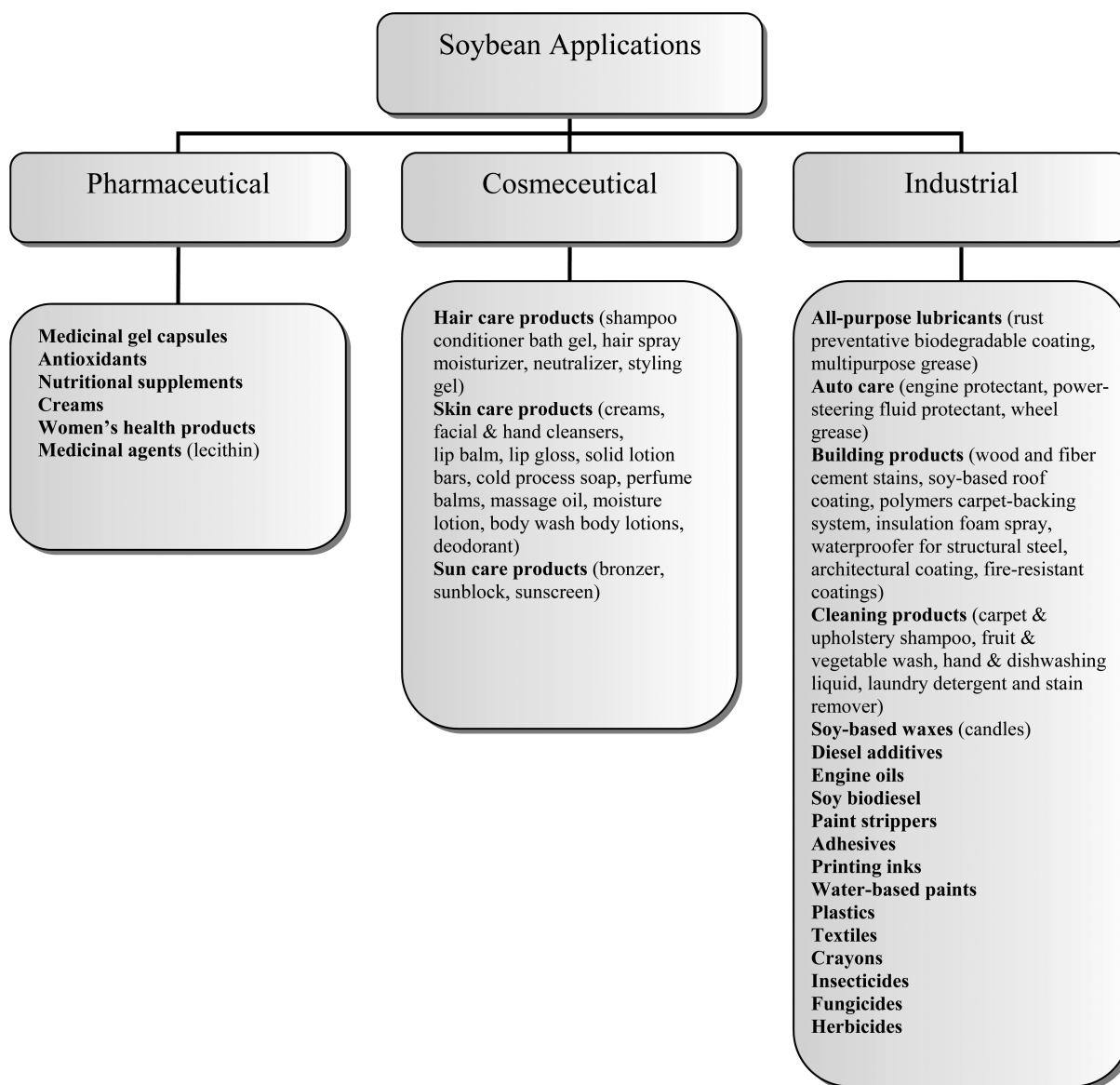


Figure 2 Pharmaceutical, cosmeceutical and industrial applications of soybeans.

product it will be listed as such, but soy present in the margarine itself may not be listed on the ingredients panel. Regulations for food allergen labelling are changing around the world (eg. North America and Europe), which should offer greater protection for allergic consumers.

Several laboratories have reported immune system responses to multiple fractions of soy protein, with no fraction being the most consistently antigenic.^{18–21} At least 16 allergenic proteins have been detected that bind to IgE of sera of soybean-sensitive patients. The identification and characterization of soybean allergens are being carried out by several research groups. While a few reviews of the dominant allergens exist in the literature,^{22,23} significant advances have been made in the last few years in the characterization of these allergens and in the identification of new allergenic proteins. The present paper provides a comprehensive review of the current body of knowledge on the identi-

fication and characterization of soybean allergens, as well as an update on current hypoallergenization techniques.

SOYBEAN PROTEINS

Since the allergenicity of soybean essentially resides in the protein fraction, it is important to provide a brief overview of the composition of the major soybean protein fractions. Approximately 90% of the soybean seed storage proteins are salt-soluble globulins, with the remainder being water-soluble albumins. Analytical ultracentrifugation studies of soy proteins have revealed four fractions with approximate Svedberg coefficients of 2S, 7S, 11S, and 15S.^{24,25} These fractions are not homogeneous but rather, mixtures of proteins (Fig. 3). They have been studied to different degrees and have been characterized.

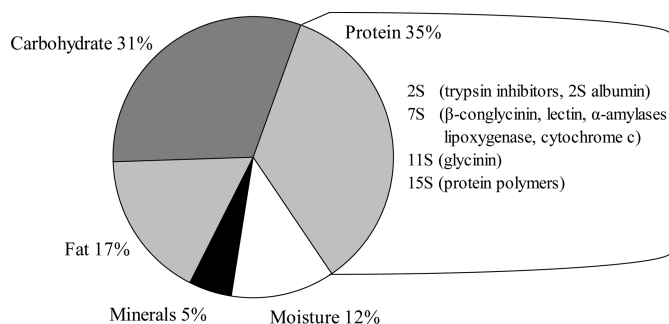


Figure 3 Average composition of soybeans.

2S Fraction

The 2S fraction contains from 8 to 22% of the extractable soybean protein and consists of a number of enzymes.²⁶ The fraction is composed of proteins with a variety of molecular weights. Probably the most studied proteins of the 2S fraction are the trypsin inhibitors: the Bowman-Birk inhibitor which has a molecular weight of 7.8 kDa and the Kunitz inhibitor with a molecular weight of about 21.5 kDa. The soybean Kunitz trypsin inhibitor (KTI) is a small monomeric and non-glycosylated protein that was first isolated and crystallized from soybean by Kunitz.²⁷

7S Fraction

The 7S fraction of soy proteins comprises about 35% of the soluble proteins. It contains the storage proteins β-conglycinin (7S globulin), which make up about 85% of the total 7S fraction. Cytochrome *c* (12 kDa), β-amylase (62 kDa), lipoxygenases (102 kDa), and lectins (hemagglutinins; 120 kDa), also occur in the 7S fraction.²⁶

β-conglycinin has been studied extensively. It is now generally agreed that β-conglycinin is a glycoprotein with a molecular weight range from 140 to 170 kDa. It is a trimer composed of three prevalent types of subunits, α, α', and β, which have molecular masses of 76, 72, and 53 kDa, respectively.²⁸ Another subunit, named β', is present only in some soybean varieties.^{29,30} All of the β-conglycinin subunits are glycoproteins and contain 4–5% carbohydrate.³¹ In addition, β-conglycinin undergoes a complicated association-dissociation phenomenon in response to changes in ionic strength and pH. According to Thanh and Shibasaki, at low ionic strength, the six forms of trimers are able to reversibly dimerize.³² The resulting 9S form is a superdimer of trimers becoming a hexamer with a molecular weight of about 280 to 350 kDa. Furthermore, native β-conglycinin (7S globulin) is heterogeneous in regard to subunit composition and six among the 10 possible combinations have been demonstrated to occur.²⁴ These are: ααα, ααα', ααβ, αα'β, αββ, and α'ββ.

11S Fraction

The 11S fraction comprises 31–52% of the soluble soy protein with about 85% of the total being 11S globulin. The 11S globulin is also known as glycinin and it accounts for over 40% of the total seed globulin.³³ In contrast to β-conglycinin, only a small portion of glycinin is glycosylated.³⁴ The currently accepted model of native glycinin is a hexamer with MW of about 320 to 360 kDa. Each of its subunits (58–69 kDa) can be dissociated under reducing conditions into acidic (A; 31–45 kDa) and basic (B; 18–20 kDa) polypeptide chains, that are linked through inter-chain disulfides.²⁶ Five major subunits have been characterized,^{35,36} namely A1aB2 (G1), A1bB1b (G2), A2B1a (G3), A3B4 (G4), and A5A4B3 (G5). The acidic polypeptide A5 had an exceptionally low MW of 10 kDa. Glycinin has a complex quaternary structure consisting of two layers of trimers. Each trimer has its three acidic and three basic polypeptides paired and held together by disulfide and hydrogen bonds, with acidic and basic alternating.³⁷ Under proper conditions, the 11S can be fractionated into units of 7S. These 7S units appear to be composed of three acidic and three basic subunits. The 11S protein can also be broken to yield 3S subunits which appear to be dimers consisting of one acidic and one basic subunit each.

15S Fraction

This fraction comprises about 5% of the total extractable protein. It is only poorly characterized and is thought to be composed of polymers of the other soy proteins.

IDENTIFIED ALLERGENS OF SOYBEAN

Identification and characterization of specific protein components linked to soybean allergy has been the focus of several studies. The results of these investigations have shown that a heterogeneous group of proteins (Table 1) are IgE-binding and are potential allergens.

Soybean Vacuolar Protein (*Gly m Bd 30 K*)

The soybean protein *Gly m Bd 30 K* also known as P34, is considered a major allergenic protein of soybean seeds.^{20,38} IgE binding assays using immunoglobulin from soybean sensitive individuals indicated that 65% of the total allergenic response was accounted for by *Gly m Bd 30 K*.^{20,39,40} This protein was characterized and identified as the soybean 34-kDa oil body associated protein,³⁹ which has also been reported as seed storage protein, P34, in the vacuoles of soybeans.⁴¹ This protein showed considerable sequence similarity (fifteen N-terminal amino-acid residues) to the thiol proteinase of the papain family, including the allergenic thiol proteinase *Der p 1* from house dust mites.

Table 1 Identified allergens of soybean

IgE-binding proteins	Allergen nomenclature	Molecular weight	Family	References
Hydrophobic proteins	<i>Gly m</i> 1: (IUIS)* <i>Gly m</i> 1.0101 <i>Gly m</i> 1.0102	7.5 kDa 7.0 kDa	Lipid transfer protein	Gonzalez et al. ^{42,43}
Unknown	<i>Gly m</i> 2 (IUIS)	8.0 kDa	Storage protein	Codina et al. ⁵⁹
Profilin	<i>Gly m</i> 3 (IUIS)	14 kDa	Profilin	Rihs et al. ⁶²
SAM22	<i>Gly m</i> 4 (IUIS)	16.6 kDa	Pathogenesis related protein PR-10	Kleine-Tebbe et al. ⁶⁴ Neudecker et al. ⁶⁴ Mittag et al. ⁶⁶
P34	<i>Gly m</i> Bd 30 K	34 kDa	Protease	Ogawa et al. ^{20,39}
Unknown Asn-linked glycoprotein	<i>Gly m</i> Bd 28 K	26 kDa	Unknown	Tsuji et al. ^{46,48}
β -Conglycinin	Not assigned	140–170 kDa	Storage protein	Ogawa et al. ⁴⁴
Glycinin	Not assigned	320–360 kDa	Storage protein	Zeece et al. ⁵¹ Helm et al. ⁵⁰
2S albumin	Not assigned	12 kDa	Prolamin	Gu et al. ⁸²
Lectin (agglutinin)	Not assigned	120 kDa	Lectin	Fu et al. ⁷⁴
Lipoxygenase	Not assigned	102 kDa	Enzyme	Baur et al. ⁵⁷
Kunitz trypsin inhibitor	Not assigned	21 kDa	Protease inhibitor	Baur et al. ⁵⁷ Quirce et al. ⁵⁸
Unknown	Not assigned	39 kDa	Unknown	Gu et al. ⁸²
Unknown	Not assigned	50 kDa	Homology to chlorophyll A-B binding protein	Codina et al. ⁶⁸
P22-25	Not assigned	22–25 kDa	Unknown	Hessing et al. ⁶⁷

*IUIS: International union of Immunological Societies (IUIS) official allergen nomenclature.

P34 was named by the authors *Gly m* 1,²⁰ and should not be confused with another soybean allergen, the hull hydrophobic protein, which has been officially designated as *Gly m* 1.^{42,43} Helm et al. determined the linear IgE specific epitopes of the 34-kDa allergen amino-acid sequence through B-cell epitope mapping.⁴⁰ Their study revealed 10 regions of IgE-binding activity using an overlapping peptide strategy of 15-mers offset by 8 amino acids throughout the P34 sequence. Smaller overlapping peptides, 10-mers offset by 2 amino acids, revealed 16 distinct linear epitopes, 9 of which were mapped to the mature protein. Using individual patient serum, 5 immunodominant epitopes were identified in this allergen. Whether there are any conformational epitopes in addition to the linear epitopes on *Gly m* Bd 30 K is unknown.

β -Conglycinin

Burks et al.¹⁹ reported IgE-binding to the three, α , α' and β subunits of β -conglycinin. Later on, Ogawa et al.⁴⁴ confirmed α -subunit of β -conglycinin to be allergenic, but showed α' -subunit, which is highly homologous to the α -subunit, to have no allergenicity. Different sensitization histories of individual patients may be one of the reasons for the discrepancy. Ogawa et al.⁴⁴ showed that the epitope(s) of the α -subunit was located on the peptide 232–383, which had no sugar moiety, suggesting that the sugar moiety of α -subunit was not essential for the binding of IgE antibodies. Beardslee et al.⁴⁵ have suggested that the IgE epitopes are confined to regions of the 170 residue N-terminal insert found in the α -subunit.

Gly m Bd 28 K

The allergenic soybean seed protein *Gly m* Bd 28 K has been isolated and characterized by Tsuji et al.⁴⁶ It has been purified by immunoaffinity chromatography and was shown to be an unknown Asn-linked glycoprotein with a molecular mass of 26 kDa.⁴⁶ The glycan moiety of *Gly m* Bd 28 K was shown to bind to IgE antibodies at Asn 20, in the sera of soybean-sensitive patients (Hiemori et al., 2000).⁴⁷ A cDNA clone encoding *Gly m* Bd 28 K has been isolated.⁴⁸ This clone was shown to encode a polypeptide composed of 473 amino acids, which exhibited high homology with the MP27/MP32 proteins in pumpkin seeds and carrot globulin-like protein.⁴⁸

Glycinin

Glycinin of the 11S fraction of soy proteins has also been implicated as a major allergen. Pedersen and Djurtoft⁴⁹ reported IgE-binding to all of the native acidic subunits of glycinin, but little or none to the basic subunits. Other studies have, however, shown that both chains of soybean glycinin proteins bind IgE from soybean allergic patients.⁵⁰ Zeece et al.⁵¹ reported that the acidic glycinin G1 subunit exhibited a much stronger IgE binding than acidic glycinin G2, even though the sequence homology was quite similar. These authors, by using *in situ* digestion, were able to narrow the region of IgE-binding on glycinin G1 to 15 kDa peptide corresponding to amino acid residues 192–306 and comprising the C-terminal of the acidic portion of the polypeptide. This fragment of 15 kDa was also

shown to have the greatest sequence variability with the G2 acidic chain. In their study, Helm et al.,⁵² by using B cell epitope mapping, identified 11 linear epitopes in the glycinin G2 that bound to IgE. Four immunodominant epitopes were identified among them. Furthermore, based on the structure of the 7S phaseolin protein from the French bean, a three-dimensional (3D) model for glycinin was developed. This 3D model was used to determine the spatial arrangement of the epitopes. Interestingly, the epitopes of G2 glycinin were all located on the exterior of the molecule, and predominantly on one surface of glycinin trimers. Later on, Xiang et al.,⁵³ by using sera from patients with known soybean allergies and sera from patients with known peanut allergies, performed epitope mapping for IgE-binding for glycinin G1 acidic chain (G1a), glycinin G2 acidic chain (G2a), and *Ara h 3* (a major peanut allergen). Their results confirmed that the acidic chains of both soybean G1 and G2 bind IgE; furthermore, they established the dominant epitope region of the G2a protein as residing on the C-terminal end and identified it as the sequence S219-N233. To further identify the amino acids that are critical to IgE binding, the authors carried out alanine scanning mutagenesis of this G2a epitope, which revealed that six amino acids contributed most towards its IgE binding. Another peptide E247-V261, which showed weak IgE-binding, was also reported. This peptide has been previously identified in G2a,^{50,52} as well as in the corresponding sequences of G1a⁴⁵ and *Ara h 3*.⁵⁴ The dominant G2a epitope identified by Xiang et al.⁵³ was, however, not among the four IgE-binding epitopes identified for soybean G2a by Helm et al.^{50,52} Interestingly, this G2a epitope was part of a corresponding region of a G1a epitope identified by the same group earlier⁴⁵ and which was also found to be adjacent to the *Ara h 3* epitope (G240-A254) identified by Rabjohn et al.⁵⁴

Kunitz Trypsin Inhibitor

Not only have reports about hypersensitivity after ingesting soybean products been published, but also some articles about inhalative sensitization to soybean flour and soybean hull. The first reports of soybean inhalative allergy occurred in workers in a soybean mill,⁵⁵ which indicated that soybean was not only a food allergen but also an occupational aero-allergen capable of inducing asthma. Moroz and Yang,⁵⁶ later demonstrated that the serum of a patient with an occupational respiratory disorder had IgE antibodies specific for soybean Kunitz trypsin inhibitor (KTI; 21 kDa). In subsequent studies, serologic tests of 14 bakers with work related respiratory symptoms who had been sensitized to soybean flour, revealed KTI to be a major soybean allergen.⁵⁷ In similar studies, Quirce et al.⁵⁸ demonstrated a positive cutaneous and IgE response, as well as a specific bronchial response to purified soybean KTI in two bakers with work-related asthma, who were occupationally exposed to soybean flour; thus confirming the soybean KTI to be an occupational inhalant allergen.

Soybean Hull Proteins (*Gly m 1.0101*, *Gly m 1.0102*, and *Gly m 2*)

Soybean hull was identified as a source of aeroallergens that caused soybean epidemic asthma.⁴² The hull components responsible for respiratory allergies were identified and officially denominated by the International Union of Immunological Society (IUIS) Allergen Nomenclature Sub-Committee as isoallergens *Gly m 1.0101* also known as *Gly m 1A* (glycoprotein S2, MW of 7.5 kDa, pI 6.8) and *Gly m 1.0102* also known as *Gly m 1B* (protein S1, MW 7.0 kDa, pI 6.1–6.2). These isoallergens were identified as soybean hydrophobic proteins belonging to the lipid transfer protein family.⁴³

Using sera of asthmatic epidemic patients from Barcelona, Codina et al.^{59,60} confirmed the allergenicity of *Gly m 1.0101* and *Gly m 1.0102* from soybean hull. The authors further identified and characterized a third allergen named *Gly m 2* (MW of 8 kDa, pI 6.0), which was shown along with *Gly m 1* (*Gly m 1.0101* and *Gly m 1.0102*) to be partially responsible for the soybean asthma outbreaks that occurred in Barcelona, Spain. The N-terminal partial sequence of *Gly m 2*, lacked homology with that reported for allergen *Gly m 1*, but had homology of 71% with a storage protein from the cotyledon of cow pea, and 64% homology with a disease response protein from green pea, suggesting that *Gly m 2* in soybeans could protect against diseases, which affect soybeans plants. These three low molecular weight soybean hull aeroallergens were found to be not responsible for the occupational asthma caused by soybean flour in bakers, where mainly high molecular weight allergens from both flour and hull were involved.⁶¹

Soybean Profilin (*Gly m 3*)

Rihs et al.⁶² isolated a soybean seed profilin by a PCR-based cDNA cloning technique that was cross-reactive with IgE antibodies in the sera of soybean-sensitive patients. This allergen was named r*Gly m 3*. Epitope mapping with recombinant profilin indicated that only the full length r*Gly m 3* was able to bind with IgE, whereas overlapping soybean profilin fragments did not show significant binding reactivity. It was concluded that IgE-binding to r*Gly m 3*, was dependent on the integrity of a conformational structure, which was not present in the overlapping profilin fragments. Furthermore, Soybean *Gly m 3* was found to be homologous to *Bet v 2*, a birch pollen allergen, with a sequence identity of 73% and to eleven other plant profilins with a 69 to 88% identity. The significance of *Gly m 3* as an allergen in soybean deserves further study.

Soybean Protein SAM22 (*Gly m 4*)

Crowell et al.⁶³ revealed that the stress-induced 16.6 kDa PR-10 protein SAM22 (starvation-associated message 22) from

soybean seed has a sequence identity of approximately 50% with *Bet v 1*, which is responsible for IgE antibody binding in more than 95% of birch pollinosis (the prevailing allergic diseases in Northern and Central Europe and Northern America). Later on, Kleine-Tebbe et al.⁶⁴ observed severe oropharyngeal and anaphylactic reactions in birch pollinotics after ingestion of a dietary powder containing 50% of soy protein isolate. Eighty-five percent of those patients had specific IgE against SAM22, which has been now designated as *Gly m 4* allergen by the IUIS.⁶⁵ More recently, Mittag et al.,⁶⁶ through a clinical investigation revealed that 96% of patients reacting to *Bet v 1* have also *Gly m 4*-specific IgE and that 64% of them recognized other soy proteins. Their results also showed that IgE binding was at least 80% inhibited by birch pollen and 60% inhibited by recombinant r*Gly m 4*. These studies confirmed that *Gly m 4* was the major soy allergen for patients allergic to birch pollen and that soybean was another birch pollen-related allergenic food.

Other Soy Allergens

In the course of investigations on the allergenicity of soybean, Hessing et al.⁶⁷ found a low molecular weight soy protein, identified as P22–25 that induces strong antibody responses in calves. The nature of this protein remains unknown, although it was shown to be different from the allergenic P34 described earlier. In addition, a 50 kDa protein with homology to chlorophyll A-B binding protein was identified as binding IgE of patient with soy allergy.⁶⁸ Baur et al.⁵⁷ demonstrated by Radio Allergosorbent Tests (RAST), that lipoxidase (lipoxygenase) was a major allergen of soybean for fourteen bakers suffering from workplace-related respiratory symptoms and sensitized to soybean. Lectin is another soybean protein considered to be a potential allergen. This protein has a MW of approximately 120 kDa and is composed of four identical subunits of 30 kDa each. Lectins are carbohydrate-binding proteins that are predominantly found in plant seeds, particularly those of legumes,⁶⁹ they have been associated with some diseases in humans and animals including IgE-type allergies.^{70–74} Plant lectins have been shown to react unspecifically with the carbohydrate moieties of IgE inducing allergy-like symptoms.⁷⁵ The peanut agglutinin, for example, was identified as a lectin that was specifically recognized by IgE from a minority of peanut-allergic patients.⁷⁶ Little is known, however, about soybean lectin allergenicity and this obviously deserves further studies.

ALLERGENICITY OF SOYBEAN OIL

The bibliography is frequently contradictory on the allergenicity of soybean oil. Montagnon et al.⁷⁷ showed that allergenic proteins (28 and 56 kDa), were present not only in cold-pressed but also in deodorized soy oil. Moneret-Vautrin et al.⁷⁸ also reported an unusual soy oil allergy in an allergic infant who reacted to milk, soybeans, and soybean oil emulsion. In contrast, in an earlier study, Bush et al.⁷⁹ reported the safe ingestion by

soybean-allergic individuals of 6 commercial soybean oil samples. More recently, the same group of researchers undertook a comprehensive study, where 30 highly refined soy oils from around the world were fed to a geographically and ethnically diverse soy-allergic people.⁸⁰ Their study demonstrated that none of the 29 tested individuals had a reaction. Based on these findings, American regulators reportedly⁸⁰ exempted highly refined vegetable oils from known allergens, such as peanuts and soybean from the new federal food allergen labelling law that will take effect in 2006. Similarly, the European Union temporarily exempted highly refined soybean oil from their new food allergen labelling regulations.

The disparity in the available data may be a result of variabilities in the processes used in industry, the conditions under which proteins are extracted in the laboratory, as well as the use of different sensitized human sera. From the bulk of existing reports it appears that highly refined soybean oil may not represent a risk of provoking allergic reactions in the overwhelming majority of susceptible people. However, it is uncertain if the minute amounts of proteins remaining in this oil could provoke reactions in very sensitive individuals. This issue continues to deserve further investigation.

ALLERGENICITY OF SOYBEAN LECITHIN

Another suspected source of allergens is soybean lecithin. The content of lecithin in soybean (1.1–3.2%) is one of the highest found in plants.⁸¹ Since extraction of soybean lecithin is a relatively easy process, they have gained importance as emulsifiers and stabilizers for the food, pharmaceutical, and other technical industries (Figs. 1 and 2). Due to their widespread use and the extremely low levels at which allergens can elicit severe reactions, soy lecithin could represent an unrecognized source of hidden allergens in many products. Müller et al.⁸¹ tested six commercial soy lecithins against sera from six soybean-allergic adults. The enzyme allergosorbent test (EAST) revealed that three among the six sera reacted with four among the six soy lecithins tested. The same lecithins were found to contain residual proteins. Two lecithins with protein content of less than 20 ppb did not bind IgE. EAST inhibition showed that the allergens found in soy lecithin were more immunologically related to allergens from heat treated soybeans than to those from raw soybeans. IgE-immunoblotting of the protein extract from these lecithins identified various allergens with molecular weights between 14 kDa and 94 kDa. A prominent allergen band of 39 kDa was identified as the acidic subunit of soy glycinin. Another, 25–28 kDa protein was identified, but was not further characterized. Recently, Gu et al.⁸² by using sera from soy-sensitive individuals revealed IgE-binding proteins in soybean lecithin with bands corresponding to 7, 12, 20, 39, and 57 kDa. The 12 kDa band was identified as a methionine-rich protein and a member of the 2S albumin class of soy proteins. The 20 kDa band was found to be the Kunitz trypsin inhibitor. The authors considered the 39 kDa band as a newly identified soybean IgE-binding protein with unknown function.

CROSS-REACTIVITY OF SOYBEAN ALLERGENS

IgE-mediated allergy to soy products might be the result of primary sensitization but could also result from cross-reactivity to a variety of legumes. A comparison of IgE immunoblots to soybean extracts with common legumes showed that soybean extracts shared several common as well as unique allergenic proteins with other members of the legume family.^{19,83}

Cross-Reactive Allergens

Extensive *in vitro* cross-reactivity has been demonstrated among different legumes. As early as 1971, Fries,⁸⁴ by using a skin test on 30 atopic children with suspected soybean allergy, showed that 90% of patients were sensitive to soybean, 90% to peanut, 80% to green pea, 53% to lima pea, and 43% to string bean. Later on, Bernhisel-Broadbent and Sampson⁸⁵ reported that among 69 legume-sensitive patients, 43% showed sensitivity to soybean, 87% to peanut, 22% to green bean, and 26% to pea. Similarly, Eigenmann et al.⁸⁶ showed that the removal of soy-binding antibodies, by antigen-affinity chromatography, from the serum of a patient allergic to peanut and soy, resulted in 73% reduction of IgE-binding to peanut allergens.

The immuno-observed cross-reactivity between soybean and peanut has been confirmed by Beardslee et al.⁴⁵ and Xiang et al.,⁵³ who showed that IgE-binding epitopes of glycinin G1 and G2 acidic chains were homologous to IgE-binding epitopes of the peanut allergen *Ara h* 3.⁵⁴ The position of these IgE-binding epitopes in the secondary and tertiary structures of soybean and peanut seed storage proteins suggests that this portion of the protein molecules may represent a particularly allergenic domain among the legume family.^{50,52–54}

The clinical significance of cross-reactivities between legumes is, however, still unclear.^{85–87} Reported clinical co-reactivity rates in peanut allergics for soy are low and range only from 1–6.5% in placebo controlled studies.⁸⁸ Foucard and Malmheden Yman,¹⁰ however, warn that soybean proteins might be underestimated as a cause of severe allergic response in the general population of peanut allergic patients.

The homology between soybean seed storage proteins and respective peanut allergens could be the basis for the development of new therapeutic intervention strategies for peanut allergy. In their recent study, Ponset et al.⁸⁹ successfully used soy immunotherapy (administration of specific doses of soybean) to desensitize/downregulate peanut-specific response in peanut-allergic mice. Cross-reactivities between soy and peanut will continue to represent an important clinical and therapeutic challenge, as both share common antigens,^{45,53} and both are widely used in food product formulations.⁸⁷

Cross-reactivity between soybean profilin (*Gly m* 3) and the well-characterized birch profilin *Bet v* 2 has been studied by Rihs et al.⁶² The two profilins are known to have 73% homology in their amino acid sequences and results show that r*Gly m* 3 and r*Bet v* 2 share similar IgE-binding epitopes, with both these

epitopes existing as discontinuous forms. Kleine-Tebbe et al.⁶⁴ also demonstrated cross-reaction between *Bet v* 1, another major birch pollen allergen and PR-10 soy protein SAM22. Patients with birch pollen allergy developed immediate-type allergic symptoms after ingesting food items containing soy protein.

Perhaps the most interesting of soy allergy cross-reactivities is the one with milk proteins. Several authors have reported co-existing clinical soy allergies in patients with cow's milk allergies, ranging from 5%⁹⁰ in a prospective study to around 50% in a selected group of patients with cows' milk enterocolitis.⁹¹ Based on a positive RAST/skin test and open or DBPCFC, Cantani and Lucenti⁹² also reported a 3–4% incidence of soy protein formula allergies in cows' milk allergic children given soy protein formulae. It is unclear whether soy allergy in these children represents a *de novo* sensitisation or a cross-reaction of a soy protein component with caseins from milk.⁹³ A 30-kD component from soybean that cross-reacted with cow's milk caseins was isolated and partially sequenced. It was found to be constituted of two polypeptides (A5 and B3) linked by a disulphide bond. The protein's capacity to bind to the cow's milk specific polyclonal antiserum and casein specific monoclonal antibodies relied on the B3 poly-peptide.⁹³

Detailed structural and molecular studies of most of the cross-reactive allergens and their epitopes mapping are missing; this is a prerequisite for a full understanding of the observed immune cross-reactivity of the different food allergens.

Unique Soy Allergens

As compared to cross-reacting allergens, few studies have been aimed at identifying unique soybean antigenic fractions. Herian et al.,⁸³ in the course of studying soy and peanut cross-reactivity, found a strong IgE-binding to a soybean protein with a MW of 20 kDa from sera of individuals allergic to soybeans but not to peanuts. Helm et al.,⁵⁰ later on extended these observations. By using serum IgE from 7 clinical soybean sensitive individuals, they demonstrated that the 22 kDa proteins, which bound to these IgE were the basic chains from glycinin subunits. Similarly, Eigenmann et al.,⁸⁶ by removing cross-reacting antibodies to soy and peanut from sera of patients allergic to both legumes, identified two unique allergenic fractions from soybean extracts that had an apparent molecular weight of approximately 46 and 21 kDa.

Although these soy allergens are currently being defined as unique, it is important to mention that the fact that they do not cross-react with a known allergen source does not rule out their potential cross reactivities to other sources.

STRUCTURAL AND BIOLOGICAL STUDIES OF SOYBEAN ALLERGENS

Allergenicity of proteins is due to numerous molecular immunoreactive structures (i.e. the IgE-binding epitopes), that are

widespread within the protein molecule. Depending on their structure, two kinds of epitopes are described. Some are linear because they are formed by a sequence of amino acid residues on the peptide chain of the protein. Other epitopes are conformational because they are associated with the secondary and tertiary structure of the protein. Characterization of epitopes, particularly IgE-binding epitopes, is necessary to help understand the correlation between the structure and physicochemical properties of a protein and its allergenicity. To date, no particular structure can be described as being solely and intrinsically allergenic. Different allergens may share similar sequence homologies and/or structural features or common epitopes. These homologies are mostly responsible for cross-reactions and usually occur in related molecular species of the same family. They involve common, highly conserved structures, which generally correspond to important biological activities and functions that are vital for the plant. They may also, however, be present among apparently unrelated proteins.⁹⁴ Just like other plant food allergens, most of the known soybean allergens have a metabolic, storage, or protective function.⁸ They belong to protein families which have conserved structural features in relation with their biological activity.

Glycinin (11S) and β -conglycinin (7S), for example are both storage proteins known as globulins, which belong to the cupin superfamily.⁹⁵ The 3D structure of proglycinin from soybean (a glycinin precursor) has been determined by X-ray studies.^{96–98} The crystal structure of soybean β -conglycinin has also been determined by Maruyama et al.⁹⁹ Both glycinin and β -conglycinin were shown to share conserved sequence motifs comprising identical or chemically similar amino acid residues and a common 3D conformation. This tertiary structure called β barrel structure is made up of series of anti-parallel β sheets associated with α helices which form a cavity (a cup, from where the term Cupin was given) comprising a binding site for hydrophobic ligands.¹⁰⁰ Interestingly, it has been also reported that many allergens of animal origin share this β barrel structure which is a characteristic of the lipocalin family involved in the transport of hydrophobic ligands (e.g. milk β -lactoglobulin). Other important allergens from the Cupin superfamily are the *Ara h 1* and *Ara h 3* from peanuts and *Cor a 9* from hazelnuts.⁹⁵

The soybean hydrophobic proteins (*Gly m 1.0101* and *Gly m 1.0102*) are lipid transfer proteins (LTPs), which are named for their ability to transfer phospholipids from liposomes to mitochondria.⁴³ LTPs are made up of low molecular weight monomeric proteins (around 7–9 kDa), that take part in plant defence because of their antibacterial and anti-fungal activities.¹⁰¹ These lipid transfer proteins have common structural features also shared by other members of the Prolamin super family. They have high amino acid sequence homologies and conserved motifs of cysteine residues involved in disulphide bonds. They have a very compact and stable tertiary structure made of associations of α helices and loops stabilized by eight disulphide bonds; this defines a central cavity which contains a lipid binding site.^{43,102} The Prolamin superfamily includes several important allergens such as the Brazil nut 2S albumin *Ber e 1* and the

sesame allergen *Ses i 2*, which belong to the storage protein group of 2S albumin.¹⁰³ The 2S albumins are also classified within the prolamin super family (LTP), because of the conserved similar 3D structure rich in α helices.¹⁰⁴

The Kunitz trypsin inhibitor belongs to the group of proteins of the plant defence system, which has inhibitory activity against various proteases, such as serine, thiol, and aspartic proteases. Like all members with inhibitory activity it contains two disulfide bridges. Allergens from the Kunitz family of trypsin inhibitors are present in a range of legume species,¹⁰⁵ but the soybean Kunitz trypsin inhibitor is the most characterized. Its primary and tertiary structures have been determined.^{106–108} Based on circular dichroism (CD) spectra, soybean KTI has been classified in the family of anti-parallel β -sheet proteins that are highly resistant to chemical and thermal denaturation.¹⁰⁸ Its allergenic epitopes, however, remain unknown.

Soybean allergens *Gly m Bd 30 K* and *Gly m 4* belong to two others groups of the plant defence system proteins, the proteases and the pathogenesis-related proteins (PR proteins). Soybean *Gly m Bd 30 K/P34* was shown to have sequence similarity with the papain-like proteases; this protein, however, appears to have lost its enzymatic activity and adopted an alternative function as a syringolide receptor.¹⁰⁹ The PR proteins represent a collection of 14 unrelated protein families that function as part of the plant defence system by resisting biotic and abiotic stress. The homology between many plant food allergens and PR proteins has been extensively reviewed.^{95,100,110–112} The major birch pollen allergen *Bet v 1* was the first to show sequence homology to PR-10 family members,¹¹³ which in turn was demonstrated to be homologous to soybean *Gly m 4/SAM22*.^{63,64,66} The 3D structure of *Gly m 4* has been recently studied using NMR techniques.⁶⁵

The soybean allergen *Gly m 3* belongs to the profilin family, which is also a group of structural and metabolic plant proteins that harbor several allergenic proteins.⁶² These cytosolic proteins (12 to 15 kDa) are found in all eukaryotic cells. They bind to monomeric actin and participate in the regulation of polymerization of actin filaments.⁹⁵ The sequence conservation among profilins is reflected by highly similar structure and profilin-specific IgE was shown to cross-react between profilins from pollen and food.¹¹⁴ They are now recognized as ubiquitous cross-reactive plant allergens. The 3D structure of *Gly m 3* remains to be determined; however, epitope mapping with recombinant profilin indicated that IgE-binding to r*Gly m 3* was dependent on the integrity of a conformational structure.⁶² This could explain the fact that they are very heat labile and susceptible to enzymatic degradation and that allergy caused by profilins are usually confined to the oral allergy syndrome.^{115,116}

METHODS OF DETECTION OF SOY ALLERGENS

Reliable detection and quantification methods for food allergens are required to ensure compliance with food labelling regulations and to minimize risks to highly sensitive consumers

from undeclared allergens in food supply. Detection of allergens in food products can, however, be very difficult, as they are often present in trace amounts and can be masked by the food matrix.¹¹⁷ This is particularly true for soybean, which is an insidious hidden allergen (eg. pastries, bakery products, infant foods, sausages, processed meats, hamburgers, and dairy products). To date, the majority of methods developed to detect soybeans in food products are based on immunochemical assays.^{118–127} These protein based methods have, however, been found to be lacking in sensitivity and are not sufficiently adequate to ensure the safety of food products for soy sensitive individuals.¹¹⁷ Presently, three enzyme linked immunosorbent assay (ELISA) kits for soybean are available commercially. The Tepnel BioSystems kit (Elisa Technologies, FL, USA) is a competitive ELISA that targets renatured soy proteins with a detection limit of <5000 mg/kg of food product. The second, the ElisaSystems kit (ELISA Systems, Windsor, Australia), is based on a sandwich ELISA and determines the soy KTI in the food sample with a sensitivity of 1 mg/kg. The third, the Veratox for Soy Flour Allergen (Neogen Corporation, MI, USA), which has recently come on the market, is a sandwich ELISA used for the quantitative analysis of minimally processed soy flour protein in food products such as cookies, crackers, chocolate bars, and cereals in the range of 2.5 to 25 mg/kg. To date the ELISA technique has been the preferred approach for allergens detection because of its high precision, simple handling and good potential for standardization;¹¹⁷ however, as with any type of analysis, there are inherent drawbacks related to food matrix interferences, and effects of food processing on the targeted immunogens. Alternative techniques to ELISA have also been developed. Among these is immunolocalization by electron microscopy of soy proteins in foods. This technique makes possible the identification and the quantification of soy proteins in food matrices by using immunohistochemical techniques associated with image analysis.¹²⁸ The process confirms the presence of soy directly by histology, which allows the areas occupied by labelled soy proteins in sections mounted on slides to be measured. The length of time for analysis and the high cost of equipment are the major disadvantages of this technique.

Other methods of detection of soy allergens use DNA identification. These techniques are based on the amplification of specific DNA fragment by the polymerase chain reaction (PCR). The presence of the DNA is an indication of the presence of the allergenic food but not of the allergen itself. The PCR-based methods have been shown to be reliable, sensitive, and specific for the detection of allergenic moieties. There is, however, a lot of controversy over the employment of DNA in the analysis of allergens, since proteins are the allergenic components and processing may affect nucleic acids differently.¹¹⁷

Alternative methods to ELISA and PCR are presently being developed for the detection and identification of other major food allergens (eg. wheat, peanut). These could possibly be applied in the future to soy. Techniques such as two-dimensional gel electrophoresis coupled with immunoblotting and mass spectroscopy are very promising but are not yet ready for routine use.^{17,129,130}

HYPOALLERGENIZATION OF SOYBEAN

Currently, for individuals with food allergy, strict avoidance of the specific food or protein is the only effective treatment for preventing food-induced allergic reactions. This can be very challenging, especially for soybean, which, along with peanut were found in approximately 6,000 foods, and in most cases were not labelled on the commodity (Helm et al., 2002).¹³¹ Since avoidance is difficult, investigations on the hypoallergenization of soybean products are being carried out by several groups and a variety of methods have been attempted. These include thermal, enzymatic, and chemical modification of the allergen, as well as traditional breeding and genetic modification of soybeans cultivars. It is difficult, however to ascertain whether the resulting hypoallergenic products are truly hypoallergenic. This has been recognized by both the American Academy of Paediatrics¹³² and the European Society for Paediatric Gastroenterology and Nutrition (ESPGAN) Committee of Nutrition¹³³ who agree to define a hypoallergenic food as one that has been shown, with 95% confidence level, to not cause allergic reactions in 90% of patients who are known to be allergic to the parent food. At present the potential of a product for use as a "hypoallergenic" or "non-allergenic" can only be determined by clinical trials using scientifically appropriate standards.¹³⁴

Thermal Treatment

Shibasaki et al.¹³⁵ examined the effect of heat treatment at 80°C, 100°C, and 120°C on the crude soybean fraction and its effect on IgE binding. Their results showed that the IgE binding of 2S fraction was enhanced with heating at 80°C, whereas, the IgE binding to other fractions were reduced by 40% to 75% compared to that of the crude product. Similarly, Burks et al.,¹³⁶ by using an ELISA technique, showed that heating crude soybean, at 100°C, and 120°C for 60 min, significantly decreased IgE binding for each protein fraction (whey, 7S, and 11S). At 80°C, the IgE binding of the 7S and 11S was also reduced, except that of the whey fraction. By using EAST inhibition technique the same authors, however, reported no significant changes in both IgE- and IgG-binding after heating soybean up to 100°C.¹³⁷ Contrasting with these observations, Müller et al.⁸¹ by using RAST method showed that cooking soybeans at 100°C for 2 h resulted in maximum inhibition of IgE-binding of 89% for the heated extract compared to 39% for the raw extract. These contradicting results signify that although heat treatment can significantly alter protein structure, the nature and extent of such changes are dependent on the temperature and duration of the thermal treatment, as well as on the intrinsic characteristics of the protein and the physicochemical conditions of its environment.¹³⁸ Lakemond et al.,¹³⁹ by studying the effect of heat treatment of soybean glycinin structure, showed that glycinin heat denaturation varied strongly with pH and ionic strength, and correlated also, with the glycinin quaternary, tertiary, and secondary structure before heating.

In addition to destroying existing epitopes on native molecules, thermal treatments may also create new allergenic epitopes, uncovered by the unfolding process resulting from the heat treatment. These new antigenic sites may no longer be recognized by antibodies of native proteins. A good example is provided in the work of Herian et al.⁸³ who showed that although roasting soybeans led to largely reduced recognition of high MW allergens by IgE from patients allergic to both soybean and peanut, it strongly enhanced binding of IgE to the low MW allergens in patients sensitive only to soybean. Similarly, Codina et al.¹⁴⁰ demonstrated an increase in allergenicity when soybean hull proteins were thermally treated. Their results showed two new IgE-binding proteins (10 and 15.3 kDa) that were possibly generated or revealed as a result of the conformational changes.

Enzymatic Hydrolysis

Hydrolysis has been generally recognized to reduce the antigenicity of proteins.¹⁴¹ Good examples can be found with traditional fermented soy products, such as miso, tempeh, natto, shoyu, and tamari that have all been shown, by RAST to be less allergenic than raw soybeans.^{142,143} Yamanishi et al.¹⁴⁴ treated autoclaved soybean grain with secretory protease from *Bacillus subtilis* at conditions similar to those of a natto fermentation procedure. The proteins of the enzyme-treated soybean were hydrolyzed into peptides of molecular weight of less than 10 kDa and the product showed no binding activity with soy-sensitive human sera and monoclonal antibodies against *Gly m* Bd 30 K. Similarly, the same group of researchers treated soybean with 8 different proteases (Newlase F from *Rhizopus niveus*; Pancreatinin from porcine pancreas; Protease N, Protease S and Proleather P from *Bacillus subtilis*; Protease A and Protease M from *Aspergillus oryzae*; and Protease P from *Aspergillus melleus*). Their results showed that both antigenicity to the *Gly m* Bd 30 K monoclonal antibody and the allergenicity to the sera of soy sensitive patients were markedly decreased by processing with all proteases. Two out of the eight proteases (Proleather and Protease N) were very effective.¹⁴⁵ Tumura et al.¹⁴⁶ selectively, used a *Bacillus* protease (Proleather FG-F) to hydrolyze both *Gly m* Bd 30 K and β -conglycinin, but not glycinin. The key point for the selective hydrolysis was based on the different denaturation temperatures between β -conglycinin and glycinin at neutral pH. The digestion of denatured soybean proteins by proteases proceeded more rapidly at 70°C. The resultant product was proven to lose its reactivity against the monoclonal antibody specific to these two allergens and sera of soybean-sensitive individuals. More recently, a fermentation process of steamed soybeans, using a combination of three microorganisms (*Lactococcus lactis*, *Aspergillus oryzae* and *Bacillus subtilis*) resulted in a soybean product with amino acids and polypeptides of less than 10 kDa. These degraded proteins were tested against a serum of a soybean-sensitive patient and no antigenicity was detected.¹⁴⁷ It is noteworthy that most reported fermentation processes used to reduce soybean allergenicity combine a preliminary heat treat-

ment step, such as autoclaving and steaming, which considerably improved proteolysis of soybean allergens.^{144,147} Hydrolyzed products vary in their residual allergenicity, and this apparently depends upon the extent of hydrolysis.¹⁴¹ Selective destruction of epitopes has been observed with a proportion of soybean allergens still maintaining their native configuration after certain treatments.^{83,142} This could be explained by the fact that, although, proteolysis could destroy some epitopes, particularly conformational epitopes, it could also unmask linear epitopes that were buried within the three-dimensional native structure and/or located in hydrophobic domains of the protein; these become available for IgE-binding upon hydrolysis.¹⁴⁷ The above observations confirm, once again, that it is crucial to know the structural characteristics of the allergen to be able to specifically and efficiently use proteases to decrease their immunogenicity.

It is rather difficult to affirm if any of these hydrolyzed soybean products are safe for the larger population of soybean-allergic individuals as the majority of them are tested against only one or few specific known soybean allergen antibodies or soybean-sensitive sera. In many of these tests, no skin or oral challenges are carried out to ensure that these products do not elicit positive responses in sensitised patients. Skin prick tests have been considered to be a suitable method for determining the residual allergenicity of hydrolyzed products.¹⁴⁹ They can also be important tools for differentiating antigenicity and allergenicity of proteins. In their study, Tsai et al.¹⁴¹ used skin prick tests, western blots and ELISA to determine the residual allergenicity of soybean hydrolysates. Their results showed no evidence of IgE specific binding with both skin tests and western blots. However, some residual IgG binding was detected by ELISA. Since there is no absolute proof that any substance is completely nonallergenic, they could only conclude that the hydrolyzed soybean proteins were safe for individuals sensitized to the native soybean proteins. Concerns, however, exist over the use of skin prick tests, as some believe that the test itself could induce sensitization.

Chemical Modification

Several reports describe reduction of soybean allergenicity by allergen conjugation with polysaccharides.^{150–153} Babiker et al.¹⁵⁰ developed a method, where soybean allergen *Gly m* Bd 30 K was attached with a galactomannan preparation (a group of mannase hydrolysate containing polysaccharides of average MW of 15 kDa) through spontaneous Maillard reactions between the ϵ -amino groups of the protein and the reducing end of the carbonyl group in the polysaccharide. The reactivity of the soy P34 protein-galactomannan conjugate to rabbit polyclonal, *Gly m* Bd 30 K-monoclonal antibodies, and human soybean-allergic sera was completely diminished. The galactomannan conjugation was found to be more effective at reducing the allergenicity of P34 protein than chymotrypsin treatment (hydrolysis) and transglutaminase cross-linkage.¹⁵¹ The latter, has been

reported to be a useful means of reducing allergenicity of some food proteins.¹⁵⁴

The method of polysaccharide attachment to proteins (ie. natural Maillard reaction vs. genetic attachment of polysaccharide) on the allergenicity of soybean protein has also been examined.¹⁵² Allergenicity of the P34 soy protein was quantified by measuring the amount of IgE and IgG produced, using mice immunized with the protein-polysaccharide conjugate or native proteins. The results indicated that the genetic attachment of polysaccharide using a yeast expression system was very efficient in decreasing both IgE and IgG levels compared to the Maillard-type polysaccharide attachment, which showed a decrease of 70% in the level of IgE, but a significant increase in the level of IgG to 125%. This finding supports the notion that the stimulation of IgG antibody responses is a reflection of protein immunogenicity, whereas protein allergenicity is associated with induction of strong IgE responses.¹⁵² More recently, Usui et al.¹⁵³ used chitosan (natural and partially deacetylated polymer of N-acetyl glucosamine prepared from chitin) as another polysaccharide for the formation of protein-polysaccharide conjugate. Their results showed that soybean protein conjugation with chitosan was more effective at reducing soybean allergenicity than that with galactomannan. Using western blotting, the band of the soy allergen (*Gly m* Bd 30 K) immunoblotted with monoclonal antibody completely disappeared. The most likely explanation for the decreased allergenicity of conjugated proteins is the masking of the allergen epitopes, which then becomes unavailable to the immune system. Polysaccharide conjugation, which results in the formation of high molecular weight proteinaceous components, may on the other hand change the processing and functional properties of the modified protein. In the case of soy protein-conjugation with galactomannan, some beneficial effects have been reported, such as improved heat stability and emulsifying properties,¹⁵⁰ which could be of interest to the food processing industry. Similarly the soy protein-chitosan conjugate exhibited enhanced antimicrobial activity and high emulsifying properties, which are also of potential interest to both the pharmaceutical and food industries.¹⁵³

Breeding and Genetic Modification

In the last two decades, various soybean mutant genes which control the production of enzymes, allergenic proteins, and storage proteins have been identified in the world soybean germplasm.¹⁵⁵ As a result, breeding and mutagenesis have both been presented as technologies that have the potential to reduce soybean allergenicity. Examples of this include the development of the soybean cultivar «Kunitz» (Illinois Agriculture Experiment Station) lacking the Kunitz soybean inhibitor¹⁵⁶ and the cultivar Tohoku 124 (Tohoku National Agriculture Experiment Station) lacking three of the known allergenic soybean proteins, the α and α' subunits of β -conglycinin, and the *Gly m* Bd 28 K.^{157,158} The removal rate of these allergens was 99.8, 100, and 100%, respectively. The soybean Tohoku 124, how-

ever, still contained the normal level of *Gly m* Bd 30 K. Another example is the wild cultivar, named QT₂, that was found by Kyushu National Agriculture Experiment Station, and which lacked all of β -conglycinin.¹⁵⁹ By back-crossing this QT₂ line with Fukuyutaka, which contains only glycinin as storage protein and which lacks α , and β subunits of β -conglycinin, as well as *Gly m* Bd 28 K, a new hypoallergenic variety, named Kyu-kei 305, was developed.¹⁶⁰

Breeding and conventional mutagenesis, although quite successful with some major soybean allergens, have not yet been able to remove the dominant allergen *Gly m* Bd 30 K. Helm et al.¹⁶¹ have reported that a single-site amino acid substitution of the IgE-binding epitopes of *Gly m* Bd 30 K with alanine resulted in the reduction of its IgE-binding activity. More recently, Herman et al.¹⁶² used recombinant techniques to eliminate *Gly m* Bd 30 K by down-regulating the expression of the gene that encodes it (gene knockout). The resultant transgenic soybean lacked any detectable *Gly m* Bd 30 K, as assayed by both a specific monoclonal antibody and cross-reactivity with IgE from soybean-sensitive persons and was also reported to be otherwise unchanged by the removal of this allergenic protein. Recently, Kinney and Jung¹⁶³ patented a hypoallergenic transgenic soybean and recombinant expression constructs technique to lower the P34, as well as other allergens. Soybean protein products made from these hypoallergenic soybeans were claimed to be substantially free of *Gly m* Bd 30 K, *Gly m* Bd 28 K, α -subunit of β -conglycinin, soybean Kunitz trypsin inhibitor, *Gly m* 2, *Gly m* 1.0101, *Gly m* 1.0102, r*Gly m* 3 and glycinin G1.

Although, it seems possible to reduce or remove some of the known soybean allergens with cross-breeding and genetic engineering, it will be difficult to remove them all (eg. glycinin, profilin (*Gly m* 4) without impairing the structural composition and physiological development of the seeds, which might in turn, have negative consequences on the nutritional and functional properties of soybean. It would also be necessary to ensure that resulting cultivars do not amplify alternative proteins or express novel proteins that might be allergenic.^{164,165}

CONCLUSION

Significant advances have been made in the last two decades to identify allergenic proteins of soy. The known soybean proteins with allergenic potential are compiled in a database of allergenic proteins and public access is available at URL: <http://www.allergome.org>. Not all of the soy allergens have, however, been identified and/or clinically confirmed, and only in a few cases, have the allergenic epitopes been fully characterized. Elucidation of the chemical structure of the allergens as well as the factors that are responsible for epitope integrity will allow the development of specific and more efficient technological approaches to decrease soybean allergenicity. Development of any such technologies would need to address the impact on nutritional, functional, and sensory quality. A combined approach using processing and genomics-based technologies may open up

new avenues to improve the safety of soybeans that could lead to the development of new products for allergenic individuals. Issues such as residual and/or possible increased allergenicity of hypoallergenicized soybean and soy products will likely remain of concern irrespective of the processes used. These issues are important as they could potentially have grave consequences for consumers and should be addressed by establishing reliable standards and methods for risk assessment. Further research is clearly needed to increase our understanding of food allergenicity, as much still remains unknown. This knowledge will be critical for the development of safe hypoallergenic products for sensitized individuals.

REFERENCES

- [1] USDA. 2003. United States Department of Agriculture. In: <http://www.soystats.com>.
- [2] Endres, J.G. 2001. *Soy protein products—Characteristics, nutritional aspects, and utilization*. Endres, J.G., Ed., AOCS Publication, Champaign, IL, 1–51.
- [3] Hefle, S.L., Nordlee, J.A., and Taylor, S.L. 1996. Allergenic foods. *CRC Crit. Rev. Food Sci. Nutr.*, **36**:S69–S89.
- [4] Zarkadas, M., Scott, W.F., Salminen, J., and Pong, A.H. 1999. Common allergenic foods and their labelling in Canada – A review. *Can. J. Allergy Clin. Immunol.*, **4**(3):118–141.
- [5] Buttriss, J., and Schenker, S. 2002. Introduction and definitions. In: Buttriss, J., Ed., *Adverse reactions to food. The report of the British Nutrition Foundation Task Force*. London: Blackwell Science Ltd., 1–10.
- [6] Mills, E.N.C., Madsen, C., Shewry, P.R., and Wichers, H.J. 2003. Food Allergens of plant origin—their molecular and evolutionary relationships. *Trends Food Sci. Technol.*, **14**:145–156.
- [7] Sampson, H.A. 2004. Update on food allergy. *J. Allergy Clin. Immunol.*, **113**(5):805–819.
- [8] Becker, W., Brasseur, D., Bresson, J.L., Flynn, A., Jackson, A.A., Lagiou, P., Mingrone, G., Moseley, B., Palou, A., Przyrembel, H., Salminen, S., Strobel, S., and van Loveren, H. 2004. Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission relating to the evaluation of allergenic foods for labelling purposes (Request N° EFSA-Q-2003-016). *EFSA Journal*, **32**:1–197.
- [9] Errahali, Y., Morisset, M., Moneret-Vautrin, D.A., Kanny, G., Metche, M., Nicolas, J.P., and Fremont, S. 2002. Allergen in soy oils. *Allergy*, **57**(7):648–649.
- [10] Foucard, T., and Malmheden-Yman, I. 1999. A study on severe food reactions in Sweden is soy protein an underestimated cause of food anaphylaxis. *Allergy*, **53**(3):261–265.
- [11] Mortimer, E.Z. 1961. Anaphylaxis following ingestion of soybean. *Pediatr.*, **58**:90–92.
- [12] Yunginger, J.W., Nelson, D.R., Squillace, D.L., Jones, R.T., Holley, K.E., Hyma, B.A., Biedrzycki, L., Sweeney, K.G., Sturmer, W.Q., and Schwartz, L.B. 1991. Laboratory investigation of deaths due to anaphylaxis. *J. Forensic Sci.*, **36**:857–865.
- [13] Vidal, C., Perez-Carral, C., and Chomon, B. 1997. Unsuspected sources of soybean exposure. *Ann. Allergy Asthma Immunol.*, **79**:350–352.
- [14] Senne, G.E., Crivellaro, M., Bonadonna, P., Dama, A., Mezzelani, P., and Passalacqua, G. 1998. Pizza; an unsuspected source of soybean allergen exposure. *Allergy*, **53**(11):1106–1107.
- [15] Bock, S.A., Munoz-Furlong, A., and Sampson, H.A. 2001. Fatalities due to anaphylactic reaction to foods. *J. Allergy Clin. Immunol.*, **107**(1):191–193.
- [16] Tamarcaz, P., Hauser, C., and Eigenmann, P.A. 2001. Soy anaphylaxis. *Allergy*, **56**(8):792.
- [17] Popping, B., Waiblinger, H.U., and Holzhauser, T. 2004. Here, there and everywhere: allergen detection and labelling—scientific and regulatory issues. *Inform.*, **15**(9):620–621.
- [18] Sampson, H.A., and McCaskill, C.C. 1985. Food hypersensitivity and atopic dermatitis evaluation of 113 patients. *J. Pediatrics*, **107**(5):669–675.
- [19] Burks, A.W., Butler, H.L., Brooks, J.R., Hardin, J., and Connaughton, C. 1988. Identification and comparison of differences in antigens in two commercially available soybean protein isolates. *J. Food Sci.*, **53**(5):1456–1459.
- [20] Ogawa, T., Bando, N., Tsuji, H., Okajima, K., Nishikawa, K., and Sasaoka, K. 1991. Investigation of the Ig binding proteins in soybeans by immunoblotting with the sera of the soybean-sensitive patients with atopic dermatitis. *J. Nutr. Sci. Vitaminol.*, **37**(6):555–565.
- [21] Lalles, J.P., and Peltre, G. 1991. Biochemical features of grain legume allergens in humans and animals. *Nutr. Rev.*, **54**(4/Part I):101–107.
- [22] Besler, M., Helm, R.M., and Ogawa, T. 1999. Allergen Data collection update: soybean (glycine max). *Internet Symposium on Food Allergens*, **2**(suppl 3):1–35.
- [23] Ogawa, T., Samoto, M., and Takahashi, K. 2000. Soybean allergens and hypoallergenic soybean products. *J. Nutr. Sci. Vitaminol.*, **46**(6):271–279.
- [24] Thanh, V.H., and Shibasaki, K. 1976. Major proteins of soybean seeds: A straight forward fraction and their characterization. *J. Agric. Food. Chem.*, **24**(6):1117–1121.
- [25] Howard, P.A., Lehnhardt, W.F., and Orthoefer, F.T. 1983. 7S, and 11S vegetable protein fraction and isolation. *US Patent*, No. 4,368,151.
- [26] Nielson, N.C. 1985. Structure of soy proteins. In: Altschul, A.M., and Wilcke, H.L. Eds., *New Protein Food, Vol. 5. Seed Storage Proteins*. Orlando: Academic Press, 27–64.
- [27] Kunitz, M. 1945. Crystallization of a trypsin inhibitor from soybeans. *Science*, **101**:668–669.
- [28] Shattuck-Eidens, D.N., and Beachy, R.N. 1985. Degradation of β -conglycinin in early stages of soybean embryogenesis. *Plant Physiol.*, **78**:895.
- [29] Coates, J.B., Medeiros, J.S., Thanh, V.H., and Nielson, N.C. 1985. Characterization of the subunits of β -conglycinin. *Arch. Biochem. Biophys.*, **243**:184–189.
- [30] Morita, S., Fukase, M., Yamaguchi, M., Fukuda, Y., and Morita, Y. 1996. Purification, characterization and crystallization of single molecular species of β -conglycinin from soybean seeds. *Biosci. Biotechnol. Biochem.*, **60**(5):866–873.
- [31] Pernollet, J.C., and Mosses, J. 1983. Structure and location of legume and cereal seed storage proteins. In: Daussant, J., Mosse, J., and Vaughan, J., Eds., *Seeds Proteins. Annual Proceedings of the Phytochemical Society of Europe*. London: Academic Press, 155–190.
- [32] Thanh, V.H., and Shibasaki, K. 1979. Major proteins of soybean seeds. Reversible and irreversible dissociation of β -conglycinin. *J. Agric. Food Chem.*, **27**(4):805–809.
- [33] Murphy, P.A., and Resurrection, A.P. 1984. Varietal and environmental differences in soybean glycinin and β -conglycinin concentration. *J. Agric. Food Chem.*, **32**:911.
- [34] Lei, M.G., and Reeck, G.R. 1987. Two-dimensional electrophoretic analysis of the protein of isolated soybean bodies and of the glycosylation of soybean proteins. *J. Agric. Food Chem.*, **35**:296–300.
- [35] Moreira, M.A., Hermodson, M.A., Larkins, B.A., and Nielson, N.C. 1979. Partial characterization of the acidic and basic polypeptides of glycinin. *J. Biol. Chem.*, **254**:9921–9926.
- [36] Staswick, P.E., Hermodson, M.A., and Nielson, N.C. 1981. Identification of the acidic and basic subunit complexes of glycinin. *J. Biol. Chem.*, **256**:8752–8755.
- [37] Badley, R.A., Atkinson, D., Hauser, H., Oladani, D., Green, J.P., and Stubbs, J.M. 1975. The structural, physical, and chemical properties of soybean protein glycinin. *Biochim. Biophys. Acta.*, **412**:214–228.
- [38] Yaklich, R.W., Helm, R.M., Cockrell, G., and Herman, E.M. 1999. Analysis of the distribution of the major soybean seed allergens in a core collection of *Glycine max* accessions. *Crop Science*, **39**(5):1444–1447.

- [39] Ogawa, T., Bando, N., Tsuji, H., Nishikawa, K., and Kitamura, K. 1993. Identification of the soybean allergenic protein, *Gly m* Bd 30 K, with the soybean seed 34-kDa oil-body-associated protein *Biosci. Biotechnol. Biochem.*, **57**(6):1030–1033.
- [40] Helm, R.M., Cockrell, G., Herman, E., Burks, A.W., Sampson, H.A., and Bannon, G.A. 1998. Cellular and molecular characterization of a major soybean allergen. *Int. Arch. Allergy Immunol.*, **117**:29–37.
- [41] Herman, E.M., Melroy, D.L., and Buckhout, T.J. 1990. Apparent processing of a soybean oil body protein accompanies the onset of oil mobilization. *Plant Physiol.*, **94**:341–349.
- [42] Gonzalez, R., Polo, F., Zapatero, L., Caravaca, F., and Carreira, J. 1992. Purification and characterization of major inhalant allergens from soybean hulls. *Clin. Exp. Allergy*, **22**:748–755.
- [43] Gonzalez, R., Varela, J., Carreira, J., and Polo, F. 1995. Soybean hydrophobic protein and soybean hull allergy. *Lancet*, **346**:48–49.
- [44] Ogawa, T., Bando, N., Tsuji, H., Nishikawa, K., and Kitamura, K. 1995. α -Subunit of β -conglycinin, an allergenic protein recognised by IgE antibodies of soybean-sensitive patients with atopic dermatitis. *Biosci. Biotechnol. Biochem.*, **59**(5):831–833.
- [45] Beardslee, T.A., Zeece, M.G., Sarath, G., and Markwell, J.P. 2000. Soybean glycinin G1 acidic chain shares IgE epitopes with peanut allergen *Ara h* 3. *Int. Arch. Allergy Immunol.*, **123**(4):299–307.
- [46] Tsuji, H., Bando, N., Hiemori, M., Yamanishi, R., Kimoto, M., Nishikawa, K., and Ogawa, T. 1997. Purification and characterization of soybean allergen *Gly m* Bd 28 K. *Biosci. Biotechnol. Biochem.*, **61**(6):942–947.
- [47] Hiemori, M., Bando, N., Ogawa, T., Shimada, H., Tsuji, H., Yamanishi, R., and Terao, J. 2000. Occurrence of IgE antibody-recognizing N-linked glycan moiety of a soybean allergen, *Gly m* Bd 28 K. *Int. Arch. Allergy Immunol.*, **122**(4):238–245.
- [48] Tsuji, H., Hiemori, M., Kimoto, M., Yamashita, H., Kobatake, R., Adachi, M., Fukuda, T., Bando, N., Okita, M., and Utsumi, S. 2001. Cloning of cDNA encoding a soybean allergen, *Gly m* Bd 28 K. *Biochim. Biophys. Acta.*, **1518**(1–2):178–182.
- [49] Pedersen, H.S., and Djurtoft, R. 1989. Antigenic and allergenic properties of acidic and basic peptide chains from glycinin. *Food Agric. Immunol.*, **1**(2):101–109.
- [50] Helm, R.M., Cockrell, G., Connaughton, C., Sampson, H.A., Bannon, G.A., Beilinson, V., Livingstone, D., Nielsen, N.C., and Burks, A.W. 2000. A soybean G2 glycinin allergen. 1. Identification and characterization. *Int. Arch. Allergy Immunol.*, **123**(3):205–212.
- [51] Zeece, M.G., Beardslee, T.A., Markwell, J.P., and Sarath, G., 1999. Identification of an IgE-binding region in soybean acidic glycinin G1. *Food Agric. Immunol.*, **11**(1):83–90.
- [52] Helm, R.M., Cockrell, G., Connaughton, C., Sampson, H.A., Bannon, G.A., Beilinson, V., Nielsen, N.C., and Burks, A.W. 2000. A soybean G2 glycinin allergen. 2. Epitope mapping and three-dimensional modeling. *Int. Arch. Allergy Immunol.*, **123**(3):213–219.
- [53] Xiang, P., Beardslee, T.A., Zeece, M.G., Markwell, J., and Sarath, G. 2002. Identification and analysis of a conserved immunoglobulin E-binding epitope in soybean G1a and G2a and peanut *Ara h* 3 glycinins. *Arch. Biochem. Biophys.*, **408**(1):51–57.
- [54] Rabjohn, P., Helm, E.M., Stanley, J.S., West, C.M., Sampson, H.A., Burks, A.W., and Bannon, G.A. 1999. Molecular cloning and epitope analysis of the peanut allergen *Ara h* 3. *J. Clin. Invest.*, **103**(4):535–542.
- [55] Duke, W.W. 1934. Soybean as a possible important source of allergy. *J. Allergy*, **5**:300–305.
- [56] Moroz, L.A., and Yang, W.H. 1980. Kunitz soybean trypsin inhibitor: a specific allergen in food anaphylaxis. *N. Engl. J. Med.*, **302**:1126–1128.
- [57] Baur, X., Pau, M., Czuppon, A., and Fruhmman, G. 1996. Characterization of soybean allergens causing sensitization of occupationally exposed bakers. *Allergy*, **51**(5):326–330.
- [58] Quirce, S., Fernandez-Nieto, M., Polo, F., and Sastre, J. 2002. Soybean trypsin inhibitor is an occupational inhalant allergen. *J. Allergy Clin. Immunol.*, **109**(1):178.
- [59] Codina, R., Locky, R.F., Fernandez-Caldas, E., and Rama, R. 1997. Purification and characterization of a soybean hull allergen responsible for the Barcelona asthma outbreaks. II. Purification and sequencing of the *Gly m* 2 allergen. *Clin. Exp. Allergy*, **27**(4):424–430.
- [60] Codina, R., Locky, R.F., Fernandez-Caldas, E., and Rama, R. 1999. Identification of the soybean hull allergens responsible for the Barcelona asthma outbreaks. *International Arch. Allergy Immunol.*, **119**:69–71.
- [61] Quirce, S., Polo, F., Figueredo, E., Gonzalez, R., and Sastre, J. 2000. Occupational asthma caused by soybean flour in bakers -difference with soybean-induced epidemic asthma. *Clin. Exp. Allergy*, **30**(6):839–846.
- [62] Rihs, H.P., Chen, Z., Rueff, F., Petersen, A., Rozynek, P., Heimann, H., and Baur, X. 1999. IgE binding of the recombinant allergen soybean profilin (r*Gly m* 3) is mediated by conformational epitopes. *J. Allergy Clin. Immunol.*, **104**(6):1293–1301.
- [63] Crowell, D.N., John, M.E., Russell, D., and Amasino, R.M. 1992. Characterization of a stress-induced, developmentally regulated gene family from soybean. *Plant Mol. Biol.*, **18**:459–466.
- [64] Kleine-Tebbe, J., Vogel, L., Crowell, D.N., Hausteine, U.F., and Vieths, S. 2002. Severe oral allergy syndrome and anaphylactic reactions caused by a *Bet v* 1-related PR-10 protein in soybean, SAM22. *J. Allergy Clin. Immunol.*, **110**(5):797–804.
- [65] Neudecker, P., Lehmann, K., and Rosch, P. 2003. Sequence-specific 1H, 13C and 15N resonance assignments of SAM22, an allergenic stress-induced protein from soy bean. *J. biomol. NMR.*, **26**(2):191–192.
- [66] Mittag, D., Vieths, S., Vogel, L., Becker, W.M., Rihs, H.P., Helbling, A., Wuthrich, B., and Ballmer-Weber, B.K. 2004. Soybean allergy in patients allergic to birch pollen: clinical investigation and molecular characterization of allergens. *J. Allergy Clin. Immunol.*, **113**(1):148–154.
- [67] Hessing, M., Bleeker, H., Tsuji, H., Ogawa, T., and Vlooswijk, R.A.A. 1996. Comparison of human IgE-binding soya bean allergenic protein *Gly m* 1 with the antigenicity profiles of calf anti-soya protein sera. *Food Agric. Immunol.*, **8**(1):51–58.
- [68] Codina, R., Arduoso, L., Locky, R.F., Crisci, C.D., Jaen, C., and Bertoya, N.H. 2002. Identification of the soybean hull allergens involved in sensitization to soybean dust in a rural population from Argentina and N-terminal sequence of a major 50 KD allergen. *Clin. Exp. Allergy.*, **32**(7):1059–1063.
- [69] Sharon, N., and Lis, H. 1972. Lectins: cell-agglutinating and sugar-specific proteins. *Science*, **177**:949–959.
- [70] Barnett, D., and Howden, M.E. 1987. Lectins and the radioallergosorbent test. *J. Allergy Clin Immunol.*, **80**(4):558–561.
- [71] Hessing, M., Bleeker, H., van Biert, R., Sleijsters Selis, H., van Duijn, G., van Westerop, H., and Vlooswijk, R.A.A. 1995. Analysis of proteinaceous antinutritional compounds in soya oilseed products. In: *Immunoassays for Residue Analysis: Food Safety: Proceedings of a Symposium*. Washington D.C.: ACS Anaheim, April 1995, 219–230.
- [72] Mills, E.N.C., Madsen, C., Shewry, P.R., and Wichers, H.J. 2001. Plant food protein allergens: the role of structure and function in allergenic potential. *Food Allergy and Intolerance*, **32**:194–209.
- [73] Freed, D.L.J. 2002. Dietary lectins and disease. In: *Food Allergy and Intolerance*, 2nd ed., London: Saunders Publishers, 479–495.
- [74] Fu, T.J., Abbott, U.R., and Hatzos, C. 2002. Digestibility of food allergens and nonallergenic proteins in simulated gastric fluid and simulated intestinal fluid—a comparative study. *J. Agric. Food Chem.*, **50**(24):7154–7160.
- [75] Shibasaki, M., Sumazaki, R., Isoyama, S., and Takita, H. 1992. Interaction of lectins with human IgE: IgE binding property and histamine-releasing activity of twelve plant lectins. *Int. Arch. Allergy Immunol.*, **98**:18–25.
- [76] Burks, A.W., Cockrell, G., Connaughton, C., Guin, J., Allen, W., and Helm, R.M. 1994. Identification of peanut agglutinin and soybean trypsin inhibitor as minor legume allergens. *Int. Arch. Allergy Immunol.*, **231**:877–887.
- [77] Montagnon, I., Errahali, Y., Metche, M., Nicolas, J.P., and Fremont, S. 2003. Allergenicity of soya oils. *Food Allergy and Intolerance*, **4**(2):153–163.
- [78] Moneret-Vautrin, D.A., Morisset, M., Flabbee, J., Kanny, G., Kirch, F., and Parisot, L. 2002. Unusual soy oil allergy. *Allergy*, **57**(3):266–267.

- [79] Bush, R.K., Taylor, S.L., Nordlee, J.A., and Busse, W.W. 1985. Soybean oil is not allergenic to soybean-sensitive individuals. *J. Allergy Clin. Immunol.*, **76**(2, 1):242–245.
- [80] Unknown. 2005. Soy oil helps shape food allergen labelling rules. In: <http://www.newswise.com/articles/view/511384>.
- [81] Müller, U., Weber, W., Hoffmann, A., Franke, S., Lange, R., and Vieths, S. 1998. Commercial soybean lecithins: a source of hidden allergens? *Z. Lebensm Unters Forsch*, **207**(5):341–351.
- [82] Gu, X., Beardslee, T., Zeece, M., Sarath, G., and Markwell, J. 2001. Identification of IgE-binding proteins in soy lecithin. *Int. Arch. Allergy Immunol.*, **126**(3):218–225.
- [83] Herian, A.M., Taylor, S.L., and Bush, R.K. 1990. Identification of soybean allergens by immunoblotting with sera from soy-allergic adults. *Int. Arch. Allergy Appl. Immunol.*, **92**:193–198.
- [84] Fries, J.H. 1971. Studies on the allergenicity of soy bean. *Ann. Allergy*, **29**:1–7.
- [85] Bernhisel-Broadbent, J., and Sampson, H.A. 1989. Cross-allergenicity in the legume botanical family in children with food hypersensitivity. *J. Allergy Clin. Immunol.*, **83**:435–440.
- [86] Eigenmann, P.A., Burks, A.W., Bannon, G.A., and Sampson, H.A. 1996. Identification of unique peanut and soy allergens in sera adsorbed with cross-reacting antibodies. *J. Allergy Clin. Immunol.*, **98**:969–978.
- [87] Sicherer, S.H., Sampson, H.A., and Burks, A.W. 2000. Peanut and soy allergy: a clinical and therapeutic dilemma. *Allergy*, **55**:515–521.
- [88] Burks, A.W., Brooks, J.R., and Sampson, H.A. 1988. Allergenicity of major component proteins of soybean determined by enzyme-linked immunosorbent assay (ELISA) and immunoblotting in children with atopic dermatitis and positive soy challenges. *J. Allergy Clin. Immunol.*, **11**:1135–1142.
- [89] Pons, L., Ponnapan, U., Hall, R.A., Simpson, P., Cockrell, G., West, M., Sampson, H.A., Helm, R.M., and Burks, A.W. 2004. Soy immunotherapy for peanut-allergic mice: Modulation of the peanut-allergic response. *J. Allergy Clin. Immunol.*, **114**(4):915–921.
- [90] Host, A., and Halken, S. 1990. A prospective study of cow milk allergy in Danish infants during the first 3 years of life. Clinical course in relation to clinical and immunological type of hypersensitivity reaction. *Allergy*, **45**:587–596.
- [91] Burks, A.W., Casteel, H.B., Fiedorek, S.C., Williams, L.W., and Pumphrey, C.L. 1994. Prospective oral food challenge study of two soybean protein isolates in patients with possible milk or soy protein enterocolitis. *Pediatr. Allergy Immunol.*, **5**:40–45.
- [92] Cantani, A., and Lucenti, P. 1997. Natural history of soy allergy and/or intolerance in children, and clinical use of soy-protein formulas. *Pediatr. Allergy Immunol.*, **8**:59–74.
- [93] Rozenfeld, P., Docena, G.H., Anon, M.C., and Fossati, C.A. 2002. Detection and identification of a soy protein component that cross-reacts with caseins from cow's milk. *Clin. Exp. Immunol.*, **130**:49–58.
- [94] Wal, J.M. 1999. Recognition of allergenic structures in 'novel' food. *Sciences des Aliments*, **19**(3/4):409–422.
- [95] Breiteneder, H., and Radauer, C.A. 2004. Classification of plant food allergens. *J. Allergy Clin. Immunol.*, **113**(5):821–830.
- [96] Gidamis, A.B., Mikami, B., Katsube, T., Utsumi, S., and Kito, M. 1994. Crystallization and preliminary X-ray analysis of soybean proglycinins modified by protein engineering. *Biosci. Biotechnol. Biochem.*, **58**(4):703–706.
- [97] Utsumi, S., Gidamis, A.B., Mikami, B., and Kito, M. 1993. Crystallization and preliminary X-ray crystallographic analysis of the soybean proglycinin expressed in *Escherichia coli*. *J. Mol. Biol.*, **233**(1):177–178.
- [98] Adachi, M., Takenaka, Y., Gidamis, A.B., Mikami, B., and Utsumi, S. 2001. Crystal structure of soybean proglycinin A1aB1b homotrimer. *J. Mol. Biol.*, **305**(2):291–305.
- [99] Maruyama, N., Adachi, M., Takahashi, K., Yakasaki, K., Kohno, M., Takenaka, Y., Okuda, E., Nakagawa, S., Mikami, B., and Utsumi, S. 2001. Crystal structures of recombinant and native soybean beta-conglycinin beta homotrimers. *Eur. J. Biochem.*, **268**(12):3595–3604.
- [100] Breiteneder, H., and Ebner, C. 2000. Molecular and biochemical classification of plant-derived food allergens. *J. Allergy Clin. Immunol.*, **106**:27–36.
- [101] Molina, A., Segura, A., and Garcia-Olmedo, F. 1993. Lipid transfer proteins (LTPs) from barley and maize leaves are potent inhibitors of bacterial and fungal plant pathogens. *FEBS Lett.*, **316**:119–122.
- [102] Baud, F., Pebay-Peyroula, E., Cohen-Addad, C., Odani, S., and Lehmann, M.S. 1993. Crystal structure of hydrophobic protein from soybean; a member of a new cysteine-rich family. *J. Mol. Biol.*, **231**(3):877–887.
- [103] Mills, E.N.C., Jenkins, J.A., and Bannon, G.A. 2004. Plant seed globulin allergens. In: Oxford Ed. *Plant Food Allergens*. London: Blackwell Science Ltd., 141–157.
- [104] Pastorello, E.A., Pravettoni, V., Calamari, A.M., Banfi, E., and Robino, A.M. 2002. New plant-origin food allergens. *Allergy*, **57**(Suppl 72):106–110.
- [105] Seppala, U., Majamaa, H., Turjanmaa, K., Helin, J., Reunal, T., Kalkkinen, N., and Palosuo, T. 2001. Identification of four novel potato (*Solanum tuberosum*) allergens belonging to the family of soybean trypsin inhibitors. *Allergy*, **56**(7):619–626.
- [106] Kim, S.H., Hara, S., Hase, S., Ikenaka, T., Toda, H., Kitamura, K., and Kaizuma, N. 1985. Comparative study on amino-acid sequences of Kunitz-type soybean trypsin inhibitors TI-A TI-3 and TI-C. *J. Biochem. (Tokyo)*, **98**(2):435–448.
- [107] Song, H.K., and Suh, S.W. 1998. Kunitz-type soybean trypsin inhibitor revisited: refined structure of its complex with porcine trypsin reveals an insight into the interaction between a homologous inhibitor from *Erythrina caffra* and tissue-type plasminogen activator. *J. Mol. Biol.*, **275**:347–363.
- [108] Roychaudhuria, R., Sarathb, G., Zeece, M., and Markwell, J. 2004. Stability of the allergenic soybean Kunitz trypsin inhibitor. *Biochim. Biophys. Acta.*, **1699**(1–2):207–212.
- [109] Ji, C., Boyd, C., Slaymaker, D., Okinaaka, Y., Takeuchi, Y., Midland, S.L., Sims, J.J., Herman, E., and Keen, N. 1998. Characterization of a 34-kDa soybean binding protein for the syringolide elicitors. *Proceedings of the National Academy of Sciences of the United States of America.*, **95**(6):3306–3311.
- [110] Midori-Horiuti, T., Brooks, E.G., and Goldblum, R.M. 2001. Pathogenesis-related proteins of plants as allergens. *Ann. allergy Asthma Immunol.*, **87**:261–271.
- [111] Hoffmann-Sommergruber, K. 2000. Plant allergens and pathogenesis-related proteins. What do they have in common? *Int. Arch. Allergy Immunol.*, **122**:155–166.
- [112] Hoffmann-Sommergruber, K. 2002. Pathogenesis-related (PR)-proteins identified as allergens. *Biochem. Soc. Trans.*, **30**:930–935.
- [113] Breiteneder, H., Pottenburger, K., Bito, A., Valenta, R., Kraft, D., Rumpold, H., Scheiner, O., and Breitenbach, M. 1989. The gene coding for the major birch pollen allergen *Bet v 1*, is highly homologous to a pea disease resistance response gene. *EMBO J.*, **8**(7):1938–1938.
- [114] Ramachandran, S., Christensen, H.E., Ishimaru, Y., Dong, C.H., Chao-Ming, W., Cleary, A.L., and Chua, N.H. 2000. Profilin plays a role in cell elongation, cell shape maintenance, and flowering in *Arabidopsis*. *Plant Physiol.*, **124**(4):1637–1647.
- [115] Jankiewicz, A., Bahes, W., Bogl, K.W., Dehne, L.I., Jamin, A., Hoffmann, A., Haustein, D., and Vieths, S. 1997. In Vitro study of the gastrointestinal stability of celery allergens. *Food Agric. Immunol.*, **9**:203–217.
- [116] Jankiewicz, A., Bahes, W., Bogl, K.W., Dehne, L.I., Jamin, A., Hoffmann, A., Haustein, D., and Vieths, S. 1997. Influence of food processing on the immunochemical stability of celery allergens. *J. Sci. Food Agric.*, **75**:359–370.
- [117] Poms, R.E., Klein, C.L., and Anklam, E. 2004. Methods for allergen analysis in food: a review. *Food Additives and Contaminants*, **21**(1):1–31.
- [118] Hitchcock, C.H.S., Bailey, F.J., Crimes, A.A., Dean, D.A.G., and Davis, P.J. 1981. Determination of soy proteins in food using an enzyme-linked immunosorbent assay procedure. *J. Sci. Food Agric.*, **32**:157–165.
- [119] Griffiths, N.M., Billington, M.J., Crimes, A.A., and Hitchcock, C.H.S. 1984. An assessment of commercially available reagents for an

- enzyme-linked immunosorbent assay (ELISA) of soy protein in meat products. *J. Sci. Food Agric.*, **35**:1255–1260.
- [120] Rittenburg, J.H., Adams, A., Palmer, J., and Allen, J.C. 1987. Improved enzyme-linked immunosorbent assay for determination of soy protein in meat products. *J. Assoc. Off. Anal. Chem.*, **70**:582–587.
- [121] Yasumoto, K., Sudo, M., and Suzuki, T. 1990. Quantitation of soya protein by enzyme linked immunosorbent assay of its characteristic peptide. *J. Sci. Food Agric.*, **61**:19–23.
- [122] Heppell, L.M., Sissons, J.W., and Pedersen, H.E. 1987. A comparison of the antigenicity of soybean-based infant formulas. *Br. J. Nutr.*, **58**:393–403.
- [123] Brandon, D.L., Bates, A.H., and Friedman, M. 1991. ELISA analysis of soybean trypsin inhibitors in processed foods. *Adv. Exp. Med. Biol.*, **289**:321–337.
- [124] Tsuji, H., Okada, N., Yamanishi, R., Bando, N., Kimoto, M., and Ogawa, T. 1995. Measurement of *Gly m Bd 30 K*, a major soybean allergen, in soybean products by a sandwich enzyme-linked immunosorbent assay. *Biosci. Biotechnol. Biochem.*, **59**(1):150–151.
- [125] Yeung, J.M., and Collins, P.G. 1997. Determination of soy proteins in food products by enzyme immunoassay. *Food Technol. Biotechnol.*, **35**(3):209–214.
- [126] Bando, N., Tsuji, H., Hiemori, M., Yoshizumi, K., Yamanishi, R., Kimoto, M., and Ogawa, T. 1998. Quantitative analysis of *Gly m Bd 28 K* in soybean products by a sandwich enzyme-linked immunosorbent assay. *J. Nutr. Sci. Vitaminol.*, **44**(5):655–664.
- [127] Macedo-Silva, A., Shimokomaki, M., Vaz, A.J., Yamamoto, Y.Y., and Tenuta-Filho, A. 2001. Textured soy protein quantification in commercial hamburger. *J. Food Compos. Analysis*, **14**:469–478.
- [128] Boutten, B., Humbert, C., Chelbi, M., Durand, P., and Peyraud, D. 1999. Quantification of soy proteins by association of immunochemistry and video image analysis. *Food Agric. Immunol.*, **11**:51–59.
- [129] Hird, H., Pumphrey, R., Wilson, P., Sunderland, J., and Reece, P. 2000. Identification of peanut and hazelnut allergens by native two-dimensional gel electrophoresis. *Electrophoresis*, **21**:2678–2683.
- [130] Sander, I., Flagge, A., Merget, R., Halder, T.M., Meyer, H.E., and Baur, X. 2001. Identification of wheat flour allergens by means of 2-dimensional immunoblotting. *J. Allergy Clin. Immunol.*, **107**:907–913.
- [131] Helm, R.M., Burks, W., and Herman, E. 2002. Hypoallergenic Foods-Soybeans and peanuts. *ISB News Report*, October Issue, 3–5.
- [132] American Academy of Pediatrics. 1990. Infant formulas and allergic disease. *American Academy of Pediatrics, Subcommittee on Nutrition and Allergic Disease*, Evanston, III, 1990.
- [133] ESPAGN Committee on Nutrition. 1993. Comment on antigen-reduced infant formulae. *Acta Paediatr.*, **82**:314–319.
- [134] Host, A., Koletzko, B., Dreborg, S., Muraro, A., Wahn, U., Aggett, P., Bresson, J.L., Hernell, O., Lafeber, H., Michaelsen, K.F., Micheli, J.L., Rigo, J., Weaver, L., Heymans, H., Strobel, S., and Vandenplas, Y. 1999. Dietary products used in infants for treatment and prevention of food allergy. Joint Statement of the European Society for Paediatric Allergology and Clinical Immunology (ESPACI) Committee on Hypoallergenic Formulas and the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Committee on Nutrition. *Arch. Dis. Child*, **81**(1):80–84.
- [135] Shibasaki, M., Suzuki, S., Tajima, S., Nemoto, H., and Kuruome, T. 1980. Allergenicity of major component proteins of soybean. *Int. Arch. Appl. Immunol.*, **61**:441–448.
- [136] Burks, A.W., Williams, L.W., Helm, R.M., Thresher, W., Brooks, J.R. and Sampson, H.A. 1990. Identification of soy protein allergens in patients with atopic dermatitis and positive soy challenges; determination of change in allergenicity after heating or enzyme digestion. In: *Nutritional and toxicological consequences of food processing: Proceedings of a symposium*. Washington D.C.: April 1990.
- [137] Burks, A.W., Williams, L.W., Thresher, W., Connaughton, C., Cockrell, G., and Helm, R.M. 1992. Allergenicity of peanut and soybean extracts altered by chemical or thermal denaturation in patients with atopic dermatitis and positive food challenges. *J. Allergy Clin. Immunol.*, **90**:889–897.
- [138] Davis, P.J., and Williams, S.C. 1998. Protein modification by thermal processing. *Allergy*, **53**(Suppl. 46):102–105.
- [139] Lakemond, C.M.M., De jongh, H.H., Hessing, M., Gruppen, H., and Voragen, A.G.J. 2000. Heat denaturation of soy glycinin: Influence of pH and ionic strength on molecular structure. *J. Agric. Food Chem.*, **48**:1991–1995.
- [140] Codina, R., Oehling, A.G., and Lockey, R.F. 1998. Neoallergens in heated soybean hull. *Int. Arch. Allergy. Immunol.*, **117**(2):120–125.
- [141] Tsai, E., Yeung, J., Gold, M., Sussman, G., Perelman, B., and Vadas, P. 2003. Study of the allergenicity of plant protein hydrolysates. *Food Allergy and Intolerance*, **4**(2):117–126.
- [142] Herian, A.M., Taylor, S.L., and Bush, R.K. 1993. Allergenic reactivity of various soybean products as determined by RAST inhibition. *J. Food Sci.*, **58**(2):385–388.
- [143] Tsuji, H., Okada, N., Yamanashi, R., Bando, N., Ebine, H., and Ogawa, T. 1997. Fate of major soybean allergen, *Gly m Bd 30 K*, in rice-, barley-, and soybean-koji miso (fermented soybean paste) during fermentation. *Food Sci. Technol. Int. Tokyo*, **3**:145–149.
- [144] Yamanishi, R., Huang, T., Tsuji, H., Bando, N., Kimoto, M., and Ogawa, T. 1995. Reduction of the soybean allergenicity by the fermentation with *Bacillus natto*. *Food Sci. Technol. Int.*, **1**:14–17.
- [145] Yamanishi, R., Tsuji, H., Bando, N., Yamada, Y., Nadaoka, Y., Tao, H., Nishikawa, K., Emoto, S., and Ogawa, T. 1996. Reduction of the allergenicity of soybean by treatment with proteases. *J. Nutr. Sci. Vitaminol.*, **42**(6):581–587.
- [146] Tumura, K., Kugimiya, W., Bando, N., Hiemori, M., and Ogawa, T. 1999. Preparation of hypoallergenic soybean protein with processing functionality by selective enzymatic hydrolysis. *Food Sci. Technol. Res.*, **5**:171–175.
- [147] Lee, J.O., Lee, S.I., Cho, S.H., Oh, C.K., and Ryu, C.H. 2004. A new technique to produce hypoallergenic soybean proteins using three different fermenting microorganisms. *J. Allergy Clin. Immunol.*, **113**(2):S239.
- [148] Wal, J.M. 2002. Cow's milk proteins/Allergens. *Ann. Allergy Asthma Immunol.*, **89**:3–10.
- [149] Niggemann, B., Sielaff, B., Beyer, K., Binder, C., and Wahn, U. 1999. Outcome of double blind placebo controlled food challenge test in 107 children with atopic dermatitis. *Clin. Exp. Allergy*, **29**:91–96.
- [150] Babiker, E.F.E., Hiroyuki, A., Matsudomi, N., Iwata, H., Ogawa, T., Bando, N., and Kato, A. 1998. Effect of polysaccharide conjugation or transglutaminase treatment on the allergenicity and functional properties of soy protein. *J. Agric. Food Chem.*, **46**(3):866–871.
- [151] Babiker, E.F.E., Matsudomi, N., and Kato, A. 1998. Masking of antigen structure of soybean protein by conjugation with polysaccharide and cross-linkage with microbial transglutaminase. *Nahrung*, **42**(3–4):158–159.
- [152] Arita, K., Babiker, E.E., Azakami, H., and Kato, A. 2001. Effect of chemical and genetic attachment of polysaccharides to proteins on the production of IgG and IgE. *J. Agric. Food Chem.*, **49**(4):2030–2036.
- [153] Usui, M., Tamura, H., Nakamura, K., Ogawa, T., Muroshita, M., Akazami, H., Kanuma, S., and Kato, A. 2004. Enhanced bactericidal action and masking of allergen structure of soy protein by attachment of chitosan through Maillard-type protein-polysaccharide conjugation. *Nahrung/Food*, **48**(1):69–72.
- [154] Zhu, Y., Rinzema, A., Tramper, J., and Bol, J. 1995. Microbial transglutaminase - A review of its production and application in food processing. *Appl. Microbiol. Biotechnol.*, **44**(3/4):277–282.
- [155] Fukushima, D. 2001. Recent progress in research and technology on soybeans. *Food Sci. Technol. Res.*, **7**(1):8–16.
- [156] Bernard, R., Hymowitz, T., and Cremeens, C.R. 1991. Registration of 'Kunitz' soybean. *Crop Sci.*, **31**:232–233.
- [157] Takahashi, K., Banba, H., Kikuchi, A., Ito, M., and Nakamura, S. 1994. An induced mutant line lacking the α -subunit of β -conglycinin in soybean (*Glycine max* (L) Merrill). *Breeding Sci.*, **44**:65–66.
- [158] Samoto, M., Fukuda, Y., Takahashi, K., Tabuchi, K., Hiemori, M., Tsuji, H., Ogawa, T., and Kawamura, Y. 1997. Substantially complete removal

- of three major allergenic soybean proteins (*Gly m* Bd 30 K, *Gly m* Bd 28 K, and the α -subunit of conglycinin) from soy protein by using a mutant soybean, Tohoku 124. *Biosci. Biotechnol. Biochem.*, **61**(12):2148–2150.
- [159] Hajika, M., Takahashi, M., Sakai, S., and Matsunaga, R. 1998. Dominant inheritance of a trait lacking β -conglycinin detected in wild soybean line. *Breeding Sci.*, **48**:386–386.
- [160] Takahashi, M., Hajika, M., Matsunaga, R., Komatsu, K., Obata, A., and Kanegae, R. 2000. Breeding of soybean variety lacking β -conglycinin by the introduction of *Seg* gene from wild soybean. In: Jpn. Soc. Food Sci. Technol., Ed. *The Proceedings of the Third International Soybean Processing and Utilization Conference*, 45–46.
- [161] Helm, R.M., Cockrell, G., West, C.M., Herman, E.M., Sampson, H.A., Bannon, G.A., and Burks, A.W. 2000. Mutational analysis of the IgE-binding epitopes of P34/*Gly m* 1. *J. Allergy Clin. Immunol.*, **105**:378–384.
- [162] Herman, E.M., Helm, R.M., Jung, R., and Kinney, A.J. 2003. Genetic modification removes an immunodominant allergen from soybean. *Plant Physiol.*, **132**(1):36–43.
- [163] Kinney, A.J., and Jung, R. 2003. Hypoallergenic transgenic soybeans. *US Patent*, No. US2003/0041350 A1.
- [164] Tada, Y., Nakase, M., Adachi, T., Nakamura, R., Shimada, H., Takabashi, M., Fujimura, T., and Matsuda, T. 1996. Reduction of 14–16 kDa allergenic proteins in transgenic rice plants by antisense gene. *FEBS Lett.*, **391**:341–345.
- [165] Yang, G., Matsuoka, M., Iwasaki, Y., and Komatsu, S. 2003. A novel brassinolide-enhanced gene identified by cDNA microarray is involved in the growth of rice. *Plant Mol. Biol.*, **52**:843–854.