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Soybean Allergens: Presence, Detection and Methods for Mitigation

Weihua Wade Yang[1], Elvira Gonzalez de Mejia[2], Huanyu Zheng[2] and Youngsoo Lee[2]
[1]*Department of Food Science & Human Nutrition, University of Florida, Gainesville, FL*
[2]*Department of Food Science and Human Nutrition, University of Illinois, Urbana-Champaign, IL*
USA

1. Introduction

As a major food ingredient, soybean provides numerous health benefits, for example, individuals who consume soybean-rich diets exhibit lower incidence of high plasma cholesterol, cancer (including bowel and kidney), diabetes mellitus, and obesity (Carroll and Kurowska, 1995; Friedman and Brandon, 2001; Duranti et al., 2004; Ali et al., 2004; Omini and Aluko, 2005; Kim et al., 2006). However, soybean is also among the "big 8" most allergenic foods, and the only possible solution currently to prevent allergenic reactions is total avoidance of the allergen-containing foods. Because soybean is ubiquitous in vegetarian and many meat based food products, avoidance has become increasingly difficult, and its prevalence will inevitably continue to rise. Due to the innumerable health benefits and economic importance of soy commercial products, there is a mounting need to remove the allergenic components contained in soybean proteins to a threshold deemed to be safe. The estimated threshold level for common food allergy is usually low and a small amount of the allergen may be enough to trigger an allergenic reaction (Poms et al., 2004). The threshold for soybean allergen is estimated to range from 88 mg to several grams of soy protein (Bindslev-Jensen et al., 2002; Fiocchi et al., 2003).

Soybean allergy is of particular importance because soybean is widely used in processed foods and represents a particularly insidious source of hidden allergens. Since finding foods that do not contain soy is difficult and total elimination of food allergens is practically impossible to attain, investigations on the hypoallergenization of soy ingredients and products are imperative. Current requirements by the labeling regulations also make it imperative to identify a processing technology that is capable of reducing or eliminating the allergens from soy containing products. Elimination or reduction of allergens in allergenic foods has been attempted for years by various investigators, which has included, among various strategies, the use of genetic engineering, thermal processing, enzyme treatment, ultrafiltration, chemical agents, microwave, irradiation, high pressure processing, pulsed ultraviolet light, power ultrasound and pulsed electric field. The reduction or elimination of allergens from soybean proteins by different processing technologies offers unique insight to the structure and biological interaction of the antigenic proteins. With allergens reduced, the industry can further profit from the economical attributes of soybean and promote the

soy products more rigorously along the health route. The consumers will be less concerned about allergenicity of the product and can enjoy the health benefits of soybean without the threat of an allergic reaction.

Nevertheless, the use of conventional processing method, alone or in combination, has found the complexity of eliminating or reducing the allergenicity of foods and also affecting their functional or organoleptic properties. Processing methods, such as heat treatment and enzymatic fragmentation, can reduce soy allergen reactivity in food products, but a major obstacle is the risk to alter unique functional attributes of soy proteins. Furthermore, not enough emphasis has been given to understanding the way the insidious protein interacts with the IgE binding sites, while such understanding is important in guiding a proper modification of these sites of the allergic protein. Emerging alternative food processing technologies may reduce protein immunogenicity by inducing changes in protein conformations, which mask, inactivate or destroy IgE binding sites, known as epitopes. Novel processing methods, such as pulsed ultraviolet light (PUV) and high pressure processing (HPP), alone or combined with biochemical treatments, or extrusion combined with inclusion of bioactive compounds, hold a great promise for the development of hypoallergenic food products with unique functional properties.

The objective of this chapter is to provide an overview of the major aspects of soy allergen research, which includes the presence of soy allergens (i.e., major allergenic proteins identified and their mechanism of reaction), methods for soy allergen detection and characterization, and thermal and non-thermal methods to mitigate soybean allergens and their status of development.

2. Food allergy

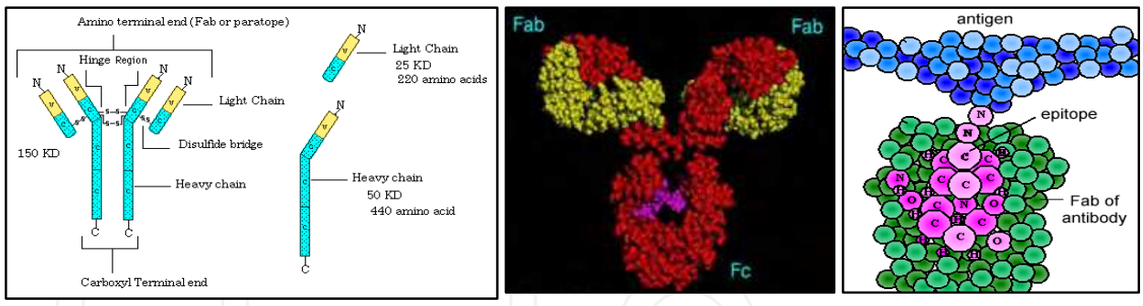
Over the past 30 years the incidences of allergies have doubled not only in industrial countries, but in developing countries as well (Pearce and Douwes, 2006). This represents an intriguing problem from a medical, epidemiological, immunological, genetic and evolutionary view. Allergic disorders are typically characterized as an abnormal or hyperactive immune response in reaction to exposure to environmental agents or allergens (Galli et al., 2008). High levels of immunoglobulin E (IgE) are a defining characteristic of such a response (Galli et al., 2008).

Immunoglobulin E is an evolutionary conserved member of the immunoglobulin (Ig) family, and the titer of IgE is very low (nano- to micrograms per ml range) in plasma of normal healthy individuals. The IgE is most prominent in epithelia and mucosa where it is bound to specific receptors on highly potent effector cells like eosinophilic granulocytes and mast cells. Bound to these cells, IgE has a long half-life (weeks to months), while free in plasma, the half-life is very short (~6 h). This suggests that IgE plays a role in local immune defense mechanisms (Gould et al., 2003). Since life-threatening, systemic reactions like anaphylactic shock may happen, the potential hazards of high systemic IgE titers are underlined.

Food allergy is a relatively rare and violent reaction of the immune system towards food proteins. It is defined as an immunologically based adverse reaction in response to dietary antigens (Beyer and Teuber, 2004). The allergen provokes an initial IgE antibody response followed by a secondary IgE antibody response, which signals an allergic reaction (Babu et al., 2001). Food proteins bind to the allergen-specific IgE molecules residing in the mast cells and basophils, causing them to release inflammatory mediators, including histamine (Beyer

and Teuber, 2004). Food allergy affects 3% of the adult population and up to 6-8% of infants. Allergenic conditions directly affect millions of people worldwide (Sampson, 1997; Goodwin, 2004), and it is believed that one in 25 Americans is susceptible to food allergies (Westphal et al., 2004; Sicherer et al., 2004). The estimated prevalence of soybean allergies is about 0.5% of the total U.S. population (Sampson, 2002; Stephan et al., 2004; Sicherer and Sampson, 2006).

Small regions of allergenic proteins known as epitopes, composed by 5-7 amino acids or 3-4 sugar residues cause the IgE-mediated allergy by reacting with an antigen (Taylor and Hefle, 2001). Specifically, the crystallizable fragment (Fc region) of IgE binds strongly to high affinity receptors on mast cells and basophils, and together with the antigen, mediates the release of inflammatory agents from these cells (Figure 1A and 1B). Then, the allergen provokes an initial IgE antibody response followed by a secondary IgE antibody response, which signals an allergic reaction in the organism (Babu et al., 2001). The level of IgE is found in extraordinarily low concentrations in the serum of humans, varying from 20 to 500 ng/ml. Therefore, IgE is important since its biological activities are greatly amplified by binding to receptors on mast cells and basophils (Tizard, 1995). The hinge region is replaced by a constant domain so that each heavy chain contains four constant domains, and as a result, IgE has a molecular weight of 190 kDa. An IgE molecule contains two identical light chains (23 kDa) and two identical heavy chains (72 kDa). Between the two antigen binding fragments (Fab region), the molecule's surface has a depression that forms the antigen-binding site (Figure 1C). Individual protein allergens can have several recognition sites (epitopes) per allergenic protein.



A. B. C.

Fig. 1. A. Structure of an immunoglobulin, B. The tridimentional molecular model of an IgE molecule showing the antigen binding site (Fab) (heavy chain in red, light chain in yellow, and carbohydrate residues in purple). C. Diagram illustrating antigen bound to fab of the antibody (reprinted with permission).

According to Lehrer et al. (1996, 2002), the epitopes are not only fully characterized by their primary structure, but also by their tertiary structure conformations.

Food allergens and their epitopes are able to resist the effects of digestion and enzymatic reactions (Taylor et al. 1987) and individual allergen systems are affected differently by the processing methods. This is because food allergens are complex mixtures of potentially immunoreactive proteins. According to Wilson et al. (2005), soybean allergens comprise proteins with molecular masses from 7 to 71 kDa.

3. Soy allergens

In humans, 34 IgE reactive proteins have been identified and characterized as related to soybean allergy (FARRP, 2008; Xiang et al., 2008). Among the numerous allergenic proteins present in soybean, the strongest immunodominant allergenic protein identified is Gly m Bd 30K, or P34 (Ogawa et al., 1993, 2000), recognized by 65 percent of soybean sensitive individuals with atopic dematitis. Gly m Bd 30K is known as a soybean oil-body associated glycoprotein that consists of 257 amino acids residues. The P34 is associated with α, α′ and β subunits of the globulin β-conglycinin by bi-sulfide linkage (Ogawa et al., 1993) and has a 30% homology to Der p 1, a major allergen of house dust mite, classified under the papain super family.

Ogawa et al. (1993, 2000) identified three major soy allergens: Gly m Bd 60K, Gly m Bd 30K and Gly m Bd 28K. Gly m Bd 60K is an α subunit of 7S β-conglycinin, well known as a major storage protein. β-conglycinin is a primarily constituent of 11S glycinin globulin fraction and includes three subunits: α (~67 kDa), α′ (~71 kDa), and β (~50 kDa) (Rihs et al., 1999). Gly m. Bd 28K is a vicillin-like glycoprotein of 473 amino acids (Xiang et al., 2004), initially isolated from soybean meal as a 28 kDa glycosylated protein. Gly m Bd 28K (Hiemori et al., 2000) constitutes a minor component fractionated into 11S glycinin globulin fraction in soybean seed flour that has been recognized by soybean sensitive patients with about 25% of incidence (Ogawa et al., 2000). Moreover, Gly m Bd 28 K protein shares sequence homology with proteins in pumpkins and carrots (Rihs et al., 1999). Studies revealed a slightly stronger IgE-binding region present in the C-terminal domain of Bd 28K (Xiang et al., 2004) than the N-terminal half of this protein (Ogawa et al., 2000). An important IgE binding region was found in the C-terminal 23 kDa polypeptide, which contains an Asn-N linked moiety with the same sugar composition as that of P34 (Hiemori et al., 2000).

Additional to the list of major soy allergens, Cordle (2004) includes soy hydrophobic protein (Gly m 1a), soy hull protein (Gly m 2), soy profiling (Gly m 3) (Klein-Tebbe et al., 2002), glycinin (320-360 kDa), β-conglycinin (140-180 kDa) and Kunitz tripsin inhibitor (20 kDa) as major soy allergenic proteins. The acidic subunit of glycinin G1 (Beardslee et al., 2000) and the basic subunit of glycinin G2 (Helm and Burks, 2000) have been classified as important allergens in patients affected by soybean allergens. Glycinin is a hexameric pure protein present in the 7S globulin fraction and each of its subunits contains an acidic and a basic polypeptide linked by a disulfide bond (Marayama et al., 2003).

Soybean processing may inactivate some antinutritional factors through heat treatment or extrusion; however, those processes may also reduce availability of amino acids, especially when the soybean product is overcooked (Danielson and Crenshaw, 1991). Fermentation has proven to improve the nutritional value of soybean by increasing the bioavailability of nutrients and reducing antinutritional factors (Hotz and Gibson, 2007; Egounlety and Aworh, 2003). Moreover, it has been demonstrated that fermentation of soybean proteins reduces its immunoreactivity toward human IgE, probably through the removal of epitopes present in the native protein (Song et al., 2008a,b; Frias et al., 2008).

4. Basic mechanisms of allergen reactions

Epitopes are generally categorized as linear or conformational, where linear epitope involves a contiguous stretch of amino acids, and a conformational epitope involves noncontiguous amino acids forming a three-dimensional or structural motif. Individual patients may differ

significantly in their sensitivity toward an allergen; however, the basis of such differential sensitivities remains to be elucidated. More than one epitope or IgE binding site is required per fragment of an allergen to cause IgE cross-linking. Therefore a molecule with a single IgE binding site must be bound or cross-linked to another molecule with an IgE binding site in order to cause histamine release. Understanding molecular properties of the epitope(s) is therefore important in learning the nature of IgE–allergen interaction. In case of linear epitopes, amino acid residues that determine whether allergen would bind with IgE or not are known as critical amino acid residues. Any modification, deletion, or substitution of such critical amino acid residues may result in loss of IgE binding and may potentially result in reduction or elimination of allergenicity. If the epitope is conformational in nature, change in epitope conformation may permit modulation of allergic activity.

Food processing, under appropriate conditions, offers opportunities to alter the nature of epitopes. For example, epitope conformation may be modified as a result of protein denaturation treatments (e.g., various thermal processing treatments) leading to reduction or elimination, or in some cases, an increase, in IgE binding. Acid or enzyme hydrolysis of an allergenic protein may help delete critical amino acids of an epitope. Whether caused by protein denaturation or hydrolysis, loss of epitope and thus loss of IgE binding may help reduce or eliminate the bioactivity of an allergen. It should be emphasized here that processing, depending on the allergen and the processing method, may not affect the allergenic properties of all allergens.

Physicochemical changes will alter the way in which allergens are broken down during digestion and may modify the form in which they are taken up across the gut mucosal barrier and presented to the immune system. Certainly, the structure of the food matrix can have a great impact on the elicitation of allergic reactions and fat-rich matrices may affect the kinetics of allergen release, potentiating the severity of allergic reactions (Grimshaw et al., 2003). Understanding the impact of food processing and food structure on allergenic potential is central to managing allergen risks in the food chain. However, our current knowledge of the impact of food processing on allergen structure indicates that there are no clear rules regarding how different allergens respond to food processing.

5. Methods for soybean allergen detection

Reliable detection and quantification methods for food allergens are required to ensure compliance with food labeling regulations and minimize risks to highly sensitive consumers from undeclared allergens in the food supply (L'Hocine and Boye, 2007; Taylor et al., 2006). 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 (Poms et al., 2004). This is particularly true for soybean, which is an insidious hidden allergen (e.g., pastries, bakery products, infant foods, sausages, processed meats, hamburgers, and dairy products).

Presently, various methods have been applied to detect soybean in food products. The majority of methods developed to detect soybeans in food products are based on immunochemical assays.

5.1 SDS-PAGE, immunoblotting and dot blotting

Several methods based on electrophoretic separation coupled with immunological detection have been developed primarily to identify soy proteins in meat products (Catsimpoolas and Leuthner, 1969). Sensitivities of these procedures are usually in the g/kg range. Janssen et al.

(1986) presented a more sensitive protocol to determine soy in meat products by SDS-PAGE/immunoblotting and dot blotting. This procedure achieved a detection limit of 0.02% (200mg/kg) soy in meat. Two-dimensional gel electrophoresis followed by immunoblotting and identification of IgE-reactive proteins are proteomic approaches to identify new allergens in foods. Protein profiling using two dimensional electrophoresis and allergens detection by IgE has become a powerful method for analyzing changes of allergen content in complex matrices during food processing (De Angelis et al., 2010).

5.2 Enzyme-linked immunosorbent assay (ELISA)

ELISA is a powerful analysis tool for detection of specific proteins. It has the advantage of simultaneously testing a larger series of samples at a high level of sensitivity. A large number of investigators have used ELISA methods for the detection of soy allergens in food products (Koppelman and Hefle, 2006). Immunochemical methods are generally limited to the qualitative screening of raw or mildly processed products, since protein denaturation often alters the antigen-antibody interaction.

5.2.1 ELISAs for soy glycinin

Ravestein and Driedonks (1986) raised antibodies against the SDS-denatured acidic polypeptides of glycinin in rabbits. An ELISA constructed with these antibodies in an immunoblotting experiment decreased the detection limit enormously, but this was not considered relevant since soy is usually used in concentrations at 1% and higher. Meisel (1993) use IgY antibodies obtained from egg yolk of immunized chickens, an enzyme-linked immunoassay (ELISA) and an immunoblotting procedure has been developed for specific determination of soy protein. The procedure for antigen preparation provides a rapid method for the isolation of SDS-denatured glycinin A from polyacrylamide gels that could be used directly for immunization. ELISA with SDS-denatured soy protein isolate was linear in the range 0.5-256 μg/ml and covers the expected levels of soy protein in simulated milk and milk products. IgY antibodies used in immunostaining of SDS-PAGE blots allowed the detection of glycinin A at nanogram levels (Meisel, 1993). Plumb et al. (1994) raised monoclonal antibodies (Mabs) against beta-conglycinin. They found that heating glycinin at pH 7.6 caused the immunoreactivity to decrease to around 50% of the original value but it increased sharply above 92°C (Plumb et al., 1994). These results are in agreement with Demonte et al. (1997) but differ from the results of Iwabuchi and Yamauchi (1984), who found that the antigenic activity disappeared after heating. Huang et al. (1998) found that the epitope identified by Plumb et al. (1994) corresponded to residues 86-104 of the acidic polypeptides of glycinin AlaB1b and lies on the C-terminus of the proteolytic intermediate known as glycinin-T. The epitope seems to be continuous in nature. Iwabuchi and Yamauchi (1984) studied the effect of ionic strength (I) on thermal denaturation of soybean glycinin. Up to I=0.7, no effects of ionic strength were found. A reduction in immunogenicity also occurred when glycinin was taken to pH 2.0 and pH 11.0 and exposed to high temperatures (Iwabuchi and Shibasaki, 1981).

5.2.2 ELISAs for soy ß-conglycinin

Plumb et al. (1994) raised specific antibodies for the native α and α′ subunits of ß-conglycinin; ß-conglycinin immunoreactivity was increased as the protein was heated, reaching a maximum at the denaturation temperature of 65°C (Plumb et al., 1995). This

phenomenon is unusual as most thermally denatured proteins have low immunoreactivity when probed with antibodies raised against native protein. This was also found at pH 7.6 at different ionic strengths (Iwabuchi and Shibasaki, 1981). The epitope of the antibody used by these researchers corresponds to the residues 78-84 in the acidic extension present in the α′ subunit of ß-conglycinin, and seems to be continuous in nature (Huang et al., 1998). A linear epitope, in contrast to a conformational epitope, is expected to be more heat stable and may become more exposed after denaturation. This may explain the increased detectability of heated ß-conglycinin.

5.2.3 ELISAs for soy trypsin inhibitors

Brandon et al. (1989) used antibodies that bind the Bowman-Birk protease inhibitor. They identified an epitope that was destroyed by heat and developed an ELISA for specific recognition of native Bowman-Birk inhibitor (BBI) in the presence of denatured forms (Brandon et al., 1989). None of these tests were optimized for sensitivity. Brandon et al. (2004) developed ELISA approaches using monoclonal antibodies against the Kunitz (KTI) and the Bowman-Birk trypsin inhibitors. This assay was able to measure 1 and 3 ng/mL for BBI and KTI, respectively in processed food. An antibody was raised against Kunitz-type soybean typsin inhibitor that worked equally well against native and denatured protein (Barkholt et al., 1994).

5.2.4 ELISAs for other soy allergenic proteins

Porras et al. (1985) developed an ELISA for the detection of soy protein in soy lecithin, margarine and soy oil, with sensitivity between 100 and 200 mg/kg. Two soy allergens were measured by a sandwich ELISA (Gly m Bd 30K and Gly m Bd 28K, respectively) with monoclonal antibodies for both the immobilized and the capturing antibodies (Tsuji et al., 1995; Bando et al., 1998). These assays were developed for soy products and processed foods that contain soybean protein isolates. Soy protein was detected in various food products within the range 140–700 mg/kg, but could not be detected in fermented products. A competitive enzyme-linked immunosorbent assay (ELISA) based on monoclonal antibody 6G4 was established to determine ß-conglycinin from soybean and soybean products and showed an IC_{50} value of 4.7 ng/mL with a detection limit of 2.0 ng/mL (You et al., 2008).

5.2.5 ELISA for total soy proteins

Several methods use antibodies raised not against just one particular protein but against the whole soy protein fraction (Song et al., 2008a,b). For example, Janssen et al. (1986) detected soy proteins in meat products up to 0.1% by gel electrophoresis, followed by blotting and dot blot. All major soy fractions were recognized by the antibody (Janssen et al., 1986).
Presently, the ELISA technique has been the preferred approach for allergens detection because of its high precision, simple handling and good potential for standardization; however, as with any type of analysis, there are inherent disadvantages related to food matrix interferences and effects of food processing on the targeted immunogens.

5.3 Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction is presently being developed for the detection and identification of the major food allergens. These techniques are based on the amplification of specific DNA

fragment by the polymerase chain reaction. DNA-based methods offer many advantages over protein-based methodologies, primarily that the target DNA is less degraded than the proteins from food matrices (Poms et al., 2004). In addition, the ELISA technique is more expensive and time consuming than PCR (Espiñeira et al., 2010).

Up to date, several assays have been developed and applied to evaluate the presence of genetically modified soy in food products. Duplex real-time PCR method was used to simultaneously detect traces of lupin and soya in processed food. Both lupin and soya at a level of 2.5 mg/kg food matrix could be detected in cookies (Galan et al., 2011). Koeppel et al. (2010) developed two novel quantitative multiplex real-time PCR systems. They simultaneously determine the DNA of peanut, hazelnut, celery, soy, egg, milk, almond and sesame, respectively. The specificity and sensitivity of test are in the range of 0.01% (Koeppel et al., 2010). Espiñeira et al. (2010) compared end-point and real-time PCR for the detection of soy protein in a wide range of foods. Real-time PCR method is better in specificity and sensitivity compared with the end-point PCR and is the simpler and more rapid process, with a higher potential for automation, therefore it is currently the most suitable screening method (Espiñeira et al., 2010).

Processing and ingredients can influence the detection limits. The lower detection limits were obtained for full-fat and defatted soybean flours than those for toasted soybean flour and soy fiber samples (Gryson et al., 2008). The detection limits for unprocessed and heat-processed pork meats are 0.01% and 0.06% (w/w) of soybean protein, respectively (Soares et al., 2010).

Comparisons of ELISA results with PCR results suggest a qualitative accordance, but a low correlation of quantitative results (Koeppel et al., 2010). The presence of the DNA is an indication of the presence of the allergenic food but not of the allergen itself. There is 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 (Poms et al., 2004).

5.4 Mass spectroscopy

Mass spectroscopy is a very promising method to quantify the allergens in soybean. Ten allergens were quantified from 20 non-genetically modified commercial soybean varieties using parallel, label-free mass spectrometry approaches (Houston et al., 2011). Also, a multi-method was developed for the detection of seven allergens based on liquid chromatography and triple-quadrupole tandem mass spectrometry in multiple reaction modes. It is based on extraction of the allergenic proteins from a food matrix, followed by enzymatic digestion with trypsin. The chosen marker peptides were implemented into one method that is capable of the simultaneous detection of milk, egg, soy, hazelnut, peanut, walnut and almond. This method has been used to detect all seven allergenic commodities from incurred reference bread material, which was baked according to a standard recipe from the baking industry. Detected concentrations range from 10 to 1000μg/g, demonstrating that the mass spectrometric based method is a useful tool for allergen screening (Heick et al., 2011).

5.5 Commercially available methods

Since the 1990s, several tests to detect soy in food have become available commercially, most of which are ELISAs (Koppelman and Hefle, 2006). Table 1 gives an overview of the available methods and their most important characteristics. Tepnel BioSystems kit (ELISA

Technologies, FL, USA) is a quantitative ELISA (<0.5% detection limit) which specifically detects soy protein in raw and cooked/canned foods and meats/meat products. The ELISA Systems kit (ELISA Systems, Windsor, Australia) is based on a sandwich ELISA and determines the soy in the food sample with a sensitivity of 2.5 ppm. Recently, ELISA Systems, Neogen Corporation and Ceogen companies launched several soy protein screening assays. The detection limit is 2.5-10ppm, and those are well suited to allergen detection. Veratox for Soy Protein and Soy Flour Allergen (Neogen Corporation, MI, USA) is a sandwich ELISA used for the quantitative analysis of minimally processed soy and soy flour protein in processed food products such as cookies, crackers and cereal.

Analyte	Type of method	Kit manufacturer	Sensitivity	Web site
Soy protein	Inhibition ELISA	Tepnel	5000 ppm	www.tepnel.com
Soy protein	Sandwich ELISA	ELISA Systems	2.5 ppm	www.elisasystems.net
Soy protein	Sandwich ELISA	Neogen	2.5 ppm	www.neogen.com
Soy lectin gene	Real-time PCR	Congen	10 ppm	www.congen.de

Table 1. Commercially available methods to detect soy.

6. Soy allergen mitigation

A number of thermal and nonthermal techniques have been researched for reducing allergens in soybean and other foods. Food allergen reactivity due to thermal processing (treatment) (i.e., moist and dry heat) has been extensively studied. Heating can alter proteins by inducing denaturation (e.g., tertiary and/or secondary conformational changes), cross-linking, aggregation or rearrangements of disulfide bonds, which may cause changes in allergen reactivity (Mondoulet et al., 2005). In some cases, the changes are shown to decrease allergen reactivity (Beyer et al., 2001; Mondoulet et al., 2005), yet in other cases, the changes caused an increase in allergen bioactivity (Leszczynska et al., 2003; Simonato et al., 2001; Pasini et al., 2001), due possibly to exposing new IgE binding sites. Thermal treatments by moist heat include boiling, frying, extrusion, autoclaving and retorting, and those by dry heat include baking, roasting, and microwaving. In this chapter, for thermal treatments, only the effect of direct heating and extrusion on soybean allergens will be reviewed.
Nonthermal processing methods, which can help preserve original characteristics, organoleptic properties and nutritional benefits compared to thermal processing, have been used more in recent years to reduce allergen reactivity of different foods including soybean. These treatments include pulsed ultraviolet light, high hydrostatic pressure, irradiation, power ultrasound, and pulsed electric field, which will be reviewed in this chapter.

6.1 Thermal methods

6.1.1 Thermal processing

Thermal processing itself was not intended to reduce allergens when it was first developed. Rather, it was aimed to enhance food safety (pasteurization or sterilization), change of physical attributes or texture (drying, gelation), or induce flavor profile modification (baking) (Davis and Williams, 1998). However, unintentionally thermal processing also

affects protein structure. Some of the protein tertiary structure can be altered by temperatures as low as 50 °C and as temperature increases the extent of structural changes becomes greater (Lee, 1992). In general, linear epitopes are more difficult to be eliminated than conformational epitopes by thermal process or any other technique, because they are more likely to withstand structural change caused by processing. Conformational epitopes, on the other hand, are relatively easy to be removed by changing the tertiary or secondary protein structure, which can occur within reasonable temperature ranges of 50 – 125 °C (Lee, 1992). However, in some cases IgE binding activity of a soybean based material increases after thermal process. Gly m Bd 30K (P34) is an example of such cases. It was reported that IgE binding activity was significantly increased after retorting at a temperature of 121.1 °C (Yamanishi et al., 1995). It was hypothesized that the native epitopes may be refolded after the thermal process. On the contrary, it was also reported that Gly m Bd 30K (P34) was not present in a texturized soy protein after extrusion (Franck et al., 2002). Both retorting and texturization processes use elevated temperatures, but the thermal energy applied is lower for texturization than for retorting. So, it can be assumed that there are other factors present than just thermal energy that are responsible for the disappearance of the allergen.

Another issues related to thermal processing is the possibility to create new epitopes. During protein structural changes, there is a potential for residual structures to form new epitopes that were not present before the process. The process and product have to be carefully examined, although there are also evidences to support the incidences of no additional epitope development after various thermal processes (Franck et al., 2002). The modification of soybean allergen potency is quite obvious after thermal process, as the protein profiles have changed. Most likely the allergen reactivity will be reduced. However, there is a consensus among researchers that thermal processes alone may not be enough to produce hypoallergenic soy foods.

6.1.2 Extrusion

Extrusion is a widely used processing method which controls heat, shear and pressure to produce texturized proteins or expanded cereals or snacks in the food industry. In addition to heat controlled by temperature of extrusion barrel, mechanical energy controlled by screw configuration, screw speed, die size and shape, and barrel fill defines the extrusion process as a whole. Combination of these parameters may help change conformational epitopes and reduce allergen potency of soy products. It is believed that the extrusion process can be effective on reducing allergens using different extrusion equipment and process parameters (Ohishi et al., 1994; Saitoh et al., 2000; Franck et al., 2002). Saitoh et al. (2000) concluded that extrusion is an effective technique to produce low soy allergen products. A twin-screw extruder with 6 kneading blocks and temperature above 100 °C could reduce soy allergen level to 1%. Franck et al. (2002) also suggested that texturized soy protein might eliminate Gly m Bd 30k (P34), one of the major allergens in soy. Although the specific extrusion parameters were not provided, a typical texturization process is less intense compared to the process that Saitoh et al. (2000) has used and still managed to eliminate Gly m Bd 30k (P34). Ohishi et al. (1994) found that twin-screw extrusion using kneading blocks with temperature higher than 66 °C could reduce the allergen level to 0.1% of native soybean due to protein configuration change. In one of the authors' laboratories, three corn-soy blend samples, 51% soy flour, 21% soy flour, and 38% soy protein isolate (SPI), were extruded using a twin screw extruder with elevated temperatures of 140 °C. The results are shown in Fig. 2.

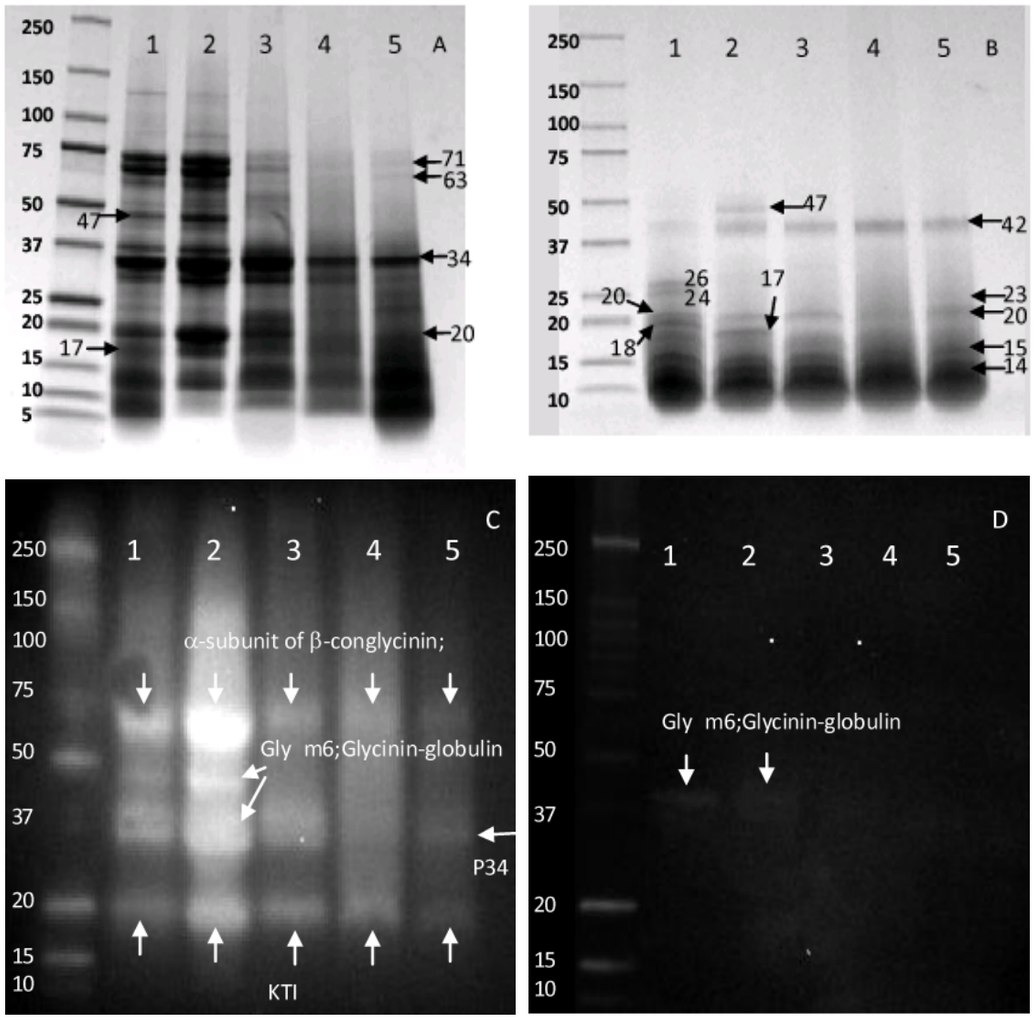


Fig. 2. SDS-PAGE profile of peptides in soybean products before (A) and after (B) hydrolysis with pepsin and Western blot band of peptides in soybean products before (C) and after (D) hydrolysis. Sample: 1: SPI; 2: soy flour; 3: 52% soy flour; 4: 21% soy flour; 5: 38% SPI.

The protein profiles changed due to different extruded samples from the soy flour and soy protein isolate (SPI). Although P34 band (Gly m Bd 30) was not completely removed, 38% SPI sample showed significant reduction of P34 compared to the other two samples. The results provide the same conclusions as in the previously mentioned studies, that is, the extrusion process is promising but not a complete solution. Additional steps such as pulsed ultraviolet light, fermentation or HPP may be required to achieve hypoallergenic soy products.

6.2 Nonthermal methods

6.2.1 Pulsed ultraviolet light

Pulsed ultraviolet light (PUV) consists of intense broad photonic spectra emitted within several nanoseconds from ionized inert gas (e.g., Xenon) under high voltage. The PUV radiation contains approximately 54% UV-C, 25% visible light, and 20% infrared light (Oms-Oliu et al., 2010; Shriver et al., 2011). The PUV light can be thousands of times more intense

than conventional, continuous mercury UV light (Dunn et al. 1995; Krishnamurthy et al., 2009).

Fig. 3 shows two types of PUV systems used for food applications: a batch unit and a continuous unit. In the batch system, foods are manually loaded to the sample rack in the treatment chamber, and the operator can adjust rack position (distance to lamp), treatment time and pulse frequency (typically 1-20 pulses/s). In the continuous system, a conveyor belt passes the food under one or multiple lamps in succession. Conveyor speed, distance to lamp (adjusted by a hydraulic mechanism), pulse frequency, treatment duration, and lamp tilt angle relative to conveyor belt direction can all be adjusted. The continuous system can also be used for batch treatment by setting the conveyor speed to zero.

Fig. 3. A. SteriPulse XL-3000 batch PUV system; B. LHS40 continuous PUV system, as manufactured by Xenon Corporation (Wilmington, MA).

The PUV treatment is a process of photophysical, photothermal and photochemical effects (Chung et al., 2008; Krishnamurthy et al., 2009; Yang et al., 2010; Shriver and Yang, 2011). Normally, PUV is regarded as nonthermal as long as the duration of exposure is short (e.g., seconds), as the temperature rise is insignificant; however, a prolonged PUV exposure (e.g., minutes) can incur significant temperature rise and moisture loss of the sample, where both the nonthermal (mostly due to the UV spectrum) and photothermal (mostly from the infrared spectrum) effects co-exist (Yang et al., 2011). Fiedorowicz et al. (2001) found that prolonged UV light treatment caused formation of insoluble complex in food, depolymerization of starch, peroxidation of unsaturated fatty acid, carbohydrate crosslinking, protein crosslinking, and protein fragmentation. Although proteins, including soybean allergens, have a peak absorbance at 280 nm wavelength (Gómez-López et al., 2005), light with shorter wavelength is more effective in changing protein molecules. The PUV treatment could not easily break the peptide bonds in protein. Photons absorbed by cystine had a higher chance of inactivating a protein than photons absorbed in the aromatic amino acids. The absorbed photons ionize the protein (Setlow, 2002). Aromatic amino acids (e.g., tyrosine and phenylalanine) can absorb UV radiation and recombine to form covalent cross-links in proteins (Gennadios et al., 1998).

The UV portion of PUV can form oxygen radicals, superoxide radicals, hydrogen peroxide and super oxide radicals. Oxygen radicals lead to generation of ozone. Super oxide radicals can induce protein cross-linking and protein fragmentation. Water molecules absorb UV photons and produce hydroxyl and hydrogen radicals (OH^- and H^+) which are powerful protein-modifying agents (Krishnamurthy et al., 2009; Davies et al., 1987). Hydroxyl radicals

are shown to induce alteration to the primary structure of bovine serum albumin (BSA). Oxygen radicals significantly alter the effects of hydroxyl radicals on protein primary structure. Protein aggregation by hydroxyl radical may involve intermolecular bityrosine formation (Davies et al., 1987). However, there may be other covalent modification involved in the process of PUV treatments.

It is believed that PUV's photothermal, photophysical and photochemical effects could alter allergen conformation (Krishnamurthy et al., 2007) or cause protein aggregation (Chung et al. 2008; Yang et al., 2010), resulting in the loss or modification of conformational epitopes. Furthermore, radicals may be induced during PUV treatment due to molecular ionization under its high-energy burst of lights. The visible and infrared waves in the PUV spectra are believed to be responsible for vibration and rotation of molecules, respectively (Krishnamurthy et al., 2009).

Apart from its microbial pasteurization or sterilization effects, PUV was applied by Chung et al. (2008) and Yang et al. (2011) to mitigate peanut allergens. The PUV radiation has also been found to significantly decrease the allergenic potency of soybean extracts (Yang et al., 2010), shrimp extracts (Shriver et al., 2010), egg extracts (Anugu et al., 2010), milk proteins (Anugu et al., 2009), and wheat extracts (Nooji et al., 2010).

For the peanut protein extracts and liquid peanut butter, PUV reduced the IgE binding by 6- to 7-fold compared to the control (Chung et al., 2008). It was found that PUV treatment caused protein aggregation of the major peanut allergens Ara h 1 and Ara h 3. It is believed protein conformation and IgE binding epitopes were altered accordingly. They found that insoluble proteins caused by PUV were insoluble in concentrated urea (2 M) or sodium chloride (1 M). In the study by Chung et al. (2008), the solubility of Ara h 2 (18-20 kDa), the most potent allergenic peanut protein (Chu et al., 2008; Dodo et al., 2005) was unaffected, but in a separate study by Yang et al. (2011) where raw peanut extracts, roasted peanut extracts and peanut butter slurry were treated with PUV under different conditions, Ara h 2 was significantly inactivated.

Specifically on soybean, Yang et al. (2010) noticed a pronounced reduction in soybean allergens (glycinin, 14-34 kDa and β-conglycinin, 50 kDa), when PUV was applied to soybean protein extracts for a duration up to 6 min with 3 pulses per second. SDS-PAGE (Fig. 4) shows a marked reduction in glycinin (14-34 kDa) and β-conglycinin (50 kDa). However, soybean proteins of higher molecular weights (e.g., 45-75 kDa) were slightly reduced, exhibiting considerably more resistance to PUV light treatment. Proteins of a larger molecular weight were formed in the region of 150-250 kDa, signifying that protein crosslinking or aggregation occurred during the PUV treatment. ELISA results (Fig. 5) show a marked decrease in IgE binding after the PUV treatment for 6 min.

Shriver et al. (2010) treated shrimp protein extracts with PUV for 4 min and noted a pronounced reduction in the potency of major shrimp allergen, tropomyosin, as probed through ELISA using pooled human sera from patients of shrimp allergy. Boiling treatment (100°C) of the samples was found to cause negligible allergen reduction. The researchers attributed shrimp allergen reduction under PUV conditions to conformational changes of tropomyosin, particularly intramolecular crosslinking.

Exposure of milk to PUV for 150 s was found by Anugu et al. (2009) to be effective in reducing its allergen levels, as evident from the undetectable level of allergenic casein and whey in SDS-PAGE after the treatment. ELISA results showed that IgE binding was reduced by 7.7 folds in whey protein and 7.4 folds in α-casein. Anugu et al. (2010) applied PUV to egg protein extracts and found PUV was effective in reducing egg allergens, for example, a 2-min PUV treatment caused all IgE binding to major allergens to be undetectable on

Western blot, except for ovalbumin that was still active in IgE binding to a certain degree. ELISA results showed that the total egg allergen reactivity was reduced by 9.5-fold at 2 min. The PUV exposure for 45 s with heat (boiling 100°C) and without heat caused the allergen reactivity of wheat gluten to be reduced (Nooji et al., 2010), as probed by SDS-PAGE and Western blotting. Although thermal treatment alone did not affect the allergen reactivity of wheat gluten, a combination of PUV and heat treatment enormously enhanced wheat allergen reduction compared to PUV alone.

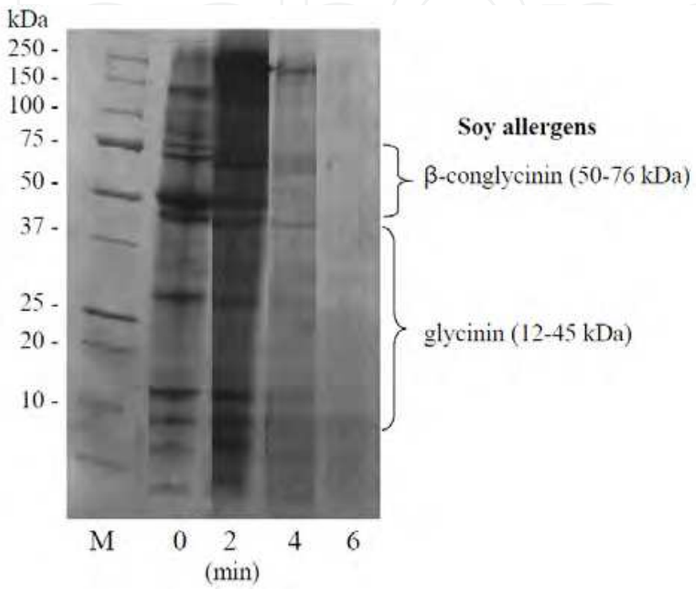


Fig. 4. SDS-PAGE of soybean protein extracts treated with PUV for 0 (control), 2, 4 and 6 m as indicated in figure. Reproduced with permission. M for protein marker.

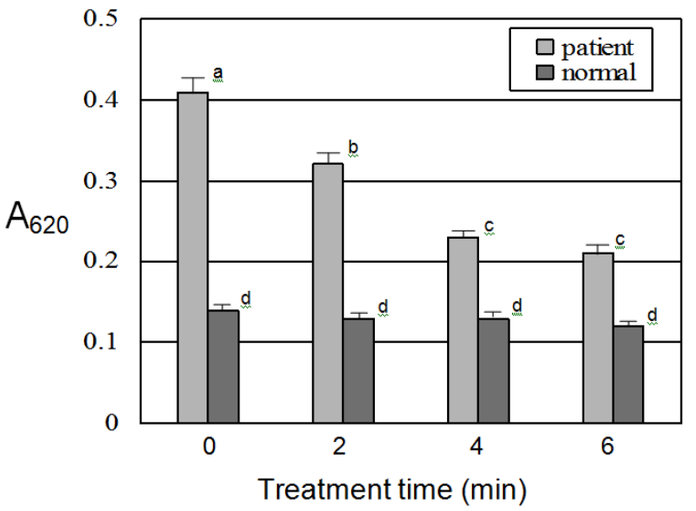


Fig. 5. The IgE binding capacity (expressed as the absorbance value A_{620} read at 620 nm) of PUV-treated and untreated raw soy extracts as probed by indirect ELISA. A620 Data represents mean of three replications. Data with same letters are not statistically different from each other ($p < 0.05$). Reprint with permission.

Although the foregoing treatments, including soybean extracts, with PUV were conducted in liquid form, the photothermal effect of PUV after an extended exposure also enables it to be applied directly to solid foods, e.g., whole almond (Li, 2011), for allergen mitigation.

6.2.2 High hydrostatic pressure

High hydrostatic pressure (HHP), also described as ultra high pressure (UHP) processing, subjects a food material, with or without packaging or supplemental heat, to pressures normally between 100 and 800 MPa for 10-30 min. During HHP treatment, the pressure is uniformly distributed in all directions (Spilimbergo et al., 2002), and it breaks non-covalent bonds (hydrogen, ionic, and hydrophobic bonds) that are present in compounds like proteins.

High hydrostatic pressure has been used to inactivate microorganisms and enzymes by protein alteration and denaturation (Mozhaev et al., 1996). Alterations of the secondary and tertiary structure of major proteins in meat and milk reportedly took place at pressures greater than 200 MPa, however, higher pressures (e.g., 300-400 MPa) are needed for egg and soy proteins to change structure (Messens et al., 1997).

Although more direct research is needed to examine the efficacy of HHP on the allergenic proteins of foods including the soybeans, HHP has been shown to induce conformational changes in proteins. Because of this, it is believed that HHP can change allergen potency by altering its epitope structures (Yamamoto et al., 2010). Kleber et al. (2007) found that the allergen reactivity of major milk allergen, β-lactoglobulin, was actually underscored by HPP from 200 to 600 MPa. With supplemental heat during pressure treatment, the allergen potency was further enhanced. The possible reason is that the conformational changes in β-lactoglobulin might have actually exposed new epitopes for IgE binding.

Kato et al. (2000) found a decreased reactivity of rice allergens and damaged endosperm cells under 300 MPa pressure treatment for up to 120 min. They also observed that apart from pressure-induced structural damage of rice grains, the buffer solution was able to permeate into the rice grain to facilitate allergen solubilization and subsequent extraction into the buffer, but the amount of allergen extraction depended on the solvent used and the solubility of the rice allergens. They also found the use of proteases might further reduce allergen reactivity.

High pressure treatment was found to cause the allergen reactivity of pasteurized liquid whole egg to decrease by 3.3 fold as detected by EAST inhibition. Combined thermal (heating to 70°C) and HHP at 600 MPa, 10 min caused a 8.9 fold reduction compared to control (70°C heating alone) that resulted in only 1.5 fold reduction in allergen reactivity (Hildebrandt et al., 2010). As demonstrated in the study, the combination of heat and HPP allowed for a greater reduction in overall food allergen reactivity.

In the case of soybeans, Penas et al. (2006) determined the effect of HHP of 100, 200 and 300 MPa on the protein content, the degree of enzymatic hydrolysis and the allergen Gly m 1 in the treated soybean whey and the hydrolysates. They found that besides enhanced proteolysis, HHP reduced the immunochemical response of soybean whey to anti-Gly m 1 monoclonal antibodies, which was further decreased after the combined treatment of high pressure and enzymatic hydrolysis. The results showed soybean whey proteins hydrolyzed at high pressure could be used as sources of peptides with low antigenicity when incorporated as food ingredients. Furthermore, sprouts which grew from HHP-treated soybean seeds had significantly reduced antigenicity compared with the ones grown from

untreated seeds. It was suggested that HHP could be used to produce hypoallergenic soybean sprouts without notable nutrient losses.

It was found soy proteins, including the two major storage protein components 7S and 11S globulins, are dissociated under pressure (Kajiyama et al., 1995), leading to new free thiol residues. Swanson et al. (2002) reported that some of the newly created fragments recombine to give –S–S– exchange reactions or new –S–S– binding by oxidation.

Denaturation of proteins under pressure is a complex process due to the disruption of both hydrophobic bonds and salt bridges. This process depends on a multitude of factors like the magnitude of pressure, pH, temperature, solvent used, ionic strength of the solution, and the protein structure (Kajiyama et al., 1995). Jung et al. (2008) found that the profile of storage proteins (7S, 11S) of the soymilks was similar to the control regardless of the pressure levels (100-700 MPa) applied to the beans.

In a pressure enhanced enzymatic hydrolysis study of soybean whey proteins, Penas et al. (2004) found that HHP increases hydrolysis, as Chymotrypsin and trypsin presented five visible peptides lower than 14 kDa after hydrolysis and pepsin about 11 peptides, and a pressure of 100 MPa presented the best condition for the three enzymes used.

Puppo et al. (2004) studied the HHP-induced physicochemical changes of the 7S and 11S soybean protein isolates at pH 3 and pH 8 in terms of protein solubility, surface hydrophobicity (Ho), and free sulfhydryl content (SHF). They found 200-600 MPa pressures caused the protein to aggregate and denature, with changes in secondary structure detected, leading to a more disordered structure. The protein Ho and aggregation were increased, SHF was reduced, and a partial unfolding of 7S and 11S fractions were observed at pH 8. A major molecular unfolding, a decrease of thermal stability, and an increase of protein solubility and Ho were also detected.

6.2.3 Ionized radiation

As a nonthermal method, ionized radiation (irradiation), such as gamma rays, x-rays and e-beams, has been reportedly used to control food antigenicity. Although the exact mechanism is still unknown, it is generally believed that irradiation changes the structure of the IgE-binding epitopes in the allergenic proteins of the foods like egg (Seo et al., 2007), milk (Lee et al., 2001) and shrimp (Byun et al., 2000). One possible way to induce such changes is through the free radicals created during irradiation, which may lead to protein fragmentation and aggregation.

Seo et al. (2007) treated the egg with gamma irradiation (cobalt-60) and found its allergenic ovalbumin was reduced at a dose of 100 kGy, which is rather high for food applications, but unaffected at a dose of 10 kGy, which is the maximal dose that has been shown to be safe for human foods (Byun et al., 2000). They noticed the occurrence of protein crosslinking during irradiation, as evident from the change in molecular weight, which caused the reduction in ovalbumin content. Irradiation has been reported to induce protein crosslinking, including the formation of disulfide bonds, and cause protein aggregation due to hydrophobic interactions (Davies and Delsignore, 1987).

Gamma irradiation (1-15 kGy) supplemented with heat (100°C) was found by Li et al. (2007) to be effective in reducing shrimp allergen reactivity by 5 to 30 fold. However, gamma irradiation or heat treatment alone did not cause a notable decrease in the IgE binding. In a similar study, Byun et al. (2000) looked at the effects of cobalt-60 irradiation on the major shrimp allergen, tropomyosin, and found that the protein band corresponding to

tropomyosin (36 kDa) was undetectable in SDS-PAGE at the irradiation dose of 7 kGy or so. The IgE binding to tropomyosin was minimal at the irradiation dose of 10 kGy. They explained that the proteins ultimately coagulated under irradiation, causing their disappearance on SDS-PAGE and the decreased allergenic potency.

Lee et al. (2002) treated the isolated egg ovomucoid with gamma irradiation at a dose of 10 kGy with or without supplement of heat. It was found irradiation supplemented with heat reduced the ovomucoid concentration to nearly an undetectable level, while irradiation alone or heat alone was not able to reduce the ovomucoid concentration to an undetectable level, with the former more effective than the latter, due possibly to the heat resistance of ovomucoid.

Lee et al. (2001) applied gamma irradiation to milk proteins (Bovine α-casein, ACA and β-lactoglobulin, BLG) and detected changes in their allergenic reactivity using milk-hypersensitive patients' IgE and rabbit IgGs individually produced to ACA and BLG by competitive indirect ELISA. Results showed a change in allergenicity and antigenicity of the irradiated milk proteins and a decrease in the solubility of irradiated proteins, which might be attributed to agglomeration of the proteins during irradiation. These results indicated that epitopes on milk allergens were structurally altered by gamma irradiation.

Shriver and Yang (2011) stated that the tree nut allergens are noticeably stable to gamma irradiation, even when combined with heat treatment. Su et al. (2004) subjected almond, cashew and walnut to gamma irradiation at the doses of 1, 5 10 and 25 kGy with or without heat supplement through one of the heating methods: autoclaving, blanching, frying, microwave, or roasting. They found none of the foregoing conditions, alone or in combination, were able to reduce the allergen reactivity.

Specifically for soybean, Manjaya et al. (2005) treated the soybean variety VLSoy-2 with 250 Gy gamma rays to induce variability to proteins including the two allergenic storage proteins 7S and 11S. A large number of mutants affecting morphological characters were identified and characterized. It was found there were 3 mutants lacking the A3 subunit of glycinin (11S) protein, and among the 3 mutants identified, 2 of them were also characterized by the lack of a 0-subunits of b-conglycinin (7S).

6.2.4 Power ultrasound

Ultrasound can be divided into high frequency, low-intensity diagnostic ultrasound in the MHz range and low frequency, high-intensity or power ultrasound in the kHz range (Feng et al., 2009). During sonication, the intermittent compression and rarefaction of the sonic waves promote formation of sonication bubbles in the sonication media, which eventually implore at critical bubble sizes, creating a localized high pressure (up to 1000 atm), high temperature (e.g., 5000 K), micro jets (e.g., hundreds of kilometers per hour in velocity), and free radicals to induce physical and chemical effects on the surrounding, including the change of allergen conformation and its reactivity. Also, the free radicals generated from water during sonication can contribute to protein modification (Soria and Villamiel, 2010).

The use of power ultrasound in the food processing has been a subject of research and development for many years, and recent applications include increasing extractability of protein for allergen detection (Albillos et al., 2011), tomato peeling (Rock et al., 2010), surface lipid removal and shelf life extension of potato chips (Wambura and Yang, 2009), expedited rice parboiling (Wambura et al., 2008), and expedited xylan extraction from corn cob (Yang et al., 2009).

So far, only a few applications of power ultrasound in food allergen reduction have been found in literature, but none has been reported on soybean allergens.

Li et al. (2006a) applied power ultrasound to change the structure of shrimp proteins and then assessed its effect on the IgE-binding capacity of major shrimp allergen Pen a 1(tropomyosin). The sonication conditions were 30 kHz, 800 W for 1.5 h at 0 and 50 °C, respectively. For comparison, samples were also boiled for 15 min. Allergen reactivity was analyzed by the enzyme allergosorbent test (EAST) and competitive inhibition ELISA (ci-ELISA) using pooled sera of 15 shrimp-allergic patients. Protein extracts treated with power ultrasound at 50°C (treated 2) was 2.2-fold lower than that of untreated shrimp, while protein extracts boiled was 1.2-fold lower than that of untreated shrimp. The results suggest that power ultrasound at 30 kHz, 800 W and 50°C treatment temperature may reduce the allergen potency of shrimp. In a separate but similar study, Li et al. (2006b) found the IgE binding to the power ultrasound treated isolated shrimp allergen decreased by approximately 81.3-88.5%. It was noted that during the treatment of the allergen isolate, formation of a new protein fraction with a low molecular weight increased in quantity as treatment time elapsed. Thus, fragmentation of the shrimp allergen may occur during high intensity ultrasound under the conditions stated (Li et al., 2006b).

Yu et al. (2010) evaluated the effects of combined ultrasound and boiling (CUB) along with boiling alone and high pressure steaming (HPS) on the degradation of crab tropomyosin and reduction of its IgE-binding reactivity. SDS–PAGE analysis indicated that boiling had little impact on the digestive stability of tropomyosin. Both the CUB and HPS accelerated the digestion of tropomyosin. Western blotting and inhibition ELISA also indicated a partial decrease in the reactivity of IgG/IgE-binding of tropomyosin after CUB or HPS treatment. It was concluded that the ultrasound combined method was inferior to the HPS that was found to be the most effective method to accelerate the digestion of tropomyosin in gastrointestinal digestion and reduce the reactivity of IgG/IgE-binding of tropomyosin.

6.2.5 Pulsed electric field

Pulsed electric field (PEF) is a nonthermal method for food processing and preservation that uses short pulses of high frequency electricity for microbial inactivation, with minimal changes on food quality attributes, of liquid or semi-liquid food products. The PEF treatment involves placing foods between electrodes subject to high voltage pulses in the order of 20–80 kV, which results in an electric field that causes microbial inactivation and other changes to food. Although PEF technology has found the most significant application in the inactivation of microorganisms, it has also been studied for other uses such as enzyme inactivation (Yang et al., 2004), drying or enhancing mass transfer rate (Lebovka et al., 2007; Gachovska et al., 2008), wine maturation (Zeng et al., 2008), and allergen reduction (Toshiko et al., 2004). So far, studies on the effects of PEF on allergens or protein structure and functionality in general have been scarce, which becomes an area of immediate research needs as the PEF technology is intensified in food processing applications and moves towards commercialization.

Toshiko et al. (2004) studied the effect of PEF on various allergen-antibody interactions as detected by ELISA. They found the ELISA value was drastically decreased when PEF at 10kV and 50Hz was applied to the ovalbumin solution. However, no significant changes in ELISA values were observed in other allergenic proteins. The ovalbumin concentration

before and after PEF treatments was also examined using the Bradford method. The results show that though the ovalbumin concentration was not changed significantly, ELISA value was decreased. This suggested that conformational changes of ovalbumin might have taken place during the PEF treatments, which resulted in lower incidences of allergen-antibody interactions.

Sun et al. (2011) studied the effect of PEF on the structure and properties of whey protein, including solubility, as it forms the whey protein isolate–dextran conjugation in aqueous solution during the PEF treatment. They found that higher pulsed electric field intensity enhanced the extent of glycosylation, and the secondary structure of whey protein isolate had a considerable loss due to the covalent attachment of dextran. Compared with initial whey protein isolate, the solubility was significantly improved. At the same time, glycosylation also inhibited heat-induced aggregation after treated at 80°C for 20 min.

Johnson et al. (2010) applied PEF, high pressure and thermal treatments to four purified allergens from peanut (Ara h 2, Ara h 6) and apple (Mal d 3, Mal d 1b) and examined their structural changes using circular dichroism spectroscopy and gel-filtration chromatography. For the PEF treatments, electric field strengths were from 0 to 35 kV/cm and specific energy inputs from 0 to 130 KJ/kg to minimize temperature rise during PEF treatment, a frequency of 2 Hz was chosen and samples were removed from treatment chamber immediately after PEF treatment. It was found that PEF did not induce any significant changes in the secondary structure of these four plant-based allergens. However, Johnson et al. (2010) commented, due to the fact there is a lack of studies of PEF effects on protein structure in general, it is difficult to predict whether other allergens may be affected under similar PEF conditions.

6.2.6 Nonthermal plasma

Application of nonthermal plasma (NTP) technology to allergen mitigation is still new. Although no literature has been found on NTP application to soybean yet, recent development on using NTP to reduce allergens in shrimp and wheat protein extracts (Nooji, 2011; Shriver, 2011) may indicate a potential application of NTP to soybean extracts.

Nonthermal plasma is electrically energized and highly energetic gaseous matter, which can be generated by electrical discharge across an electrical field. The NTP species can include electrically neutral gas molecules, free radicals, photons, negative or positive ions, and electrons. A similar principle of bacterial killing by NTP, i.e., oxidation of the reactive oxygen species (Montie et al., 2002), UV radiation, and free radicals that come along with plasma generation (Desmet et al., 2009), may also be applicable to explain allergen reduction.

Shriver (2011) and Nooji (2011) exposed shrimp extract and wheat extract, respectively, to the plasma generated at 30 kV voltage and 60 Hz frequency for 1, 3, and 5 min at ambient temperature. Results showed that the IgE binding to tropomyosin in the shrimp samples was decreased to undetectable levels after 3 and 5 min NTP exposure. Dot blot and ELISA confirmed a decrease in tropomyosin reactivity following the NTP treatments. It was also found 5-min NTP treatment of wheat protein extracts gave a pronounced decrease in allergen reactivity as probed by both ELISA and dot blot.

6.3 Chemical and biological methods

Combination of chemical reaction such as carbohydrate conjugation during Maillard reaction showed some evidences of reducing soy allergenicity (Babiker et al., 1998). Conjugated galactomanan by Maillard reaction was believed to mask the structure of soy

protein, P34, and decreased allergenicity, although the same method was not investigated with other soy allergens. Immunoreactivity of soy protein can be altered through control of pH and ionic strength showing that pH 6 displayed less IgE binding than at pH 2.2 or pH 7.2 (L'Hocine et al., 2007). These researchers investigated the effect of ionic strength and pH on immunoreactivity of pure soy glycinin and found that pH has significant effect on immunoreactivity, possibly due to protein's tertiary structural changes. High immunoreactivity was shown at low pH (2.2) and at neutral pH (7.2). It was suggested that conformational structure change of protein is the main reason at pH 2.2 by revealing epitopes for IgE binding. At pH 7.2 due to possibly highly ordered structure of protein, epitopes may be positioned ideally for binding with antibodies.

Protein hydrolysis and fermentation also show effectiveness on reducing soy allergenicity (Wilson et al., 2005; Penas et al., 2006; Frias et al., 2008). Although the allergen reactivity was reduced substantially, functional properties of resulting soy proteins were also significantly altered. Several studies have confirmed the degradation of soybean allergens during fermentation by microbial proteolytic enzymes in soy sauce, miso products, soybean ingredients and feed grade soybean meals (Yamanishi et al., 1996; Lee et al., 2004; Kobayashi, 2005). Fermentation has the capacity to improve nutritional and functional properties compared to original products. Fermentation may entail the ability to hydrolyze soy protein into smaller peptides. Soy sauce possesses microbial proteolytic enzymes able to degrade soybean proteins, including major soybean allergen (Gly m Bd 30K), into peptides and amino acids (Kobayashi, 2005).

As a biological method, genetic modification has gained much attention despite its controversial nature. It prevents the translation of selected allergens using post-transcriptional gene silencing or co-suppression (Shewry et al., 2001). Herman et al. (2003) used transgene-induced gene silencing to prevent the accumulation of Gly m Bd 30 K (or P34) protein in soybean seeds. It was found the Gly m Bd 30 K-silenced plants and their seeds lacked any compositional, developmental, structural, or ultrastructural phenotypic differences when compared with control plants. The silencing of Gly m Bd 30 almost completely inhibited IgE binding (Herman et al., 2003). Experimental evidences by electron microscopic-immunocytochemical assays, two-dimensional protein analysis, and tandem mass spectrometric identification all indicated no significant differences between the modified soybean and the control in respect to structural morphology and protein composition. Furthermore, comparative testing showed that no new allergens were formed during silencing. Nethertheless, genetic modification method is still under scrutiny about the stability of hypoallergenic foods produced by it, therefore food safety risks exist with foods genetically modified for allergen silencing. Also, the allergen removal process may still accompany the alteration of the foods' functional and physical properties (Shewry et al. 2001), because many allergenic proteins are parts of plant development and metabolism. Genetic modification of IgE epitopes instead of the protein fraction may be a better option for genetic modification to reduce allergen.

Combination of physical (novel technologies), chemical and biological techniques may be an effective approach to maximize the alleviation of allergen and minimize the change in functionality.

7. Future trends

Effective fighting with food allergy, including soy allergy, necessitates blocking any of the critical steps leading towards allergy development. One of the critical steps is preventing

allergen from entering human body. In the current practice, this is achieved through total avoidance of the allergenic foods with increasing difficulty to effectuate, but novel processing technologies, which may significantly reduce allergen reactivity while maintaining the product functionality, offer a hopeful prospect in minimizing allergen level in soy products, thus preventing allergens from entering human body. More research is needed in this area, especially on pulsed UV light that has been shown promising in mitigating allergens in soybean, peanut, shrimp, almond, milk, egg and wheat (Shriver and Yang, 2011).

Application of "hurdle technology" concept in allergen mitigation, where one method is combined with other methods or conditions, may prove quite effective in producing truly hypoallergenic soy products where allergens are down to a significantly low level safe to most allergenic patients. As discussed earlier, combination of physical, chemical and biological techniques may be an effective processing strategy to reduce maximally the soy allergens and yet affect minimally the product functionality.

The accurate detection and identification of soy allergens are still rather difficult, especially when they are hidden in different products including bakeries, infant foods, dairy foods and processed meats. A robust detection method is desired that can meet the foregoing demand and yet identify a multitude of allergens in the food product. Mass spectroscopy is a potential method for this purpose, which deserves more intensified research. In addition, in order for the results to be comparable, the specificity, selectivity and reliability of different methods also require a continued study for addressing.

8. Concluding remarks

Soybean is one of the most healthy and yet allergenic foods. Soybean allergens consist of proteins with molecular weights ranging from 7 to 71 kDa. Among the 34 allergic soy proteins identified, Gly m Bd 60K, Gly m Bd 30K and Gly m Bd 28K are categorized as major soy allergens, while soy hydrophobic protein (Gly m 1a), soy hull protein (Gly m 2), soy profiling (Gly m 3), glycinin (320-360 kDa), β-conglycinin (140-180 kDa) and tripsin inhibitor (20 kDa) are listed as major soy allergenic proteins. The strongest soy allergen among others is Gly m Bd 30k, also known as P34. Like any allergens in a food system, soy allergen reactivity is dominated by epitopes or IgE binding sites, categorized as linear or conformational. The epitopes are not only fully characterized by their primary protein structure, but also by their tertiary structural conformations, which offer opportunities for different thermal and nonthermal food processing technologies to alter the nature of epitopes and thus allergen reactivity with IgE antibody.

As one feasible approach towards blocking the steps of allergic reaction sequence, removing or reducing allergens in soybean and its derivatives, thermal and nonthermal methods have been gaining momentum worldwide. Postharvest processing techniques, especially nonthermal methods, are especially attractive. Several nonthermal processing techniques, such as PUV, HHP, power ultrasound, ionized radiation, pulsed electric filed and nonthermal plasma, have been tested on a number of food products including soybean to alter allergen reactivity without changing the inherent properties of the products. Nonthermal techniques cause changes to allergen structures and modifications to IgE binding epitopes, including promoting the aggregation or crosslinking of proteins. Pulsed ultraviolet light, among other novel methods, has been outstanding in terms of allergen mitigation, as it has been reported to significantly reduce allergens in soybean, as well as other products like peanut, shrimp, almond, milk, egg and wheat.

Nevertheless, before an effective allergen mitigation approach is securely in place for soybeans, total avoidance is still likely to be the most effective policy for soy allergy control. Because of this, reliable allergen detection methods are critical for ensuring the public health and compliance with food labelling regulations. To date, the majority of detection methods developed for soybean allergens are immunochemical based. The ELISA technique has become a preferred approach for soy allergen detection due to its high precision, simple handling and good potential for standardization. As a newly developed allergen detection technique, the PCR method offers unique features including less cost, faster turnaround and stability of targeted DNA over processing conditions, as compared to ELISA, but its quantitative correlation to ELISA results has so far been low due probably to the fact that nucleic acids are affected differently from allergenic proteins. In contrast, mass spectroscopy has shown a great potential over ELISA and PCR for detection and quantification of soy allergens due to its wide detection concentration range and its capability of detecting a multitude of allergens within one food species or over a number of different species in a composite food system.

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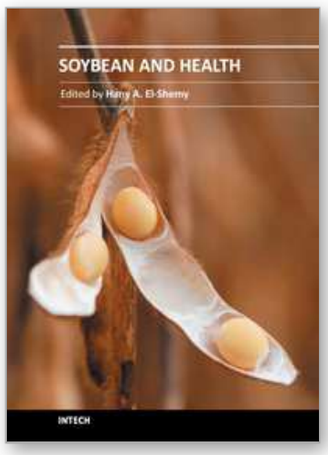

Soybean and Health

Edited by Prof. Hany El-Shemy

ISBN 978-953-307-535-8
Hard cover, 502 pages
Publisher InTech
Published online 12, September, 2011
Published in print edition September, 2011

Worldwide, soybean seed proteins represent a major source of amino acids for human and animal nutrition. Soybean seeds are an important and economical source of protein in the diet of many developed and developing countries. Soy is a complete protein, and soy-foods are rich in vitamins and minerals. Soybean protein provides all the essential amino acids in the amounts needed for human health. Recent research suggests that soy may also lower risk of prostate, colon and breast cancers as well as osteoporosis and other bone health problems, and alleviate hot flashes associated with menopause. This volume is expected to be useful for student, researchers and public who are interested in soybean.

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
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InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
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