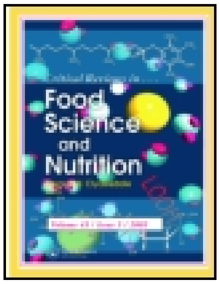

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Advances of Research on Glycinin and β-conglycinin: a Review of two Major Soybean Allergenic Proteins

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Advances of Research on Glycinin and β-conglycinin: a Review of two Major Soybean Allergenic Proteins

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Summary: Being an important crop, soybean is widely used in the world and plays a vital role in human and animal nutrition. However, it contains several antinutritional factors (ANFs) including soybean agglutinin, soybean protease inhibitors, soybean allergenic proteins, etc. that may result in poor food utilization, decreased growth performance and even disease. Among these ANFs, soybean allergenic proteins can lead to allergic reactions in human and animals which has become a public problem all over the world, but our knowledge on it is still inadequate. This paper aims to provide an update on the characteristics, detection or exploration methods, and *in vivo* research models of soybean allergenic proteins; especially glycinin and β-conglycinin are deeply discussed. Through this review, we may have a better understanding on the advances of research on these two soybean allergenic proteins. Besides, the ingredient processing used to reduce the allergenicity of soybean is also reviewed.

Keywords: allergenicity, detection or exploration methods, glycinin, β-conglycinin, *in vivo* research models, ingredient processing

As one of the major crops in the world, soybean (Glycine max) is a good source of high-quality protein, fiber, essential fatty acids, as well as vitamins, minerals, etc (FAO). Soybean is traditionally consumed by human in Asian countries (Table 1). Due to its high nutritive value and numerous beneficial effects, soybean's usages greatly increase, especially in European and North American countries (Hari *et al.*, 2000). Nowadays, soybean is widely used in foodstuffs/feedstuffs industries (John *et al.*, 1989; Rajni *et al.*, 2003; Victor *et al.*, 2007).

However, soybean possesses a certain amount of antinutritional factors, such as soybean agglutinin, soybean protease inhibitors, soybean allergenic proteins, etc. (Table 2). Soybean agglutinin is a glycoprotein with a molecular mass about 120 kDa and an isoelectric point near pH 6.0. It preferentially binds to oligosaccharide structures with terminal α or β-linked N-acetylgalactosamine, and binds to galactose residues to a lesser extent. Soybean agglutinin can lead to poor food utilization and impaired growth (Lotan *et al.*, 1974). Soybean protease inhibitors contain Kunitz soybean trypsin inhibitor (22.5 kDa) (Kunitz 1947), Bowman-Birk trypsin- and chymotrypsin-inhibitor (8 kDa) (Birk 1985), and glycine-rich trypsin inhibitor (17 kDa) (Tan-Wilson *et al.*, 1987). As their names suggest, these protease inhibitors can inhibit the activity of trypsin or both trypsin and chymotrypsin. Just like soybean agglutinin, soybean protease inhibitors also result in impaired growth and poor food utilization. Soybean allergenic proteins belong to the cupin super-family (Stanley *et al.*, 1999; Karen and Chandra, 2002; Mills and Breiteneder, 2005; Heimo and Mills, 2005), they can cause allergic reactions in human (Sampson, 2004; Makio, 2005; Ballmer-Weber *et al.*, 2007) and animals (Li *et al.*, 1990; Lalles *et al.*, 1996; Sun *et al.*, 2008). So far, various soybean allergenic proteins have been discovered (Table 3; Figure 1) (Ogawa *et al.*, 1991; Wilson *et al.*, 2008; Amnuaycheewa *et al.*, 2010) especially Glycinin and β-conglycinin are most researched (Shibasaki *et al.*, 1980; Burks *et al.*, 1988; Ogawa *et al.*, 1998; Adachi *et al.*, 2008; Sun *et al.*, 2008). Sometimes, soybean agglutinin (Wilson *et al.*, 2008) and Kunitz soybean protease inhibitors (Moroz and Yang, 1980; Burks *et al.*, 1994) are also considered as soybean allergens. For the reasons given above, soybean is known as one of the so-called "big eight" allergens foods (Food Allergen Labeling and Consumer Protection Act of 2004).

Soybean allergy affects approximately 0.4% of children (Savage *et al.*, 2010). It is most common for infants and usually develops at the age of three months. Most infants outgrow their soybean allergy by the age of two but a severe soybean allergy can last a lifetime (Soy: one of the nine most common food allergens). Soybean allergy has a variety of symptoms involving the skin, gastrointestinal tract, respiratory tracts, etc. (Table 4). Therefore, soybean allergy has become a public health problem that continues to challenge both the consumer and the food/feed manufacturer all over the world (Bennis, 1998; Gisele *et al.*, 2001; Graham *et al.*, 2003; Scott and Hugh, 2006; Scott *et al.*, 2007; Ballmer-Weber *et al.*, 2007; Frias *et al.*, 2008; Allergen Data Collection: Soybean (Glycine max); Holzhauser et al., 2009).

In this paper, the characteristics, detection or exploration methods, *in vivo* research models of soybean allergenic proteins are deeply discussed. The ingredient processing used to reduce the allergenicity of soybean is also reviewed.

Characteristics of main soybean allergenic proteins

Glycinin and β-conglycinin are the two major globulins in soybean that account for about 70-80% of the total seed globulin fraction (Krishnan *et al.*, 2009). They are referred to as 11s and 7s according to their sedimentation coefficients (Catsimpoolas and Ekenstam, 1969). The ratio of 11S to 7S is about 0.5-1.7 in soybean and it varies among cultivars (Tulloch *et al.*, 1985; Karen and Chandra, 2002; Victor *et al.*, 2007). In this part, a survey is made on the characteristics of glycinin and β-conglycinin.

Glycinin

Glycinin is the predominant soybean seed storage protein which accounts for over 50% in most varieties (Staswick *et al.*, 1981). In general, glycinin has a molecular mass ranged from 320 kDa to 360 kDa. So far, five subunits, A1aB1b, A1bB2, A2B1a, A3B4 and A5A4B3, encoded by a

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small gene family have been identified and considerable variations among these subunits have been discovered (Savithiry *et al.*, 2007). Based on the amino acid sequence homology, these subunits can be divided into three groups: group I (A1aB1b, A1bB2, and A2B1a), group IIa (A3B4) and group IIb (A5A4B3) (Kazuhiro *et al.,* 1997; Motoyasu *et al.,* 2001; Zhang *et al.,* 2002). Information of proglycinin A1aB1b homotrimer (Adachi *et al.*, 2001) and A3B4 homohexamer (Adachi *et al.,* 2003) including crystal structure, disulfide bonds location, potential N-glycosylation sites and the possible degradation mechanism during germination are reported.

"A" is an acidic polypeptide with molecular mass ranging from 35 kDa to 43 kDa and pI value about 4.8-5.5, while "B" is a basic polypeptide with a molecular mass about 20 kDa and pI value about 6.5-8.5. They are linked by disulfide bond (Badley *et al.,* 1975; Moreira *et al.,* 1979; Staswick *et al.*, 1981; Utsumi *et al.*, 1981; Staswick *et al.,* 1984a; Staswick *et al.,* 1984b; Mervyn *et al.*, 1985). There are some essential features of the primary structures of acidic polypeptides. A3 is a 46 kDa acidic polypeptide composed of 410 amino acids. It has a sequence which is highly homologous to the NH2-terminal sequence of A4 (Moreira *et al.*, 1981; Hirano *et al.*, 1984). A7, a 24 kDa acidic polypeptide consisting of 212 amino acids, is also identified to be highly homologous to A4. However, comparing with A4, A7 has a deletion of 45 residues in C-terminal region and 4 residues are substituted. Furthermore, A7 is not covalently linked to other subunits by disulphide bond (Kagawa *et al.*, 1988).

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Antiserums to native glycinin do not react with isolated polypeptides, and antibodies to the purified polypeptides do not react with native glycinin neither. When native glycinin is denatured, the anti-glycinin antibodies lost their ability to react with the denatured glycinin. These phenomena suggest that some substantial structural rearrangement occur when glycinin is denatured or disaggregated (Moreira *et al.,* 1981).

IgE-binding epitopes of glycinin G1 acidic chain locate on G217-V235 (GGSI LSGFTLEFLEHAFSV) and G253-I265 (GAIVTVKG GLSVI) (Beardslee *et al.,* 2000), while the IgE-binding epitopes of glycinin G2A situate at S219-N233 (SGFAPEFLKEAFGVN) (Xiang *et al.*, 2002). Moreover, eleven IgE-binding epitopes of glycinin G2 are found in 6 regions: 1-23 (soybean specific), 57-111, 169-215, 249-271, 329-383 and 449-471 (Helm *et al.,* 2000 a*;* Helm *et al.,* 2000 b). Most of these IgE-binding epitopes are generally buried and hydrophobic. And due to the homology of IgE-binding epitopes, cross-reactions may occur between soybean and other leguminous plants, such as peanut and lupin (Moneret-Vautrin *et al.,* 1999; Xiang *et al.,* 2002).

β-conglycinin

β-conglycinin, a glycoprotein with 180 kDa, contains 5% of carbohydrates moieties which may relate to its immunoreactivity (Amigo-Benavent *et al.,* 2009). It is made up of α′ (76 kDa), α (72 kDa) and β (53 kDa) subunits, and isoelectric points of these subunits are 4.90, 5.18, and 5.66-6.00 (Koshiyama et al., 1976; Breiteneder *et al.,* 2006). And considerable similarity of amino

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acid composition exists between α and α′ subunit. Comparing with α and α′ subunit, β subunit is devoid of cysteine and methionine, but has a higher content of hydrophobic amino acids (Thanh *et al.*, 1976; Hirano, 1986).

β-conglycinin can be fractionated into six distinct components including α′β (B_1-), αβ (B_2-), αα′β (B_3-), αβ (B_4-), αα′ (B_5-), and α (B_6-conglycinin). β subunit is a major constituent of B_1- and B_2-conglycinin, whereas B3- to B6-conglycinin are predominantly compose of α subunit (Thanh *et al.*, 1976; Coates *et al.*, 1985).

α′, α and β subunits are all potential food allergens (Krishnan *et al.*, 2009). α subunit known as Gly m Bd 60K of β-conglycinin can be recognized by serums of soybean-sensitive patients. And the IgE-binding site of α subunit locates at the peptide 232-283 (Ogawa *et al.*, 1995).

Soybean allergenic proteins detection or exploration methods

In order to further investigate soybean allergenic proteins, effective tools for detecting or exploring soybean allergens are necessary. In recent years, researches about soybean proteins detection have been rapidly expanded (Table 5). Taking into account the practicality, two currently using methods Enzyme linked immunosorbent assays and Immunoblotting are discussed in this part. In addition, Two-dimensional gel electrophoresis (2-DE) which is frequently used in Proteomic research is also reviewed.

Enzyme linked immunosorbent assays (ELISA)

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As a powerful tool for detecting proteins, ELISA such as Sandwich ELISA, Competitive ELISA and Indirect ELISA are widely used to determine soybean proteins in food products. They can conduct a larger series of samples simultaneously (Koppelman, 2004). Performing an ELISA needs at least one antibody, but human serums used in ELISA are not easy to get, so serums getting from animals such as rabbits or mice are frequently used.

Sandwich ELISA is used to detect glycinin, β-conglycinin and P34 (soybean vacualor protein). During the detection of glycinin, IFRN 0025 is taken as the capture antibody and rabbit polyclonal serum $R103b_3$ is taken as the detector (Plumb *et al.*, 1994). In the process of detecting β-conglycinin, rabbit polyclonal serum $R195b_3$ serves as the capture antibody and monoclonal antibody Mab 0089 serves as the detector (Plumb *et al.*, 1995). When detecting p34 in processed foods, sandwich ELISA shows a high specificity (Morishita *et al.*, 2008).

Competitive ELISA is another conventional ELISA which is effectively used to detect soybean proteins (Yasumoto *et al.*, 1990; Pedersen *et al.*, 2008). In the course of the experiments, some polyclonal antibodies have cross-reactivity (Zhao *et al.*, 2008) which makes their specificity debatable in some extent. So monoclonal antibody like Mab 6G4 to β-conglycinin (You *et al.*, 2008) and Mab 4B2 to glycinin (Ma *et al.*, 2010) are developed. Besides, Indirect ELISA can be used to quantify soybean protein in food products too (Bittencourt *et al.*, 2007; Yang *et al.*, 2010; Amnuaycheewa and deMejia, 2010).

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Those ELISA methods discussed above are widely used in soybean research. However, the preparations of ELISA are sometimes time-consuming and it will take much longer time when preparing antibodies by oneself. There are still some important considerations. First, antibodies should have high specificity because some plants may cross-react with soybean such as birch pollen and other legumes especially peanuts. Second, antibodies should possess high titer and high affinity for the sake of obtaining sufficient test sensitivity. Even antibodies are made successfully; they are still not suitable for all conditions. Just like antibodies against raw protein may not react with denatured proteins in processed foods. Thus commercial ELISA kit using in determining soybean protein in processed foods has been developed, and it shows high precision and reliability (Sakai *et al.*, 2010). In addition, homogeneous immunoassay which uses gold nanoparticles and light scattering detection can detect soybean proteins too. And comparing with commercial ELISA kit, its assay time is significantly shorter and the detection limit is about 10 times lower (Sánchez-Martínez *et al.*, 2009).

Immunoblotting

Immunoblotting is a powerful research tool which can indicate molecular mass and immunoreactivity of allergenic proteins (Burks *et al.*, 1988; Ogawa *et al.*, 1991). It can detect glycinin A at nanogram level (Meisel, 1993). By using immunoblotting, two soybean allergenic proteins glycinin G1 acidic chain and a 22 kDa G2 glycinin are found (Beardslee, 2000; Helm *et al.*, 2000a).

Immunoblotting can be used to investigate the digestion of glycinin and β-conglycinin in preruminant calves and rats (Lalles *et al.*, 1999; Perez *et al.*, 2000) and there are some interesting results. In the early abomasal outflow of preruminant calves, nearly intact basic polypeptides and partially degraded acidic polypeptides are found, and intact β-conglycinin exists in most samples. In ileal digesta, intact basic polypeptides and partially digested acidic polypeptides still exist up to 8-10 h after meal, whereas β-conglycinin's immunoreactivity cannot be detected (Lalles *et al.*, 1999). In rats, glycinin and β-conglycinin are digested rapidly. Immunoreactive and semi-intact globulins are found in both gut contents and gut tissues (Perez *et al.*, 2000). Besides, immuoblotting is also used to inspect the immunoreactivity variation of fermented soybean products (Frias *et al.*, 2008).

Applications of Immunoblotting promote the development of soybean allergenic proteins research. But during the operation, it is necessary to choose the fit membrane, try to find the appropriate first and second antibody concentration, and use the right developing regents. Furthermore, specialized equipments and trained staffs are also needed.

Two-dimensional gel electrophoresis (2-DE)

Two-dimensional gel electrophoresis is a powerful method which can separate protein complex according to their isoelectric point, molecular mass, solubility, and relative abundance. Since 1975 when it was firstly introduced (O'Farrell, 1975), 2-DE has been spread quickly and been widely used in scientific research. In 1987, 2-DE was used to examine the protein components in isolated soybean protein bodies, and it was found that a small portion of glycinin was glycosylated (Lei and Reeck, 1987). From then on, using 2-DE to investigate soybean proteins has been largely reported (Zarkadas et *al.*, 2007; Krishnan *et al.*, 2007; Brandão *et al.*, 2010).

2-DE leads to the establishment of soybean proteome maps with high-resolution, and enables the assessment of genetic variability among soybean cultivars become possible (Zarkadas *et al.*, 2007; Krishnan *et al.*, 2007). Through using 2-DE, it is found that Glycinin expression differences exist between transgenic and non-transgenic cultivars (El-Shemy *et al.*, 2007) or among different soybean genotypes (Natarajan *et al.*, 2007)and the differences of allergen levels among different soybean lines are also exist (Rouquié *et al.*, 2010). Moreover, using 2-DE can investigate the effects of β-conglycinin on proteome expression of pig intestinal cells, and can find that 16 different spots in cultured intestinal epithelial cells and 14 different spots in jejunal mucosa of piglets exist (Chen *et al.*, 2011).

Four different protein extraction/solubilization methods, urea, thiourea/urea, phenol, and trichloroacetic acid/acetone methods are compared in order to determine their efficacy in separating soybean proteins. And results suggest that thiourea/urea and trichloroacetic acid /acetone methods are more efficient and reliable (Natarajan *et al.*, 2005). Comparing the separation efficiencies of different immobilized pH gradient strips shows that most β-conglycinin subunits are well-separated in pH range 3.0-10.0, while glycinin are well-separated in pH range 4.0-7.0 and 6.0-11.0 (Natarajan *et al.*, 2006).

2-DE provides significant insight into soybean proteome. But there are some matters needing attention in the test, such as choosing an efficient and reliable extraction/solubilization method, using suitable immobilized pH gradient strip, designing a right running procedure, selecting right staining solution and proper staining time, etc. However, those conditions used in one experiment are not suitable for all cases.

Research using *in vivo* animal models

For reasons of ethics and efficacy, soybean allergy research using humans are impossible sometimes. Therefore, research using *in vivo* animal models becomes an alternative that provides important information for soybean allergy (Table 6). In this part, *in vivo* models using murine animals and piglets are reviewed.

Murine animals

Murine animals are widely used in soybean allergy research not only because they can simulate human health and disease status but also because they can be treated at the same time and be handled easily. Testing expenses are also quite low.

Using Wistar rats, the digestion of glycinin and β-conglycinin *in vivo* is investigated (Perez *et al.*, 2000). In Brown Norway rats, the negative effects of β-conglycinin on animals' growth and immune function are assessed (Guo *et al.*, 2007). And in Sprague-Dawley rats, it is found that a low dose of lipoic acid can attenuate allergies induced by soybean glycinin (Ma *et al.*, 2010) or β-conglycinin (Han *et al.*, 2010).

In Kunming mice, effects of steam-processing on glycinin and β-conglycinin's allergenicity are evaluated (Sun et al., 2006). In BALB/c mice, it is discovered that 7S soybean protein can elicit allergic reactions (Bittencourt *et al.*, 2007). In BALB/c mice, after gavage with glycinin or β-conglycinin, increased soybean-specific IgE, IgG1 levels, high serum histamine, severe mast cells degranulation, and small intestine epithelium damage are observed (Liu *et al.*, 2008).

Piglets

Piglets are used in the research of soybean allergy because they closely resemble humans in gastrointestinal physiology and immunity system. Moreover, they are born with immunocompetence which makes the immune responses assessment become possible (Pastoret *et al.*, 1998; Helm *et al.*, 2003; Kimber *et al.*, 2003).

After feeding soybean meal, decreased villus height and increased serum IgG titers to soybean proteins are detected in early weaned pigs (Li *et al.*, 1990). After feeding heat-treated soybean, both B and T lymphocytes increase in the duodenal mucosa of 28 day old piglets (Dreau *et al.*, 1995). After adding 4% glycinin or 4% β-conglycinin in diets, proliferative index, apoptotic index, and relative enterocyte migration rate increase in duodenum of piglets (Zhao *et al.*, 2010). In addition, diarrhea symptoms, skin wheal and flare responses are observed in Wuzhishan minipigs after ingestion of 4% glycinin or 4% β-conglycinin (Huang *et al.*, 2010).

Pigs in different physiological stages are used to investigate the immunoreactivity and structural variation of glycinin and β-conglycinin *in vivo*. Residual immunoreactive glycinin or β-conglycinin tends to decrease with the growth of age and the descending of digestive tract, and β-conglycinin especially β subunits is more indigestible than glycinin (Zhao *et al.,* 2008; Wang *et al.,* 2009; Wang *et al.,* 2010). In young pigs, it is found that *Forsythia suspensa extract* a kind of herbal extract can inhibit anaphylaxis induced by purified soybean β-conglycinin (Hao *et al.,* 2010).

Through *in vivo* animal models, considerable progresses in soybean allergy research have been made (Helm *et al.,* 2003). Meanwhile, *in vitro* cell models using mouse (Xu *et al.,* 2010a) or porcine intestinal epithelial cells (Chen *et al.,* 2011) are also reported. These *in vivo* and *in vitro* models improve our understanding on soybean sensitivity in human and provide a sound basis for designing a quality evaluation of soybean products safety. However, there still have some limitations when apply these results to human. Moreover, none of these models has been evaluated rigorously or validated formally yet (Kimber *et al.,* 2003).

Ingredient processing

Soybean has become an edible food for a long time due to its high trophism. It is often added into the alternative formulas to feed infants who are not breastfed or unable to tolerate milk-based formulas. However, for the children at high risk of soybean allergies, soybean formulas are not a better choice unless the soybean is low allergenicity. Therefore it is necessary to find economical and feasible ingredient processing that can reduce or eliminate soybean's allergenicity. In this part, the main ingredient processing used to reduce soybean's allergenicity but not to lower its nutritional values are discussed.

Physical processing

Extrusion

Extrusion processing may reduce the allergenicity of glycinin and β-conglycinin, because it can decrease skin-fold thickness, increase growth performance and benefit intestinal morphology of pigs (Hancock *et al.,* 1990; Li *et al.,* 1991; Friesen *et al.,* 1993). Meanwhile, extrusion processing can improve the nitrogen and energy utilization of diets which are deficient in lysine (Rodhouse *et al.*, 1992), reduce the antigenicity of defatted soybean meal (Ohishi *et al.*, 1994) and soybean hypocotyls (Saitoh *et al.,* 2000). Besides, comparing with roasted soybean, the availability of selenium in extruded soybean is much higher (Li *et al.*, 2009).

High pressure

After high pressure processing ≥300 MPa, glycinin is dissociated into subunits and the conformations of these subunits are changed; and more sulfhydryl groups, hydrophobic regions and amino acid residues that have ultraviolet absorbance are found. When processing at 400 MPa for 10 min, glycinin is denatured completely; while processing at 500 MPa for 10 min, some α-helix and β-sheet are destroyed and convert into random coil (Zhang *et al.,* 2003). Besides, β-conglycinin can also be denatured and dissociated into subunits after high pressure processing ≥300 MPa (Zhang *et al.*, 2010).

Under the combined effect of 60 °C and 400 MPa, flocculation and gelation in β-conglycinin emulsions increase, but there has a negligible change in glycinin (Puppo *et al.*, 2011). Moreover, high hydrostatic pressure can reduce the antigenicity of soybean that can be used in the hypoallergenic soybean sprouts production (Peñas *et al.*, 2011).

NaCl, which is used extensively in food formulations, plays an important role in high pressure processing. During high pressure treatment, 0.2M/L NaCl can promote the formation of more stable structures in glycinin. At 200 MPa, 0.2M/L NaCl can protect β-conglyicnin; while at 600 MPa, it can enhance the denature degree of β-conglyicnin (Speroni et al., 2010).

Heating

Although glycinin and β-conglycinin are thermostable, appropriate heat processing can reduce their allergenicity to some extent. Soybean protein retains its allergenicity after heating even up to 65 °C for 30 min, but loses its allergenicity rapidly in the temperature range between 70 °C and 90 °C (Catsimpoolas and Ekenstam, 1969). After heating at 80 °C for 30 min, the allergenic potentials of 2S globulin fractions slightly increase, but the allergenic potentials of 7S and 11S reduce to 39-75% of the initial values (Shibasaki *et al.*, 1980).

The denature temperatures of glycinin group I, IIa, and IIb are 92.8, 96.0 and 97.9 °C (Tezuka *et al.*, 2004). And glycinin has the propensity to form large aggregates after heating in excess of 95°C (Mills *et al.*, 2003). When heating in excess of 75°C, β-conglycinin begins to denature, but only partial secondary and tertiary structure lose (Mills *et al.*, 2001). Over heating will make soybean indigestible, while under heating can not reduce its allergenicity, so an appropriate temperature is important. Besides, Pulsed ultraviolet light, a non-thermal food processing technology, can reduce the allergenic potency of soybean extracts too (Yang *et al.*, 2010).

Roasting

Roasting is a simple soybean processing method. It can improve the soybean's utilization in early lactation cows (Faldet *et al.*, 1971) and young calves (Prasad *et al.*, 1976), increase the milk yield of cows (Tice *et al.*, 1992), fatty acid digestibility of steers (Aldrich, *et al.*, 1995) and growth performance of broiler chickens (Hamilton *et al.*, 2000). There has no detrimental effect on growth performance and carcass composition of female broiler turkeys when adding 15% roasted soybeans into starter diets or replacing the soybean meal with roasted soybeans in grower and finisher diets (MacIsaac *et al.*, 2005). And the optimal growth performance can be obtained when the ratio of roasted full-fat soybeans to soybean meal is about 2:1 in the starter feeds of broiler chickens (Hamilton and McNiven, 2005).

After roasting at 108 °C for 10 min or 30 min, the residual antitryptic activity, immunoreactive glycinin and immunoreactive β-conglycinin in fullfat soybean are 40%, 17%, 5% or 20%, 6%, 3% (Ouedraogo *et al.*, 1998). The antitryptic activity of soybean reduces to 13.33% of the initial value after microwave roasting for only 2 min (Barać and Stanojević, 2005). Processing by oil-roasting and dry-roasting, the moisture, lipid content, and free amino acids composition of roasted soybeans are different (Boge *et al.*, 2009). In addition, research finds that roasting at 200° C for 1 min is the best combination in sattu production (Mridula *et al.*, 2007).

Chemical processing

Ethanol (alcoholic) extraction

Ethanol extraction can improve the quality of soybean protein, especially when the soybean protein is under- or over-processed. Soybean products extracted with <30% or >96% ethanol at 70-80°C still remain antigenic activity, but have very little antigenic activity when extracting with 55-76% ethanol at 70-80°C (Sissons *et al.*, 1982). In soybean protein concentrate extracted by hot aqueous ethanol, the content of glycinin and β-conglycinin are under 30mg/g and 5mg/g (Russett *et al.*, 1998). Moreover, alcoholic treatment also can improve the growth performance of growing pigs (Dreau *et al.*, 1994).

In vitro glycation

Glycation not only can increase the foaming property and emulsifying activity of 11S-rich glycinin fraction (Achouri *et al.*, 2005) but also can regulate the thermal aggregation of glycinin and β-conglycinin (Xu *et al.*, 2010b). The allergenicity of soybean protein isolates glycated with fructose or fructooligosaccharide is 90% lower than that of the unglycated SPI (Lagemaat *et al.*, 2007).

Enzymatic hydrolysis

Food allergens are usually indigestible *in vitro*. Soybean β-conglycinin especially β-subunit is stable to pepsin (Astwood *et al.,* 1996; Moreno *et al.,* 2007) and Glycinin is resistant to hydrolysis by papain, Alcalase and fungal protease (De La Barca *et al.,* 2005). In some cases, pancreatin can reduce the allergenicity of defatted soybean flour (Govindaraju *et al.,* 2007). During the hydrolysis by pepsin and chymotrypsin, acidic polypeptide is hydrolyzed more easily than basic polypeptide, and the fragments less than 20 kDa has no immunoreactivity, while a 20 kDa peptide has highly immunoreactivity (Lee *et al.,* 2007). After being hydrolyzed with pepsin, trypsin or pepsin+trypsin in the enzyme/substrate ratio of 1:100, the residual immunoreactive glycinin and β-conglycinin persistently decreases. Comparing with β-conglycinin, glycinin is more easily hydrolyze by pepsin, but more hardly hydrolyze by trypsin. And the combination of pepsin and trypsin seems to be the most effective hydrolysis pattern (Figure 2; Zhao *et al.,* 2010). Besides, Microbial transglutaminase can decrease the solubility and emulsifying activity of β-conglycinin and glycinin, and improve their gelling and heat stability (Hu *et al.,* 2011).

Biological processing

Fermentation

Fermentation represents one of the oldest known uses of biotechnology. It not only can improve the sensory quality, nutritional value, digestability and preservation of soybean, but also can eliminate some harmful ingredients from soybean (Herian *et al.,* 1993; Han 2003; Song *et al.,* 2010; Kwon *et al.,* 2010). In soybean fermentation processing, various zymocyte are isolated and employed (Table 7). *Lactic acid bacteria* can improve the nutritional value of soybean white flakes (Refstiea *et al.*, 2005) and degrade α subunit, α′ subunit and acidic polypeptides (Aguirre *et al.*, 2008). Soybean meal fermented with *Aspergillus oryzae* can improve piglets' growth performance (Liu *et al.*, 2007) and increase broilers' digestive enzyme activities (Feng *et al.*, 2007). Soybean meal fermented with *Saccharomyces cerevisiae* has low immunoreactivity (Song *et al.*, 2008). After being fermented with *Lactococcus lactic subsplactis, Aspergillums oryzae and Bacillus subtilis* together, steamed soybean proteins degrade into peptides less than 10 kDa and have no antigenicity (Lee *et al.*, 2004). Besides, *Bifidobacterium lactic, Lactobacillus plantarum and Saccharomyces cerevisiae* can reduce soybean profilin's immunoreactivity by 68.3% to 72.7% (Amnuaycheewa and de Mejia, 2010). Some natural fermented soybean products, such as yogurt, miso and tempeh also have low antigenicity (Song *et al.*, 2008). Because fermentation can decrease soybean allergenicity effectively, it is widely used in the hypoallergenic soybean products manufacture (Frias *et al.*, 2008a; Frias *et al.*, 2008b).

Conclusions

From the above, we can see that glycinin is composed of acidic and basic polypeptides linked by disulfide bonds, and β-conglycinin is a glycoprotein made up of α′, α and β subunits. Among soybean protein detection or exploration methods, ELISA, Immunoblotting and 2-DE seem to be the most used ones. Although none of the *in vivo* or *in vitro* models has yet been evaluated rigorously or validated formally, these models do have made considerable contributions to the soybean allergenic proteins researches. The ingredient processing reviewed in this paper can reduce soybean's allergenicity more or less, and fermentation seems to be a better choice. However, our knowledge on glycinin and β-conglycinin especially their allergy mechanisms and medical treatments are still lacked. So it is necessary to conduct much more studies on these aspects to improve the life quality of soybean allergic consumers, help food/feed producers to concern about food safety and develop relevant strategies.

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Table 1 Traditional soybean foods/ingredients

Fermented soybean foods	Non-fermented soybean foods/ingredients
Doenjang, Empeh, Kenima, Kecap, Lobster sauce, Miso, Natto, Preserved beancurd, Soybean yogurt, Soybean sauce, Sufu, Tempeh, Tungrymbai	Bean curd sticks, Bean curd sheet, Bean sprout, Edamame, Okara, Soybean flour, Soybean milk, Soybean oil, Soybean protein isolate, Soy protein concentrate

Table 2 Anti-nutritional factors in soybean products

Soybean products	Trypsin Inhibitor	Soybean Agglutinin	Glycinin	β-glycinin	References

Extracted soybean protein[1]	2.9% (Kunitz)	ND[2]	36.5%	27.8%	Sato *et al.*, 1986
Soybean	45-50 mg/g	3.5 ppm	180 ppm	>60 ppm	Peisker, 2001
Soybean meal	1-8 mg/g	10-200 ppm	66 ppm	16 ppm	Peisker, 2001
SPC[3] enzyme treated	1 mg/g	<1 ppm	<100 ppm	<10 ppm	Peisker, 2001
SPC alcohol extracted	2 mg/g	<1 ppm	<100 ppm (<3 soycomil)	<10 ppm	Peisker, 2001
Protein isolate	<1 mg/g	0 ppm	ND[2]	ND[2]	Peisker, 2001
Soybean total protein	ND[2]	ND[2]	10-20% (immunocompetence)	1-2% (immunocompetence)	Li, 2003
Full-fat soybean powder□	42.50 TUI/mg	16.75 mg/g	44.36 mg/g	10.42 mg/g	Zhao *et al.*, 2007
Full-fat soybean powder□	51.00 TUI/mg	19.86 mg/g	167.67 mg/g	11.30 mg/g	Zhao *et al.*, 2007
Defatted soybean powder	32.00 TUI/mg	12.11 mg/g	161.01 mg/g	20.30 mg/g	Zhao *et al.*, 2007
Heated soybean powder□	3.00 TUI/mg	1.36 mg/g	31.91 mg/g	6.21 mg/g	Zhao *et al.*, 2007
Heated soybean powder□	3.40 TUI/mg	2.97 mg/g	44.65 mg/g	6.20 mg/g	Zhao *et al.*, 2007
Extruded full-fat soybean	0.90 TUI/mg	1.55 mg/g	21.74 mg/g	1.77 mg/g	Zhao *et al.*, 2007

Soybean meal	0.82 TUI/mg	5.66 mg/g	164.70 mg/g	18.46 mg/g	Zhao *et al.*, 2007
Dehulled soybean meal	0.30 TUI/mg	1.14 mg/g	48.34 mg/g	12.45 mg/g	Zhao *et al.*, 2007
SPC[3]	10.80 TUI/mg	2.87 mg/g	1.99 mg/g	6.74 mg/g	Zhao *et al.*, 2007
Soy protein isolate	9.00 TUI/mg	16.88 mg/g	0.32 mg/g	8.63 mg/g	Zhao *et al.*, 2007
SPC[3]	1-3 mg/g	<1 ppm	<20 ppm	ND[2]	Robert *et al.*, 2007
Soybean hulls	ND[2]	12.2	25.3	41.4	Wang *et al.*, 2008

Note: Extracted soybean protein[1] means proteins extracted with both water and 0.5M sodium chloride; ND[2] means not detected; SPC[3] means soy protein concentrate.

Table 3 IgE-binding soybean allergens (Adapted from Wilson *et al.*, 2005)

Molecular weight (kDa)	Fraction and name of soybean allergens
7.0	Gly m 1a; Hull protein
7.5	Gly m 1b; Hydropobicprotein; Hull protein
8.0	Gly m 2; Hull protein
12~15	rGly m 3; Profilin
17	2S-Globulin fraction
20	Kunitz trypsin inhibitor; 2S-Globulin
18~21	Whey fraction
22	Glycinin G2; Basic chain of glycinin; 11S-Globulin
28	Gly m Bd 28 K; 7S Globulin

30~34	Gly m Bd 30 K, P34; Immunodominant allergen
29~31	Whey fraction
32	Soy lectin; Soybean agglutinin
33~35	7S-Globulin
35~38	7S-Globulin
35~40	Glycinin G1; Acidic chain of glycinin; 11S-Globulin
40~41	7S-Globulin
42	β Subunit of β-conglycinin
47~50	7S-Globulin
52~55	7S-Globulin
63~67	α Subunit of β-conglycinin; Gly m Bd 60 K
71	α’ Subunit of β-conglycinin

Table 4 Symptoms of soybean-induced allergic reactions

Skin-related	Gastrointestinal tract-related	Respiratory tracts-related	The other symptoms
Acne Angioedema Atopic dermatitis Eczema Itching Urticaria (Soya-Information about Soy and Soya Products)	Colitis Diffuse small bowel disease Enterocolitis Vomiting (Soya-Information about Soy and Soya Products)	Asthma Bronchospasm Dyspnea Laryngeal edema Rhinitis Wheezing (Soya-Information about Soy and	Conjunctivitis Lethargy (Soya-Information about Soy and Soya Products)

Atrophic dermatitis (Symptoms of Soy Allergies)	Diarrhoea (Symptoms of Soy Allergies)	Soya Products) Nasal congestion (Symptoms of Soy Allergies)

Table 5 Overview of soybean protein detection methods

Methods	**References**
Disc immunoelectrophoresis	Catsimpoolas *et al.*, 1968
Capillary electrophoresis	García-Ruiz *et al.*, 1999; García-Ruiz *et al.*, 2006
Immunoblotting	Burks *et al.*, 1988; Ogawa *et al.*, 1991
Sandwich ELISA[1]	Song *et al.*, 2008; Pedersen *et al.*, 2008

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Competitive ELISA[1]	You *et al.*, 2008; Ma *et al.*, 2010
Indirect ELISA[1]	Bittencourt *et al.*, 2007; Yang *et al.*, 2010
Commercial ELISA[1] kit	Pedersen *et al.*, 2008; Sakai *et al.*, 2010
Immunohistochemical method	Pospiech *et al.*, 2009; Zhang 2009
Radio allegro-sorbent test inhibition	Herian *et al.*, 1993; Hefle *et al.*, 2005
Enzyme allego-sorbent test inhibition	Müller *et al.*, 1998; Pedersen *et al.*, 2008
Histamine release	Kleine-Tebbe *et al.*, 2002; Pedersen *et al.*, 2008
Polymerase chain reaction	Hefle *et al.*, 2005; Yamakawa *et al.*, 2007
Mass spectrometry	Lee *et al.*, 2010; Houston *et al.*, 2011
High-performance liquid chromatography	García *et al.*, 2000; Saz and Marina, 2007

Note: ELISA[1] means Enzyme-linked immunosorbent assay.

Table 6 Soybean allergy research *in vivo* animal models

Animals	**References**

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Mouse	Bittencourt *et al.*, 2007; Liu *et al.*, 2008
Rat	Perez *et al.*, 2000; Guo *et al.*, 2007
Guinea pig	Cordle, 2004; Cantani, 2008
Rabbit	Eastham *et al.*, 1982; Cordle, 2004
Dog	Jeffers *et al.*, 1991 ; Helm *et al.*, 2003
Swine	Huang *et al.*, 2010; Chen *et al.*, 2011
Calf	Lalles *et al.*, 1996; Lalles *et al.*, 1999

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Table 7 Zymocyte employed in soybean fermentation

Zymocyte	References
Aspergillus oryzae	Liu *et al.*, 2007; Feng *et al.*, 2007
Bacillus subtilis	Inatsu *et al.*, 2006; Frias *et al.*, 2008b
Bifidobacterium lactic	Wang *et al.*, 2002; Lee *et al.*, 2004
Lactobacillus plantarum	Frias *et al.*, 2008b; Amnuaycheewa and de Mejia, 2010

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Lactococcus lactic subsp. lactis	Lee *et al.*, 2004
Rhizopus oryzae	Frias *et al.*, 2008a; Frias *et al.*, 2008b
Saccharomyces cerevisiae	Song *et al.*, 2008; Amnuaycheewa and de Mejia, 2010

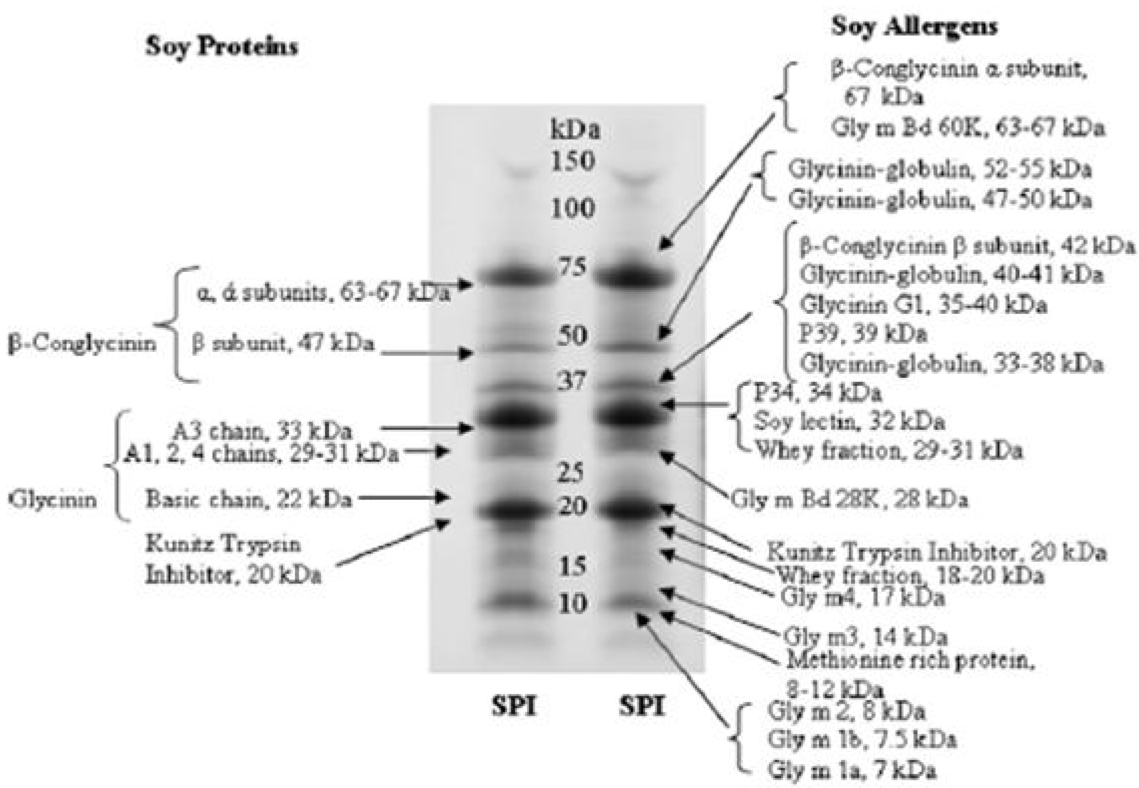

Figure 1 Electrophoretic profile of Soybean Protein Isolate with indication of major soybean proteins and reported soy allergens (Adapted from Amnuaycheewa and de Mejia, 2010)

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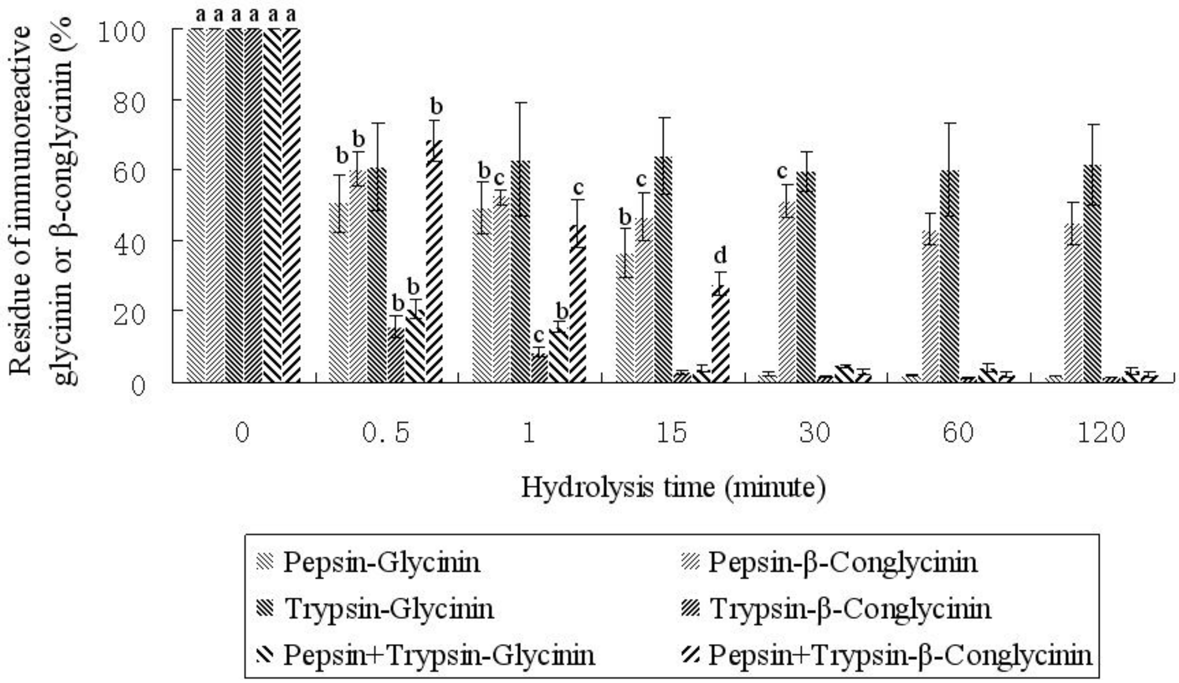

Figure 2 Residue of immunoreactive glycinin or β-conglycinin after enzymes hydrolysis in the ratio of 1:100 (enzyme/substrate) during different time (Adapted from Zhao *et al.*, 2010)

Note 1: [a,b,c,d,] Means in the same group followed by different superscripts differ at the *P* values indicated ($p<0.05$). Each value is the mean of 4 replicates.

Note 2: in Pepsin+Trypsin-Glycinin and Pepsin+Trypsin-β-conglycinin groups, the hydrolysis time is double (e.g 120 minnute means 120 minnute pepsin hydrolysis + 120 minnute trypsin hydrolysis).

Note 3: generally, either hydrolysis with pepsin, trypsin or pepsin+trypsin, the residue of immunoreactive glycinin or β-conglycinin is decreased from 0 to 120 minutes. The residual proportion of immunoreactive glycinin is only 2.43±0.36% after hydrolyzed with pepsin for 30 min, while it is still 61.41±11.36% after hydrolyzed with trypsin for 120 min; the residue proportion of immunoreactive β-conglycinin is 44.92±5.81% after hydrolyzed with pepsin for 120min, while immunoreactive β-conglycinin is nearly vanished after hydrolyzed with trypsin for 15 min (2.71±0.46%); when hydrolyzed with both pepsin and trypsin, the residual proportion of immunoreactive β-conglycinin is higher than glycinin (before 15 (15+15) min).

ACCEPTED MANUSCRIPT