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# Important Minor Soybeans Proteins: Soybean Allergens and Enzymes Inhibitors

Lenka Vorlová

*Department of Milk Hygiene and Technology,  
Faculty of Veterinary Hygiene and Ecology,  
University of Veterinary and Pharmaceutical Sciences Brno,  
Czech Republic*

## 1. Introduction

### 1.1 Overall significance

Unfavourable organism response to food can be divided into toxic and non-toxic reaction with abnormal clinic response of the organism. As allergy is called such an organism response mediated always by the immune system in contrast to food intolerance. Food allergy can be further divided into reaction of the organism bound to IgE and without IgE reaction. Hypersensitiveness of the immune system can be classified into types I - IV. Type I is reaction of mast cells with bonded IgE antibodies for the presence of antigen (Gray and Chan, 2003). The prevalence of allergic reaction varies geographically and may depend of the frequency of consumption, the age of introduction into the diet and the genome (Ötles, 2005).

### 1.2 Physiological function

In case of food allergies mediated by IgE it is possible, especially according to the clinical symptoms, to define two forms of allergic reactions. Food allergic reactions can have a different manifestation in localization, in time horizon as well as in the importance of symptoms, which represent a wide scale of evidence from the most simple up to anaphylactic shock. The first form appears already shortly after birth and in the early infancy. The sensitization is elicited by a reaction in the gastrointestinal tract and is most frequently manifested as atopic dermatitis. The second form appears in particular at a later age as a respiratory allergy, in case of which are often the sensitising agents also inhalation allergens (cross reactive allergens) (Metcalf et al.2003).

From the acute symptoms is often present nausea, emesis and diarrhoea, and in serious cases even bloody diarrhoea. The pathological-anatomical picture of the allergic reaction is characterized by inflammatory damage of intestinal mucosa with the affection of the respiratory tract, particularly by bronchoconstriction (Gray and Chan, 2003).

### 1.3 Nomenclature and legislation

The official list of allergens was created by the World Health Organization and International Union of Immunological Societies. A substance is classified as an allergen if it causes an

allergic reaction with the prevalence of IgE reactivity above 5%. According to the percentage of activating IgE the allergens are further divided into major and minor. The name of the allergen is created from the abbreviation of the Latin name of the species from which the substance originates and an Arabic number (Mills and Tatham, 2003).

#### 1.4 Occurrence and properties

Allergens represent a wide group of substances, antigenic molecules present in food. Most food allergens are proteins. Epitopes of food allergens have various tertiary and quaternary structures. However, their defining is not easy, as their conformation can be during food technologies modified (Mills and Tatham, 2003). Allergens of vegetal food mostly belong to compounds which are to protect the plant organism particularly against pathogenic microorganisms; they are so-called pathogen-related (PR) proteins. Other allergens are inhibitors of proteases, alpha-amylases, profilins, seed storage proteins, proteases and lectins. A number of these compounds are located in seeds (Mills and Tatham, 2003).

Soya and soya protein is increasingly used as raw material and food. Following this fact there is an increasing potential for atopic subjects to become sensitised. Soya belongs among five most significant kinds of food inducing type I allergy in children (Breiteneder and Ebner, 2000).

#### 1.5 Structure

Soya allergens are low-molecular weight proteins or peptides. The cause of the protein allergenicity is still little explored. The allergen activity is proportionate to its resistance to food technology, namely to digestion in the gastrointestinal tract. A fundamental role plays the conformation of protein epitope. In some cases has a significant role also posttranslational modifications such as glycosylation, phosphorylation or termination modification and most food allergens are really glykoproteins. Soya contains several allergens. All of them belong to the glycoproteins group. Inducing dose for allergic patients is from 0.0013 to 500 mg (Ballmer-Weber et al., 2007; Becker et al., 2004; Mills and Tatham, 2003).

The overview of soya allergens is summarized in chart 1.

Allergen	Biochemical Name	MW(SDS-PAGE)	Subunits
Gly m 1	Hydrophobic protein	7	
Gly m 2	Defensin	8	
Gly m 3	Profilin	14	
Gly m 4	PR-10 protein	17	
Gly m 5	Beta-conglycinin (vicilin, 7S globulin)	subunits	$\alpha, \alpha', \beta, \beta$
Gly m 6	Glycinin (legumin, 11S globulin)	subunits	G1,G2,G3,G4,G5,G6

Chart 1. Soybean allergens (<http://www.allergen.org/index.php>)

**Gly m 1- Plant lipid transfer proteins**

Soya, besides a range of food of vegetal origin, contains an allergen from the group of so-called plant lipid transfer proteins, whose name is derived from the capability to transfer phospholipids from liposomes to mitochondria. This whole protein family plays a role in the plant protection by its antifungal and antibacterial activity. These allergens feature a high content of aminoacid cysteine in the molecule and with this related presence of 4 disulphide bridges. In soybean hulls it was identified as the main protein responsible for several asthma outbreaks in Spain. It is a low-molecular protein Gly m 1, which consists of two isoforms 1A and 1B, both of which are allergenic (Breiteneder and Ebner, 2000). Of 32 patients who suffered from asthma attacks during unloading of soybean at the seaports, 90% showed serum IgE binding to an 8 kD shell protein on immunoblot. This protein was later identified as gly m 1 (medline 95326747) (Baltes, 2000, <http://www.allergen.org/index.php>).

**Gly m 2 - Defensin**

Purified Gly m 2 was recognized by serum IgE of patients suffering from soybean asthma on immunoblot. (<http://www.allergen.org/index.php>).

**Profilin Gly m 3**

Profilin is another potential soya allergen. Profilins regulate polymerisation of actin into filaments through the formation of profilactin. It is a thermolabile protein sensitive to digestive enzymes. (Lucas, Cochrane, Warner, & Hourihane, 2008; Scadding, 2008). The allergenic potential of this protein is reduced in the course of technological processing of soya. The principle of the reduction is a change of the binding epitope during heating, enzymatic hydrolysis, and fermentation (Amnuaycheewa and de Mejia, 2010). Of 12 subjects with soy bean allergy, 8 (67%) showed IgE binding to Gly m 3 on immunoblot (Baltes, 2000, <http://www.allergen.org/index.php>).

**Gly m 5 -  $\beta$  - Conglycinin (vicilin, 7S globulin)**

$\beta$  - Conglycinin belongs together with glycinin among the most important soya allergens. It is a seed storage protein. The structure of  $\beta$  - Conglycinin is composed of trimer, consisting of subunits  $\alpha$ ,  $\alpha'$ ,  $\beta$  glycinin in various combinations (Baltes, 2000, <http://www.allergen.org/index.php>).

**Gly m 6 - Glycinin (legumin, 11S globulin)**

Glycinin is also a soya bean storage protein. It occurs in the form of hexamer or dimer (Snyder, 2003) and it is allergenic even after the heat treatment of soya beans. It can be the cause of allergies from soya lecithin (Baltes, 2000).

**Kunitz trypsin inhibitor**

Kunitz trypsin inhibitor is a minor allergen, but it is at the same time an important antinutritional substance. However, the Kunitz soybean trypsin inhibitor was also reported to induce food anaphylaxis. (Breiteneder and Ebner, 2000).

**Lectins**

Lectins are known as plant agglutinin that binds to specific sequences of sugar determinants on glycoproteins. It occurs in seeds, particularly in leguminous plants. Some lectins react unspecifically with saccharide units of IgE, induce histamine-release and thus induce allergy-like symptoms (Breiteneder and Ebner, 2000).

A great problem of food are so-called hidden allergens. In case of food it means some functional ingredients added in the food. Soya can be added to bread and bakery products, sauces and soups, desserts and sweets and meat products for their moisture sorption and emulsifying properties. (Mills and Tatham, 2003).

### 1.6 Determination in food

Detection of allergenic components can be used to estimation in raw material and finished products.

Testing for food allergens should become part of the food industry's Good Laboratory Practise (GLP) and Hazard Analysis and Critical Control Points (HACCP) plans. Testing is a requirement for routine government inspections and other regulatory actions (Williams et al., 2005).

#### 1.6.1 Immunochemicals methods

There are numerous immunoassay formats, all of them based on the binding of an antibody to the target analyte. Immunoassays have been widely used to analyze complex of food proteins because of their specificity, sensitivity, and the ability to analyze complex food samples without the need for preassay purification steps.

Immunochemical methods can be qualitative, semiquantitative, or quantitative. For qualitative assays, only one cut-off value is needed and samples are compared to negative and positive controls. Two-level positive controls are required for semiquantitative assays. In the case of quantitative assays, sample values are compared to serial calibrators as well as a negative control (Williams et al., 2005).

#### Enzyme-Liked Immunosorbent Assay (ELISA)

Sandwich format ELISA is the most common immunoassay for the detection of food allergens like almond, peanut, hazelnut, casein, egg, soy and others.

The method uses antibodies bound to a solid support – the allergen is detected by a second antibody, which is conjugated to an enzyme. There is direct, indirect or enhanced (biotin-streptavidin system) assay formats, further divided into competitive or noncompetitive (Williams et al., 2005).

- **Lateral Flow (Strip test)** is the variation of the dipstick ELISA, but analyte is first recognized by the second or detector antibody, which is coupled to a color reactant and is embedded on one edge of a nitrocellulose strip. This method is used for determination of soybean, gliadin and peach.
- **Biosensors.** Surface Plasmon Resonance (SPR) immuno(bio)sensors are being developed for food allergen detection, it measures changes of the refractive index value. One of the most important features of the technology is the real-time monitoring of the samples. Quantitative methods have been developed for peanut, soy, ovomucoid and others (Williams et al., 2005).

#### 1.6.2 DNA-based methods

DNA-based methods do not analyze the protein directly, but detect instead the gene which encodes for that protein.

#### Polymerase Chain Reaction (PCR)

PCR allows the selective amplification of a specific DNA sequence. The two outstanding features of this technique are its specificity and sensitivity. PCR has been widely used to

characterize, clone, and produce recombinant food allergens using a DNA copy of the allergen-encoding mRNA. PCR assays are qualitative or quantitative. For qualitative analyses, conventional or traditional PCR uses a thermal cycler to amplify target DNA with the resulting amplicons visualized by agarose gel electrophoresis and ethidium bromide staining (Williams et al., 2005).

### **Real-time PCR (RT PCR)**

RT PCR allows quantifying the initial template concentration and the elimination of detection as a separate step, the product is detected during each cycle.

At this time, there are only several methods developed for the detection of food allergens by PCR, most being qualitative. There are tests for the detection of peanuts, soy and hazelnut allergens (Williams et al., 2005).

### **PCR-ELISA**

PCR-ELISA is similar to traditional PCR methods in that specific DNA sequences are amplified using pairs of primers and DNA polymerase. R-Biopharm has commercialized a SureFood ELISA-PCR for almond, peanut, soy and hazelnut (Williams et al., 2005).

## **2. Enzymes inhibitors**

### **2.1 Overall significance**

The enzymes inhibitors belong among significant antinutritional substances of some food. Many of these substances can be found in vegetabilia. They have different physico-chemical properties, such as isoelectric point, different activity and thermal stability and different specificity towards the inhibited enzymes. The enzymes inhibitors mostly belong to the group of hydrolase. Some of them have already been mentioned before, taking into account that they can be also potential allergens. In case of legumes they are in principle divided into protease inhibitors and amylase inhibitors.

Protease inhibitors occurring in legumes seeds belong to two families:

- Kunitz and Bowman-Birk inhibitor: both types are present in soya.
- Amylase inhibitors (salivary and pancreatic  $\alpha$ -amylase). This type of inhibitors is not accounted in soya.

### **2.2 Occurrence and properties and physiological function**

The effect of the inhibitors in relation to influencing the consumer's health consists in inhibition of intestinal protein digestion. To other manifestations belong inflammatory diseases of the pancreas after feeding animals with soybean powder leading up to hypertrophy and hyperplasia of the pancreas with the hypersecretion of digestive enzymes. (Guillamona et al. 2008) The increased secretion of the pancreatic juice leads to higher secretion of the fecal nitrogen and possible deficiency of some aminoacids, the sulphur aminoacid in particular. (Belitz, Grosh, 1992; Guillamona et al., 2008). Many proteinase inhibitors show the same active centre as is the part of the peptide enzymes binding, whose function they inhibit. Such an example is the Kunitz and Bowman-Birk soya inhibitor, which belong to the low-molecular fraction of soya proteins. The Kunitz inhibitor affects against the enzymes of trypsin and chymotrypsin and the Bowman-Birk inhibitor only against trypsin. It is interesting that the Kunitz inhibitor is inactivated by the gastric juice, but is it

not in case of the Bowman-Birk inhibitor. As regards the stability of these inhibitors, the Bowman-Birk inhibitor is protein that is by its tertiary structure predetermined to higher stability towards denaturation. In a molecule it contains 7 disulphide bridges and a circular arrangement of reactive sites on inhibition and by that it gains higher thermal stability, stability towards acids as well as proteolysis by pepsin. It was demonstrated that the daily discarded amount of trypsin and chymotrypsin is completely inactivated by the intake of 100 g soya beans (Guillamona et al., 2008).

The positive or negative impact of the protein inhibitors is given by their received amount, but also by the kind of soya cultivar. For instance, in case of the Bowman-Birk inhibitor is described a great amount of isoforms with different inhibitory properties. The Kunitz and Bowman-Birk inhibitor isoforms are divided in five sub-groups, on the basis of different representation of aminoacids, molecular weight and immunological cross reactivity. This knowledge has led to breeding of soya cultivars with low up to zero activity of the Kunitz and Bowman-Birk inhibitor. (Guillamona et al., 2008).

The Kunitz and Bowman-Birk soya inhibitor increase secretion of cholecystokinin (CCK) by intestinal mucosa. In case of Bowman-Birk inhibitor was, though, in vitro discovered a positive function, namely by demonstrating the carcinogenesis reduction in animals. (Guillamona et al., 2008) however, this situation is not imminent, as the inhibitors are deactivated by heat treatment.

### 2.3 Possibilities of inactivation

A number of studies deal with the possibility of inhibitors inactivation. In principle, the protease inhibitors are thermolabile, but a lot of heat treatment factors enter, which influence the inactivation. An example is soaking. Heat treatment of soya beans at 100 degrees Celsius for 9 minutes destroys 87% of inhibitors (Belitz, Grosh, 1992).

Reduction of the trypsin-inhibitory effect of soya proteins is possible by the means of chemical or physical processes. From the physical technological steps is effective high temperature, however, under these conditions emerges during the Maillard reaction a risk of degradation of important, especially basic essential or semi-essential aminoacids. Chemical methods represent chemical modification of the reactive spots of the trypsin inhibitors, but there is still a remaining problem of possible residuals of used chemicals. A favourable alternative represents ultrasound treatment. This process can be used with the result that the reduction happens not only in case of trypsin inhibitors, but it also leads to decreasing some enzymes (Huang et al. 2008). A great advantage is maintaining the nutritional value of the product. From other technological interventions are effective e.g. extrusions, which was proved on animal models by Marsman et al. (1995). In a feeding experiment was observed the influence of extruded soya flour and was found out that by the extrusion process these inhibitors are reduced by 25-41 %. Huang et al. (2008) studied the impact of the ultrasound treatments on Kunitz and Bowman-Birk soybean trypsin inhibitors (KTI and BBTI). They concluded that ultrasound inactivates KTI by inducing a reduction in the disulfide bonds and thus the changes in the secondary structure of both proteins. The Kunitz inhibitor was more sensitive, which can be explained by a number of disulphide bonds, in contrast to BBTI (7 bonds), the molecule of the Kunitz inhibitor contains just 2 disulphide bonds (Huang et al., 2008).

Another possible technological step leading to reduction of the soya allergens activity is fermentation, as was demonstrated in a work by Song et al. (2008), who proved, using *L. plantarum*, a reduction by 96% to 99%.

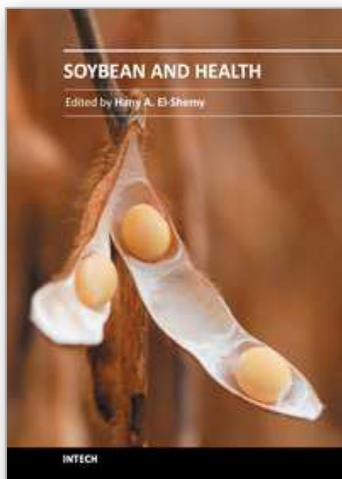
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Worldwide, soybean seed proteins represent a major source of amino acids for human and animal nutrition. Soybean seeds are an important and economical source of protein in the diet of many developed and developing countries. Soy is a complete protein, 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.

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