

SOYBEAN: FEED QUALITY AND SAFETY PART 1: BIOLOGICALLY ACTIVE COMPONENTS A REVIEW

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(Received July 3, 2003; accepted October 28, 2003)

A large number of soybean components have diverse biological activities. These include hormonal, immunological, bacteriological and digestive effects. The presently known allergens are listed. The divergence between chemical evaluation and biological value is highlighted. The following components are discussed: Kunitz inhibitor, Bowman-Birk inhibitor, saponins, soyacystatin, phytoestrogens (daidzein, glycitein, genistein), Maillard products, soybean hydrophobic protein, soy allergens, lecithin allergens, raffinose, stachyose, 2-pentyl pyridine. The studies describing the effects of the isolated components are reviewed.

Key words: Soybean, protease inhibitors, phytoestrogens, saponins, soybean allergens, lecithin allergens

Soybean is a major feed and food ingredient (Fig. 1). Its quality traits are defined in terms of chemical composition of the raw or processed products. Quality control is focused on processing industry requirements, on the assumption that suitable chemical characteristics make a good final product. The same quality argument is used in the debate on genetically modified (GM) soybean. Proponents argue in favour of technically improved varieties claiming chemical identity, while critics argue that single gene changes may have multiple, and unknown, consequences in the biological activity of components.

Soybean is a seed which contains several biologically highly active compounds, the effects of which on vertebrates are still far from being completely understood. For this reason data on biologically active constituents of either traditional or GMO soybean may contribute to a better quality assessment of raw materials and products (OECD, 2001).

Post-harvest soybean processing starts with cracking, dehulling, conditioning, flaking, oil extraction, toasting and grinding. The innumerable soy preparations used by the food, feed and other industries are the result of further processing. During the whole process quality control disregards changes in biologically active components.

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In addition, due to the large number of varieties, the different geographical production zones, the effects of climate on product composition and processing interactions make it almost impossible to see soybean as a single product with uniform quality traits at any point of the processing line.

In the poultry and swine industries the rapid progress of management and breeding resulted in standardised practices and genotypes. In this way the genotype and environment factors' variability was limited. On the other hand, feed variability, including soybean quality variability, remains high. This may pose potential economic risks to producers and biological risks to consumers. The food chain in modern societies has become endless. The traditional seasonal variation in food composition is over, and the population consumes food from the same sources 365 days a year. In human medicine this changed consumption pattern is reflected by the increasing number of allergic diseases.

In animal production, data on feed-related disease conditions, especially in the case of soybean, are also available. The large number of publications dealing with individual components warrants a review in an attempt to summarise the available data and to provide a comprehensive model on possible biological actions of individual soybean components. This may provide incentive to define a quality control scheme both for soybean raw materials and processed products.

This requirement was addressed by the European Commission by drafting a report on the risk assessment of genetically modified plants (EC, 2000). The GM feeds should be traced back to their production origin and their suitability for feeding should be established beyond reasonable doubt. This is especially the case with plant products which are the by-products of industrial raw material producing GM plants, which in EU terms are considered hazardous wastes. However, before grading soybean and soybean products, one has to revisit the biologically active components of soybean, in order to collect baseline data.

The present review collects published data on biologically active individual soybean components and animal models.

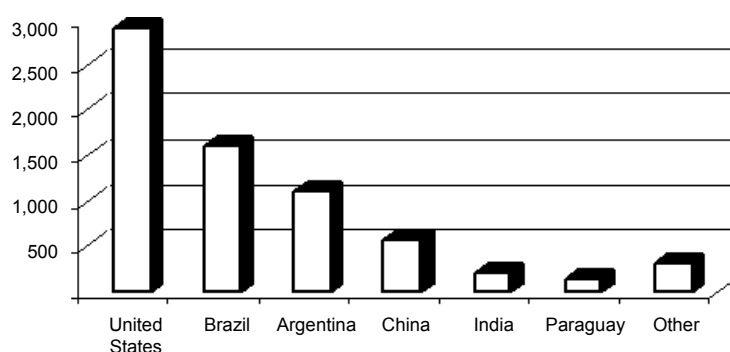


Fig. 1. Global soybean production 2001/2002 (million bushel)

Most of the soybean harvest is used for oil extraction and the by-product is further processed to animal feed and human food and industrial products.

Soy protein production

Soy protein isolates are produced by isoelectric precipitation through manipulating the pH value of a water-based soybean meal slurry. Soy proteins have been produced for non-food uses (adhesives, paper coatings) since 1930. For food purposes experiments have been going on since the late 1950s. The procedures for isolation of food proteins are based on water extraction over different pH ranges. The proteins in the extract are precipitated by lowering the pH to 4–5, then the precipitate is centrifuged and spray-dried. The process uses reducing agents (to bring proteins into suspension by disrupting disulphide bonds), membrane filtration, peroxide treatment and steam cooking. Since the procedures are described by manufacturers' licences, practically no data are available on the end products of the different manufacturers.

In order to review the major biologically active components, first a list is drawn up with the major component groups, followed by pathology data.

Soybean lectins

Liener and Rose (1953) were the first to isolate soybean lectin, which they named soyin. Most of the lectins agglutinate red blood cells through their carbohydrate-binding domain. Lectins differ in their carbohydrate and glucosamine content and share common amino acid sequences. Soybean lectins are globulins with sedimentation constants of 6.5–7.5 S. Lis et al. (1964) were the first to determine the glucosamine and mannose content of soybean lectins with a molecular mass of 110,000–120,000 kDa, composed of four subunits, two of them sharing a common N-acetyl-D-galactosamine binding site (Lis and Sharon, 1973; Lothan et al., 1974).

Soybean lectins agglutinate rabbit red blood cells but not sheep and cattle red blood cells. Soybean lectin levels are usually expressed as haemagglutinating units per milligram protein (Kakade et al., 1972).

In young rats the LD₅₀ of soybean lectin is 50 mg/kg per os. Autoclave-treated soybean with added 1% lectin reduced rat growth rate to 75% of the control, and in rats 50% of the growth-inhibiting activity is attributed to lectins. According to Liener and Rose (1953), soybean lectin content changes between 1 and 3%, which means that innocuity and toxicity levels are close. Soybean lectin binds to the intestinal epithelium and can be reisolated from the faeces. In rats raw kidney bean lectin binds to epithelial cells in the stomach, the brush border

membrane in the small intestine, and epithelial cells of the caecum and colon (King et al., 1986; Bardocz et al., 1995). Kidney bean lectin caused shortening of small intestinal villi and epithelial damage (King et al., 1980).

Douglas et al. (1999) fed chickens on 7.2 mg/kg lectin-containing soybean meal and compared the animals' performance to those receiving genetically modified lectin-free soybean meal. It was observed that lectin-free soybean meal resulted in non-significantly higher (5–8%) amino acid digestibility. It seems that chicken growth on lectin-free soybean meal is better than on Kunitz trypsin inhibitor-free soybean meal, but both meals provided better performance than raw soybean meal.

According to Grant et al. (1987), lectins exert their effect on the pancreas by causing hypertrophy, which is indistinguishable from the effect of soybean trypsin inhibitor. The inhibitor is reported to have influence on pancreas stimulation, but not on small bowel growth (Herzig et al., 1997). This effect was blocked by cholecystokinin receptor antagonist, despite the fact that soybean trypsin inhibitor failed to elevate cholecystokinin plasma levels. Jordinson et al. (1997) reported that soybean lectin stimulated pancreatic exocrine secretion. The effect is mediated by cholecystokinin, since the administration of cholecystokinin receptor blocking agent prevents this effect. In humans raw soybean flour increases cholecystokinin release (Calam et al., 1987). Jones and Freter (1976) reported on a bacterial lectin from *Vibrio cholerae* which induced cholecystokinin release upon infection in rabbits. Jordinson et al. (1997) observed cholecystokinin release in rats upon feeding peanut, soybean and wheatgerm lectins, but not kidney bean (*Vicia faba*) lectins. All three effective lectins have common sugar binding specificity for galactose, N-acetyl-D-galactosamine and N-acetylglucosamine.

Lectins with similar sugar specificity bind to the apical membrane of enterocytes and damage microvilli (Koninkx et al., 1992). Soy lectin causes increased polyamine uptake and mitotic index in enterocytes. Peanut lectin induced colon epithelial cell growth (Ryder et al., 1994).

The role of lectins in bacterial enteric conditions was first demonstrated by Banwell et al. (1988), whose results were later confirmed and extended by Pusztai et al. (1993), Bardocz et al. (1995) and Pusztai et al. (1997). In their view, intestinal bacterial counts are higher in lectin-fed animals, but lectins also may exert beneficial influence by enhancing epithelial layer replacement.

Soy lectin is resistant to proteolytic digestion as it was demonstrated by Pusztai et al. (1990), as 60% of the lectin could be recovered from the duodenum 60 min after feeding in rats.

Dietary lectins induce sustained release of several intestinal hormones and even insulin secretion (Bardocz et al., 1996; Grant et al., 2000).

Soyacystatin

Misaka et al. (1996) isolated a cysteine proteinase inhibitor from soybean, called soyacystatin. The protein consists of 245 amino acid residues and has a molecular mass of 26 kDa, suggesting the presence of extension sequences. These sequences do not affect the enzyme inhibitory activity of soyacystatin.

Soybean hydrophobic protein

This 9000 kDa protein can be isolated in 60% ethanolic extract of soybean seeds. The molecule contains 4 disulphide bonds and 80 amino acid residues, lacking methionine, phenylalanine, tryptophan, lysine and histidine. It aggregates to form 80 kDa molecules. The molecule shows similarity to rat prolactin. It is present in 200 mg/kg seed level in soybeans (Odani et al., 1987). No data are available on its role in animal nutrition.

Soybean allergens

Soybean storage proteins make up 90% of the salt-soluble globulin fraction. The principal globulins are β -conglycinin and glycinin. β -conglycinin, a member of the cupin superfamily, is a glycoprotein with a molecular mass around 180 kDa. It is made up of three subunits, α , α' and β . Glycinin is made up of 6 subunits with a molecular mass of 320 kDa. Each subunit is composed of one acidic and one basic polypeptide chain linked by a disulphide bond. In 25% of atopic dermatitis patients antibodies to the α -subunit of β -conglycinin have been detected (Breiteneder and Ebner, 2000).

Another soybean allergen, 8 kDa Gly m 2, shows 64% similarity to the disease response protein of pea (*Pisum sativum*). Both glycinin and β -conglycinin are susceptible to pepsin proteolysis, but they are insoluble between pH 3.5 and 6.5. Trypsin and chymotrypsin digestion results in glycinin-T or glycinin-C in which quaternary structures are largely retained. The stability results in immunologically active fragments during digestion and explains their resistance to thermal inactivation and retained allergenicity (Mills et al., 2002).

The Kunitz inhibitor also possesses allergenicity (Moroz and Lang, 1980). According to Isamu et al. (2000), in addition to Gyl m Bd 30K, Kunitz soybean trypsin inhibitor is the major food allergen in Japan and is responsible for urticarial contact dermatitis.

The Gly m Bd 30K allergen was identified as a thiol protease of the papain family. Helm et al. (1998) localised the allergen in the protein storage vacuoles of seed cotyledons. The allergen has 10 regions of IgE binding activity (Table 1).

Table 1

Soybean allergens

Gly m Bd 60K	Alpha subunit of beta-conglycinin (major storage protein) (Ogawa et al., 2000)
Gly m Bd 28K	Vicilin-like glycoprotein (26,000 kDa)
Gly m 1 8 Kd	Hydrophobic protein, homology with rat prolactin (Odani et al., 1987)
Gly m 2	Soybean hull protein: 71% homology with cow pea storage protein (<i>Vigna radiata</i>), 64% homology with 'disease response protein' from green pea (<i>Pisum sativum</i>)
Gly m 3	Soybean profilin binds to actin, affects structure of cytoskeleton profilin family, cross reaction to birch pollen allergen Bet v2 (Rihs et al., 1999)
G2 glycinin allergen	21 kDa basic chain of glycinin (Helm et al., 2000)
50 kDa and 39 kDa allergens	(Müller et al., 1998)
14 kDa/pI 8 allergen	(Baur et al., 1996)
16 minor allergens (21–42 kDa)	(Ogawa et al., 1995)
Major allergens 14 kDa/pI 6	(Swanson et al., 1991)
5–6 kDa and 15.5–17 kDa allergens	(Garcia-Ortega et al., 1998)
50–60, 20 and 14 kDa allergens	(Herian et al., 1993)
Gly m 4 SAM 22 allergen	Stress protein, 70% homology to <i>Pisum sativum</i> stress protein, homology with Bet v 2 birch pollen allergen (Crowell et al., 1992)
Basic 7S globulin precursor	Binds bovine insulin (Barbashov et al., 1991)
Kunitz trypsin inhibitor	32% homology with <i>Lolium perennis</i> Lol pXI allergen (Van Ree et al., 1995)

Soy lecithin allergens

Soy lecithin is widely used as an emulsifier in processed foods, pharmaceuticals and cosmetics. Soy lecithin is a by-product of soy processing. It is obtained by precipitation and may contain proteins at 100 to 1,400 ppm levels. The proteins may react with sera from allergic patients. Sera from soy-sensitive individuals showed IgE binding to bands corresponding to 7, 12, 20, 39 and 57 kD. The 12-kD band was identified as a methionine-rich protein (MRP) and a member of the 2S albumin class of soy proteins. The 20-kD band was found to be soybean Kunitz trypsin inhibitor. The 39-kD band was matched to a soy protein with unknown function (Gu et al., 2001).

Phytoestrogens

Soybeans contain several isoflavone compounds which possess oestrogenic, anti-oestrogenic and hypocholesterolaemic effects in mammals. These compounds are in the focus of controversy regarding their benefits and risks. Generally phytoestrogens are considered antinutrients. Isoflavone contents in soybean are unpredictable showing extremely large differences depending on variety, climate and agronomic practices leading to fivefold differences. The three major isoflavons are daidzein, genistein and glycitein. They occur in conjugated form and as aglucons. The glycon moieties of phytoestrogens, genistein, daidzein and glycitein, are present as β -glucosides, malonylglucosides and acetylglucosides, as well as aglycons. There are 12 isomers of these molecules and their metabolism is not well known. Published data on product isoflavone content are sometimes confusing since some of the data refer to conjugated, others to aglucon forms leading to almost twofold differences in concentration if data are not converted to aglucon values (Magee, 1963). Genistein levels range between 44 and 910 mg per 100 g of raw seeds. Toasted soybean meal has a similar level of phytoestrogens as raw seeds (Wang and Murphy, 1994).

Genistein is an inhibitor of tyrosine protein kinase and competitive inhibitor of ATP in other protein kinase reactions. As antiangiogenic agent, it downregulates the transcription of genes involved in controlling angiogenesis (Sigma-Aldrich Life Science Catalogue, 2003–2004).

In addition to oestrogenic effect on immature rat uterus enlargement, the compound also inhibits the growth of male rats. Genistein is metabolised by the enterohepatic circulation (Sfakianos et al., 1997) and may accumulate in the intestinal tract. Fed at 250 ppm level, genistein enhances carcinogenesis in induced colon cancer (Rao et al., 1978). In testis cell line it inhibits cell growth and division in a dose-dependent manner (Kumi et al., 1998). Added to bull semen it inhibits the binding of sperm cells to the zona pellucida (Hagele et al., 1998). In rats, genistein causes relaxation of the mesenteric artery (Nevala et al., 1998). Genistein also stimulates the growth of oestradiol-dependent human breast cancer *in vivo* in mice (Allred et al., 2001). The effect is dose dependent.

Soy marketing protagonists frequently claim that isoflavones are beneficial in preventing colon cancer in humans. This claim could not be confirmed in an animal model (Sorensen et al., 1998). In an extensive study Yellayi et al. (2002) concluded that the thymic effect of genistein was in part mediated through an oestrogen receptor mechanism: genistein decreased thymocyte numbers and thyroid size in a dose-dependent manner, doubled apoptosis in thymocytes, affected maturation of thymocytes, and caused systemic lymphocytopenia. In normal premenopausal women the administration of 60 g soy supplement (containing 45 mg genistein/day) significantly increased breast lobular epithelium proliferation (McMichael-Phillips et al., 1998). The supplementation also

significantly increased the expression of progesterone receptors. In a rat feeding trial, exposing the animals to those genistein levels which are comparable to human exposure, Declos et al. (2001) showed decreased body weight during pregnancy, in male offspring decreased ventral prostate weight, in male and female offspring mammary duct hyperplasia, abnormal ovarian follicles, abnormal uterine cell maturation, and in both sexes renal tubular mineralisation. According to Lee and Lee (2001) genistein also has alpha-glucosidase inhibitor activity.

In ovariectomised mice, Song et al. (1999) observed a 150% increase in uterine weight with daily dosing of mice with 3 mg/day glycitein for 4 days. In cultured rat myogenic cells (L8) Ji et al. (1999) demonstrated a dose-dependent inhibition of cellular protein synthesis by genistein. The growth-depressing effect of crude soybean meal may also be attributed to reduced myofibrillar protein synthesis.

Reviewing the biological effects of soybean isoflavons, Doerge and Sheehan (2002) reported a relationship between soy consumption and goitre, which is independent of oestrogenic activity. This antithyroid effect is dose dependent and acts by the inactivation of the thyroid peroxidase which is responsible for thyroxine iodination. Iodine deficiency is a predisposing factor of thyroid toxicity, but other dietary components are also possible contributing factors. The anti-oestrogenic and antithyroid effects may cause concern both for food and feed soy products. Thyroid hormone synthesis inhibition can induce goitre and thyroid neoplasia in rodents (Divi et al., 1997). In addition to thyroid activity, genistein seems to influence hippocampal and cerebellar tyrosine kinases in those regions of the brain which are responsible for long-term memory, by blocking long-term potentiation (O'Dell et al., 1991).

In humans the elimination of genistein and daidzein was studied by Fanti et al. (1999) who observed that in patients with end-stage renal disease both phytoestrogens reached 4–5 times higher levels than in healthy volunteers upon the consumption of soy food. The half-life of the compounds was ten times longer in renal disease patients than in healthy subjects.

Trypsin inhibitors

Han et al. (1991) reported that raw Kunitz-inhibitor-free soybean (KFSB) has better nutritional value than conventional raw soybean but lower than commercial soybean meal in chicks. The raw KFSB has 16 times lower trypsin inhibitor content than commercial soybean meal, but the Birkman inhibitor contents were similar (Friedman et al., 1991). This means that KFBS requires the same heat treatment as commercial soybean meal. In a chick digestibility assay Anderson-Haferman et al. (1992) concluded that an 18-minute-long autoclaving of KFSB was insufficient to reach the 0.2 pH unit drop in the urease test. Their

data show that even after autoclaving, pancreas weights were 25% higher than with commercial soybean meal diet, which included 9.1% soybean oil (without autoclaving the pancreas weight was 100% higher). Zhang et al. (1993) observed that commercial soybean extruded at 121 °C resulted in significantly lower amino acid digestibility values than that extruded at 154 °C (30% lower). However, overheating may reduce lysine, cysteine and arginine digestibility.

The Bowman-Birk inhibitor, which is found in KFSB varieties, is claimed to have anti-carcinogenic activity. Wan et al. (2000) found the inhibitor in the urine of human volunteers after consuming soy products containing 105–175 mg inhibitor, which means that this relatively small protein crosses not only mucosal but also renal barriers. Urinary excretion lasted 24 h after soymilk ingestion.

McLeod et al. (1972) compared the effect of soybean and egg-white trypsin inhibitors on pancreatic enzyme production in rats. Feeding soybean trypsin inhibitor (0.87% in feed) significantly reduced amylase and trypsinogen levels in the pancreas. Chymotrypsinogen levels increased by 93%, trypsinogen decreased by 46%, amylase by 42%, while lipase remained unchanged. Soybean trypsin inhibitor significantly increased pancreas weight.

Bowman-Birk (protease) inhibitor (BBI)

The BBI is an inhibitor of elastase, cathepsin G, mast cell chymase, and trypsin. It is a reversible inhibitor of serine proteases, with chymotrypsin-like specificity. BBI has two distinct inhibitory domains. The first of these domains inhibits trypsin, which ‘prefers’ positively-charged amino acid side chains at the P1 location. The second domain inhibits chymotrypsin and other serine proteases, which prefer large non-polar side chains. BBI places a leucine in the P1 position of the active site of these proteases. BBI-type inhibitors are found in essentially all legumes, such as soybeans, chick peas, and navy beans. BBI inhibits proteases associated with inflammation, including neutrophil elastase, mast cell chymase, and cathepsin G, and reduces superoxide anion radicals from polymorphonuclear leukocytes (PMNs). BBI also inhibits proteases associated with cancer. Forty to 50% of ingested BBI is taken up into the bloodstream or into the epithelial cells of the gastrointestinal tract within 3 h of ingestion, with 50% to 60% faecal excretion. There are high levels of proteolytic activity in involved colonic regions in patients with ulcerative colitis (Lichtenstein et al., 2002).

Raffinose series of oligosaccharides (RSO)

Soybean meal contains relatively large amounts of raffinose and stachyose (3–8%). They are collectively known as raffinose-series oligosaccharides (RSO) or galacto-oligosaccharides. RFOs are compounds made up of sucrose and one,

two or three galactoses. Raffinose is made up of D-galactose, D-glucose, D-fructose. Alpha-galactosidase splits the molecule to galactose and sucrose.

Humans and monogastric animals (swine, poultry) do not possess the α -galactosidase enzyme to break down these oligosaccharides, therefore these pass undigested to the large intestine for bacterial fermentation by gas-producing bacteria. Rackis (1974) attributed the flatulence associated with legume consumption to these oligosaccharides. In young pigs, poultry and preruminant calves high levels of feed RSO can cause digestive problems by interfering with protein digestion. RSO are usually removed from soybean products by ethanol treatment. These compounds are present in defatted toasted soy meal, as well as in raw soybeans (Padgett et al., 1996). On fresh weight basis soybean stachyose content varies between 1.1 and 4.1%, while raffinose levels are within the range of 0.1 to 0.9 g/100 g basis. RSO levels are not part of standard soybean quality assessment procedures in food and feed products. However, the addition of α -galactosidase enzyme to poultry rations has been recommended by several authors (Coon et al., 1990; Irish et al., 1995; Leske et al., 1995).

Maillard reaction products

During heat treatment the reducing carbohydrates of glucose and RSOs may react with the amino groups of proteins. The major amino acids involved in the reaction are lysine, arginine, histidine and tryptophan. The resulting products are glycosylamines, causing off-flavour and off-colour in soybean meal. The reaction renders these amino acids unavailable for digestion and utilisation, despite the fact that amino acid analysis confirms their presence in the feed. The resulting compounds are absorbed, but not metabolised. The formation of Maillard reaction products leads to lower protein solubility in KOH.

Another by-product of soy protein isolate manufacturing is 2-pentyl pyridine, which is responsible for grassy flavour in different products (Zhou and Boatright, 1999)

Saponins

There are several saponins in commercial soy products. There is hardly any information on their role and metabolism in animals. Soy saponins co-fractionate with the isoflavones because of their similar solubility in ethanol. As amphoteric surfactant compounds, they may play a role in cholesterol absorption and protein antigenicity.

Saponins may enhance the transfer of macromolecules across the mucosal barrier. Gee et al. (1997) studied beta-lactoglobulin sensitised rats and observed significant enhancement of beta-lactoglobulin transfer in the presence of *Gyp-*

sophila saponins. In saponin allergen primed animals the intestinal villi were damaged after challenge. The role of saponins in the development of antibodies to orally administered antigens (ovalbumin) has also been demonstrated by Atkinson et al. (1996), who could significantly increase anti-ovalbumin antibody levels by giving 1.5 mg/ml g *Gypsophila* saponin to Brown Norway rats.

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