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Review

BIOLOGICALLY ACTIVE COMPONENTS OF SOYBEANS AND SOY PROTEIN PRODUCTS - A REVIEW

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Soybeans provide a source of low-cost protein with good nutritional and physico-chemical properties. Recently, soybean has received much attention because of its potential role in preventing and treating several diseases, including cancer and other human chronic diseases. Health benefits of soy diet are attributed to the minor soybean constituents (called phytochemicals). Soybean contains a variety of phytochemicals with demonstrated anticancer activity, including bioactive proteins and polypeptides (trypsin inhibitors and the most recently discovered peptide lunasin), isoflavones, phytic acid, phytosterols and saponins. The present review provides an overview of recent knowledge about biologically active components of soybean.

KEY WORDS: Soybean; biologically active compounds; protein; health benefits

INTRODUCTION

Soybean is an abundant source of proteins that have long been recognized for high nutritional value and excellent physico-chemical properties in food. Also, soybean and soy products are rich sources of minor non-nutritive components with potential health benefits, in the literature often called phytochemicals.

Soybean contains many unique biologically active components including isoflavones, biologically active proteins and peptides, phytosterols, phytic acid and saponins. In traditional nutritional theory, many of these components have been considered as antinutrients. During the last two decades it has been found that they may exert beneficial health and therapeutic effects. The purpose of this work was to highlight recent knowledge about biologically active components of soybean, especially biologically active proteins and polypeptides.

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BIOLOGICALLY ACTIVE PROTEINS AND POLYPEPTIDES FROM SOYBEAN

Soybean is a good source of biologically active proteins and polypeptides, including protease inhibitors, lectins, low molecular weight polypeptides and the most recently discovered peptide lunasin. Recently, there has been an increased interest in their potential health benefits.

Protease inhibitors

Protease inhibitors, most commonly referred to as trypsin inhibitors, are the best known and the most studied biologically active proteins in soybean. Trypsin inhibitors are low molecular weight proteins that bind trypsin and interfere with protein hydrolysis during digestion (1). Soybean contains two major types of trypsin inhibitors: Bowman-Birk (BBI) and Kunitz inhibitors (KTI). Depending on the cultivar soybean has three Kunitz isoinhibitors and 5-12 Bowman-Birk inhibitors. Raw soybean contains 1.67% KTI and 0.4 % BBI (2). They are partially responsible for low digestibility of raw soybean. About 40% of the adverse effect of raw soy consumption is the result of their affects (3). Kunitz inhibitor (Figure 1a) consists of 181 amino acid residues with two disulfide bridges. One of them is essential for inhibitor activity. KTI stoichiometrically binds and inhibits only trypsin. The Bowman-Birk (Figure 1b) inhibitor has 70-80 amino acid residues with molecular weight of 7-8 kDa. The isoelectric point of BBI is 4.2 (5,6). This protein contains seven disulfide bridges and it is a rich source of amino acid residues with sulfur. BBI has two binding sites: a trypsin binding site (Lys^{16}-Ser^{17}) and a chymotrypsin reactive site (Leu^{43}-Ser^{44}). Thus, BBI can simultaneously bind both trypsin and chymotrypsin (7).

Trypsin inhibitors in raw soybean cause growth inhibition, pancreatic hypertrophy and hyperplasia in experimental animals (3). For a long time in the traditional nutritional theory trypsin inhibitors (TI) have been considered as typical antinutritional factors. Consequently, to reduce their activity, several treatments have been used. Thermal treatment is the most common method used for this purpose. The level of residual TI activity depends on several factors including treatment mode (level of temperature, time of heating), initial content of moisture, pH conditions and the presence of reducing agents (2, 8-12). It is well known that dry heat have no significant influence on TI activity, while moist heat treatments with steam at over the range of 0.5-2.0 bar (2, 8, 13, 14, 15), autoclaving (16) and steam jet cooking (17,18) were more effective. Additionally, the investigation conducted in our laboratory (19) showed that microwave roasting could be an effective method for the reduction of inhibitor activity. The type of inhibitor responsible for TI activity depends on treatment mode. Both types of inhibitor are responsible for the activity of microwave treated flour (19) and traditional soy protein concentrates (20). In opposite, our earlier investigation (2, 21) showed that the residual activity of moist heat treated flour is only the result of KTI.

In opposite to the traditional theory of nutrition, numerous investigations (22-29) have shown that protein inhibitors, especially BBI, had anticarcinogenic properties. Since 1992, BBI has achieved Investigational, New Drug status. BBI has been shown to be very effective in preventing carcinogenesis in the liver, lung, and gastrointestine in mouse models (22, 23). Kennedy and coworkers (24) conducted clinical trials to evaluate BBI as an anticarcinogenic agent in human populations. The mechanism of prevention is not clear. It has been suggested that protease inhibitors suppress both initiation and promotion stages of carcinogenesis. Due to the beneficial effects in nutrition, Kennedy and Szuhaj

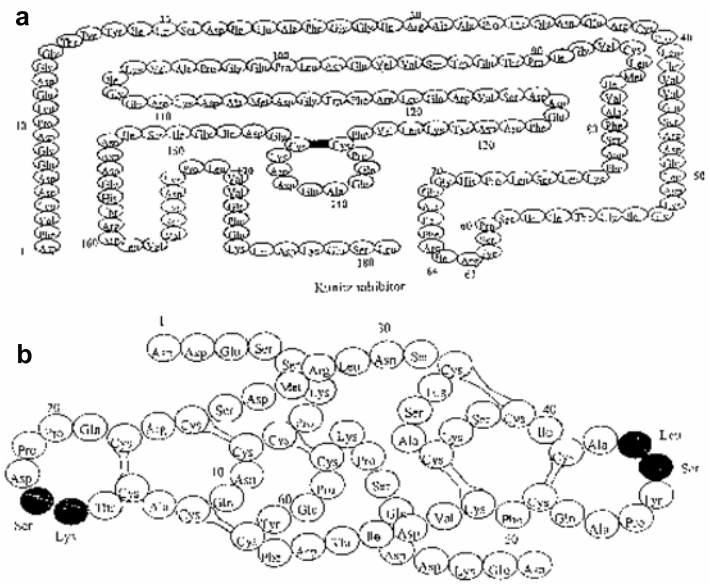

Fig. 1. Kunitz (a) and Bowman-Birk inhibitor (b) (4)

(30, 31) patented the isolation of Bowman-Birk concentrate from acidic aqueous extracts of hexane defatted soybeans. Also, Sessa and Wolf (32) prepared BBI concentrate from seed coats with considerably higher BBI activity than the Kennedy and Szuhaj patented concentrates.

Lectins

Lectins are a significant group of bioactive proteins found in almost all organisms, including plants, bacteria and viruses (33). Lectin originating from soybean seed is a tetrameric glycoprotein that accumulates during embryogenesis (34) and recognizes terminal α-linked 2-acetoamido-deoxy-D-galactosyl or α- or β—D-galactosyl sugar residues (35). Molecular weight of tetrameric form is 120 kDa. It normally constitutes 1-2 % of the seed protein mass. Lectins express ability to agglutinate red blood cells. It is a well recognized physiologic effect that is dependent on their specific high–affinity binding to particular carbohydrate moieties on the cell surface (36). SBA exists as multiple isolectins having similar binding and immunochemical properties. Native SBA consists of at least five isolectins: SBA-I, SBA-II and SBA-III (Figure 2).

The ingestion of pure lectins in the diet of animals has several biochemical, physiological, and nutritional implications such as agglutination of cells, stimulating pancreatic enzyme secretion (38-42). Once ingested lectin tends to stimulate intestinal cells, and thus can interfere with intestinal absorption of nutrients. Due to these facts lectin has been for a long time considered as antinutritient. In opposite, in the past 50 years it has been reported that plant lectins may have antitumor and anticarcinogenic activities that could be beneficial in cancer treatment (43-46). The exact mechanism(s) of the antitumor effect of plant lectins is not clear, although several have been proposed, including reduction of cell divi-

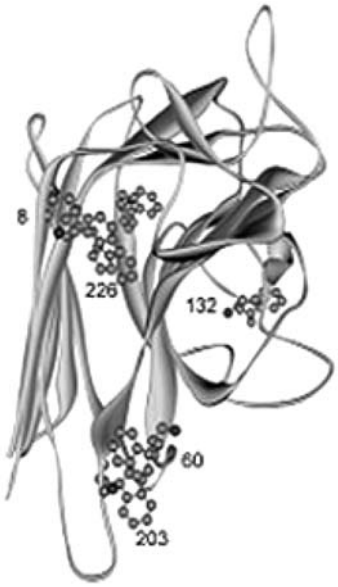

Fig. 2. The monomer of SBA showing the disposition of the tryptophan residues (37)

sion, increasing the number of macrophages, increasing the susceptibility of tumor cells to macrophage attack, serving as a bridge between tumor cells and macrophages, and improving the immunocompetence of tumor-bearing animals (34).

Soybean cultivars contain 6.5 g of lectin per kg of defatted soybean meal (47). Due to their protein structure, soy lectin is heat unstable and can be inactivated by different heat treatments. According to the heat stability soy lectins are similar to trypsin inhibitors and urease. Fasina and co-wokers (48) showed that the level or urease activity and TI activity can be reliable parameters of the degree of lectin activity reduction during soy meal autoclaving. Our earlier investigation (49) showed that moist heat treatments at 1.5-2.0 bar for 15 minutes completely destroy soy lectin polypeptides.

Bioactive peptides

Bioactive peptides may exist naturally or be derived from soy protein hydrolyzates. Usually, these peptides have common structural properties such as a relatively short peptide residue length (2-9 amino acid residues) and hydrophobic amino acid residues in addition to proline, lysine or arginine groups (50). Bioactive peptides are resistant to the action of peptidases (34, 51). These peptides act as physiological modulators during gastrointestinal digestion of soy products. Peptides derived from tryptic hydrolyzates of soybean proteins stimulate superoxide anions, which trigger nonspecific immune defense systems (52). Also, soybean peptides obtained from hydrolyzates express antioxidant activity (53), antiobesity effects (54). Furthermore, lunasin and hydrophobic peptides obtain from defatted proteins exhibit anticancer activity (50, 55).

Lunasin. Lunasin is the most recently discovered bioactive polypeptide originally isolated from soybean. While searching for methionine-rich proteins from midmaturation

soybean seed, researchers from the University of California, Berkley (56) isolated and cloned gene for small peptide termed as lunasin. More recently, lunasin has been isolated from barley (57). It is unique 43 amino acid peptide, whose carboxyl end contains nine residues of aspartic acid, an Arg-Gly-Asp cell adhesion motif, and a helix with structural homology to a conserved region of chromatin-binding proteins (58, 59). Now it is known that lunasin is a major component of Bowman-Birk protease inhibitor (34). Precisely, it is a linker peptide of BBI (60) with relative molecular mass of 5.45 ± 0.25 kDa detected by SDS-PAGE (52).

During seed development, lunasin peptide appears 5 weeks after flowering and persists in the mature seed. The content of lunasin in soybean seed varies with varieties and environment (51). Also, commercial soy protein products contain different amounts of lunasin. Soy isolates and hydrolyzed soy proteins contain the highest concentrations of lunasin. Soy protein concentrate, isolate, and hydrolyzate contain 2.81 ± 0.30, 3.75 ± 0.43, and 4.43 ± 0.59 g/100 g respectively, while soy flour and soy flakes contain 1.24 ± 0.22 g lunasin/100g (51). The content of lunasin detected in physiological phosphate buffer (PBS, O.1M pH 7.4) is shown in Table 1 (59).

Table 1. Lunasin content in some commercially available soy proteins (59)

Soy protein	**Lunasin (mg /g of protein)**	**Lunasin (% in product)**
Deffated soy flour	5.48 ± 0.17	0.054 ± 0.0017
Soy concentrate (alcohol washed)	8.72 ± 0.19	0.065 ± 0.0015
Soy isolate	6.92 ± 0.16	0.059 ± 0.0014
Soy concentrate (water washed)	16.52 ± 0.23	0.091 ± 0.0013

Synthetic lunasin is heat stable, surviving temperatures up to 100°C for 10 minutes (34).

According to Galvez et al. (58) transfection of mammalian cells with the lunasin gene leads to mitotic arrest and cell death characterized by cell lysis and chromosome fragmentation. This effect is attributed to binding of its poly-aspartyl carboxyl end to regions of hypoacetylated chromatin like that found in centromeres (59).

Lunasin is a promising chemopreventive peptide from soybean. Galvez et al (55) reported *in vitro* and *in vivo* chemopreventive properties of chemically synthesized lunasin against chemical carcinogenesis. This may be a result of the RGD-cell adhesion motif and chromatin-binding properties (60). Also, animal studies showed that lunasin inhibited skin tumorigenesis in a mouse skin cancer model when applied topically (55). In all of these experiments synthetic peptide was used. Due to the high cost of synthetic lunasin, there is a need to isolate, characterize and demonstrate the biological activity of natural polypeptide from soybean.

Hydrophobic peptides. During processing of soy protein products, especially during fermentation or heat treatment, many peptides can be formed. Some of these peptides

exert various physiological activities (61). Kim et al. (62) reported that low molecular weight peptides derived from soybean proteins exhibited cytotoxicities on several cell tumor lines. Kitts et al. (52) isolated and characterized nonapeptide with cancer reducing activity of thermoase-hydrolyzed defatted soybean. Anticancer peptide was extracted with ethanol. The molecular weight of this peptide was 1157 Da and the amino acid sequence was X-Met-Leu-Pro-Ser-Tye-Ser-Pro-Tyr.

NON-PROTEIN BIOACTIVE COMPONENTS FROM SOYBEAN

Phytic acid. Phytic acid (PA, myo-inositol 1,2,3,4,5,6-hexakis-dihydrogen phosphate, Figure 3) is the storage form of phosphorus in soybean seeds. Soybean seed and soy protein products have relatively high content of phytic acid. The phytic acid content of soy protein products is determined by the conditions employed for its preparation. Commercial soy protein products contain 1.4-3% PA (64), while the content of the laboratory prepared isolates and concentrates are 2.89% and 4.81-4.95 %, respectively (64, 20). The major portion of phytate is in the 7S protein fraction, while glycinin (11 S-protein) contains only about 0.07% phytate (65).

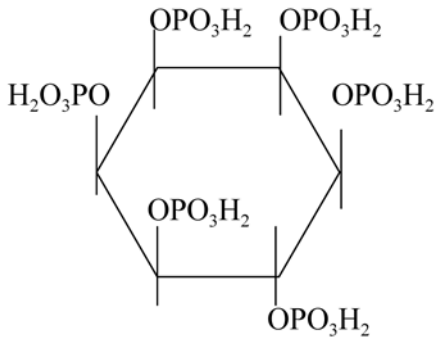

Fig. 3. Phytic acid (63)

Phytic acid can interact with minerals, proteins and starch. The degree of interaction of phytic acid and proteins is affected by the protein charge, conformation and ionic strength of the solution at a given pH. The interaction of PA with proteins and minerals is shown in Figure 4.

Due to the abillity of chelating of minerals and binding of proteins and starch PA can reduce their digestibility (66). Consequently, it has been considered as an antinutritient. On the contrary, it is well known today that low concentration of PA has some beneficial effect in nutrition, which include controling dental caries, improving oxygen–providing ability of red blood cells and cancer preventing activity, preventing of cardiovascular diseases and diabetes (1,67,68). According to Wang and Wixon (1), anticarcinogenic effect of phytic might be result of their interaction with starch, protein and minerals.

Soy isoflavones. Soybeans and soy protein products are abundant source of isoflavones. Isoflavones are class of phenolic components. The major isoflavones of soybeans are daidzein, glycitein, and genistein. Each of them is found in four chemical forms: the unconjugated form or aglycone; the conjugated form or glucoside (daidyin, genistin, and glycitin); acetylglucoside; and malonylglucoside (69,70,). Structures of soy isoflavone aglucones are shown in Figure 5.

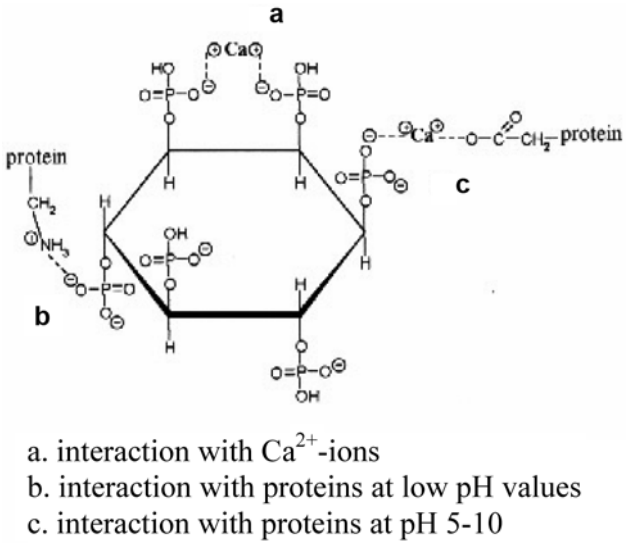

Fig. 4. The interaction of PA with proteins and Ca-ions (62)

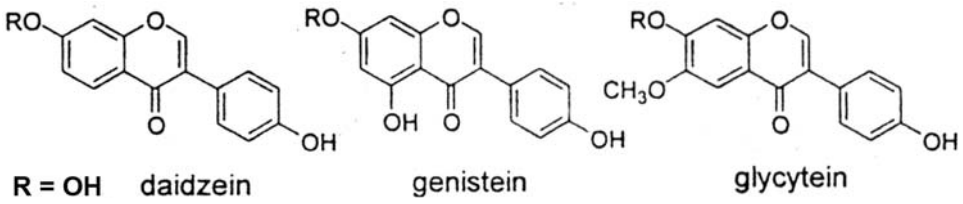

Fig. 5. Structure of soy isoflavone aglucones (69)

The concentration of isoflavones in soybean seed and soy ingredients significantly varies due to genetic variation of soybean cultivars, environment conditions where the soybean is rised, and processing conditions used for soy ingredient preparation. For example, only 23% of the total isoflavones in soy flour remaind after the soy flour had been processed to soy protein isolates (1). According to Kurzer and Xu (71), soybean-based products contain approximatly 0.2-1.6 mg of isoflavones per g dry weight.

Isoflavones have multi-biological and pharmacological effects in animals and humans including estrogenic and antiestrogenic effects (72, 73), cell signalling conduction (74-76), as well as cell growth and death. Soy isoflavones have been associated with reduced incindences of breast, prostate and lung cancer (77-79) cardiovascular diseases (80) and osteoporosis (77, 81). The mechanism through which isoflavones may exert beneficial effects are not only based on their estrogenic properties, but also on their role as protein tyrosin kinase inhibitors, as regulators of gene transcription, antioxidants, as well as by altering some enzyme activities (70).

Phytosterols. Soybeans are rich source of phytosterols. The total content of these components is 0.3-0.6 mg/g. β-sitosterols, campesterol and stigmasterol (Figure 6) are

three major phytosterols in soybean. Although these components are structurally related to cholesterol, they have many "anticholesterol" properties (82). Phytosterols decrease absorption of cholesterol due to the inhibition of bile cholesterol micelle formation and competition for the enzyme required for cholesterol uptake. Also, *in vitro* animal studies have demonstrated that these components have anticarcinogenic properties. Namely, phytosterols competitively inhibit cholesterol dehydrogenase and other bacterial enzymes in the colon. Consequently, they reduce the production of the secondary biles that may cause colon cancer (83).

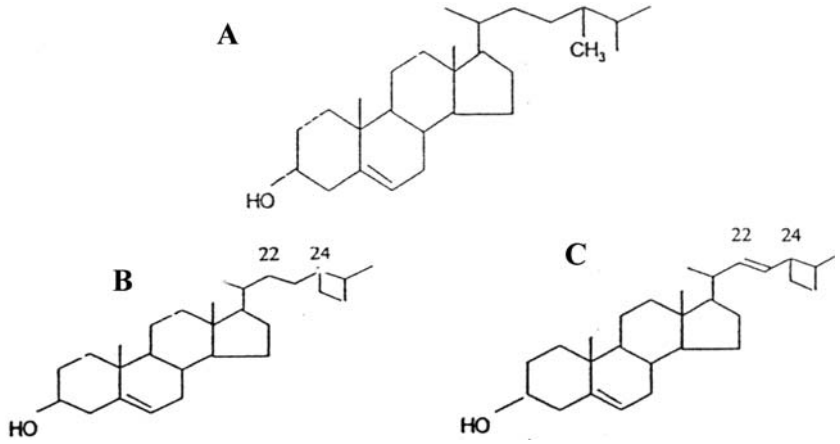

Fig. 6. Structures of three major phytosterols in soybeans (82)
A.campesterol, B.sitosterol, C.stigmasterol

Saponins. Saponins are a group of heat-stable glycosides of triterpenoids or steroids. Soybeans are rich source of saponins. Also, saponins are present in all of the soy protein products except for those that are extracted with alcohol. Soybean saponins are classified into three groups A, B, C. Group A saponins, known as bis-desmosides contain two ether-linked shugar chains, while groups B and C, known as mono-desmosides, contain one shugar chain. There are three dominant forms of saponins in raw soybean: soyasaponin ag, soyasaponin βg, and soyasaponin βa, in concentrations ranging from 1 to 3 mg/g (1). Due to the presence of triterpene or steroid part of their molecule, which is hydrophobic, and the presence of hydrophilic sugar chains, these compounds are amphiphilic. As a result of surface characteristics, saponins have hemolytic, hypocholesterolemic, immunostimulatory and antitumor activities (84, 85).

CONCLUSION

Soybeans are not only excellent sources of high-quality vegetable proteins but also contain appreciable amounts of biologically active components. Soybeans have many unique biologically active components including trypsin inhibitors, lectins, isoflavones, phytosterols, saponins, phytic acid and the most recently discovered peptide called lu-

nasin. For a long time, many of these components have been considered as antinutrients. However, the current literature contains numerous reports that most of these components exert beneficial health and therapeutic effects at low concentrations. Due to their role in preventing and treating several diseases, including cancer and other chronic diseases, with the careful selection of processing conditions soy-based products may become the "superfoods" of the millennium.

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БИОЛОШКИ АКТИВНЕ КОМПОНЕНТЕ ЗРНА СОЈЕ И ПРОТЕИНСКИХ ПРОИЗВОДА ОД СОЈЕ

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Соја представља добар извор нутритивно високовредних протеина погодних физичко-хемијских особина. Данас се соји поклања велика пажња као потенцијално значајном извору компоненти које могу имати улогу у превенцији и лечењу неких хроничних и тешких болести, као што су болести срца и различити облици канцера. Позитивни ефекти исхране сојом приписују се мање заступљеним биолошки активним компонентама сојиног семена. Ове супстанце у литератури се често називају и «фитохемикалијама». Соја садржи цео спектар биолошки активних супстанци које испољавају антиканцерогена својства попут биоактивних протеина и полипептида (инхибитори протеаза, лектин и најновије детектовани полипептид - лунасин), фитинска киселина, изофлавони, фитостероли и сапонини. Овај рад даје преглед данашњих сазнања о биолошки активним компонентама зрна соје и протеинских производа од соје.

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