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Review

# SOY PROTEIN MODIFICATION - A REVIEW

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*Soy protein products such as flour, concentrates and isolates are used in food formulation because of their functionality, nutritional value and low cost. To obtain their optimal nutritive and functional properties as well as desirable flavor different treatments are used. Soybean proteins can be modified by physical, chemical and enzymatic treatments. Different thermal treatments are most commonly used, while the most appropriate way of modifying soy proteins from the standpoint of safety is their limited proteolysis. These treatments cause physical and chemical changes that affect their functional properties. This review discusses three principal methods used for modification of soy protein products, their effects on dominant soy protein properties and some biologically active compounds.*

KEYWORDS: Soybean protein; hermal treatment; limited hydrolysis; functional properties; biologically active compounds

## INTRODUCTION

Soybean is becoming the most important vegetable source of proteins. The increased acceptance of soy proteins is due to soybean manyfold qualities, good functional properties in food applications, high nutritional value, availability and low cost. The major soybean storage proteins referred as glycinin (11S) and ß-conglycinin (7S) are globulins and the functional properties of soy based protein products (such as flour, concentrates and isolates) are reflected on their composition and the structure. The utilization of soybean based protein products is limited due to the presence of certain antinutritional factors. Among these are protease inhibitors, hemagglutinins, phytic acid, saponins and isoflavones. On the other side, the current literature contains numerous reports that some of these compounds might exert beneficial health and therapeutic effects at low concentra-

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tions. Therefore, manipulation of processing conditions is necessary to reduce the activity or content of these components to the level that will have beneficial effects and obtain desirable functional properties of soy based protein ingredients. The scientific literature contains numerous references to soy protein functionality research. The objective of much of this research is to develop systematic approach that would provide better understanding of the physicochemical properties of the individual and total soy protein system. This information is essential for manufacturing and utilizing soy protein ingredients that will meet the food industry‘s functionality requirements. The purpose of this paper is to highlight three principal methods used to regulate nutritional and functional properties of soy protein products.

## STORAGE SOYBEAN PROTEINS

Major storage proteins of soybean (Glycine max) are globulins. There are four protein fractions that are classified according to their sedimentation properties. They are 2S, 7S, 11S and 15S fractions and comprise 8%, 35%, 52% and 5% of the total protein content respectively (1). The composition and nomenclature of storage soy globulins are shown in Figure 1.

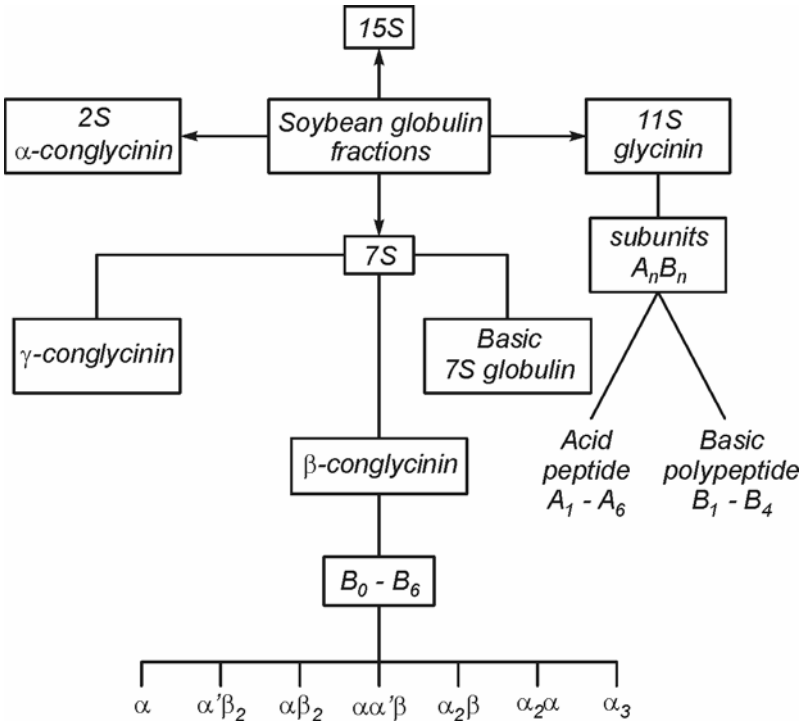


**Fig. 1.** The nomenclature and composition of reserved soybean globulins (2)

The principal storage proteins are glycinin (11S) and β-conglycinin (7S) and constitute over 70 % of soluble protein (3). Their content, ratio and dynamics of biosynthesis vary with soybean varieties and environment (4-8). Glycinin (m.w ~360 kDa) is a protein with compact quaternary structure stabilized via disulfide, electrostatic and hydrophobic interactions. It is made up of six A-SS-B subunits. Each subunit is composed of an acid (m.w. ~38 kDa) and basic polypeptide (mw~20 kDa) (9) linked by single disulfide bond, except for the acid polypeptide -$A_4$- (10). Subunits are packed into two hexagons placed one over the other to form a hollow oblate cylinder (11, Figure 2.). According to Marcone et al. (12) and Lakemond et al. (13), basic polypeptides are placed in the interior of the glycinin molecule.

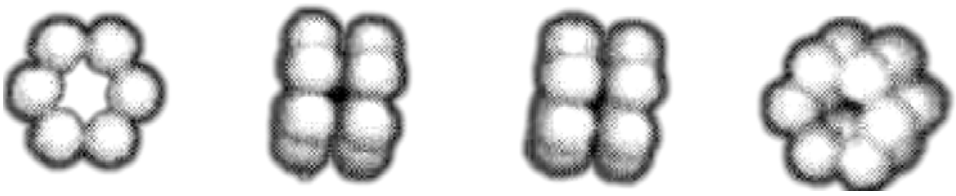

**Fig. 2.** Model of glycinin (12)

β- Conglycinin is a major protein of 7S fraction with molecular weight of 150 -180 kDA (14). It is composed of three subunits, -α`-, -α- and -β- which interact to produce seven isomers ($B_0$-$B_6$). Molecular weight of these subunits is 72kDa, 68 kDa and 52 kDa, respectively. The original β-conglycinin is a glycoprotein and it contains the carbohydrates as one unit attached to the aspartic acid residue at the N-terminal end of the molecule. The carbohydrate moiety consists of 38 mannose and 12 glucosamine residues per molecule of protein.

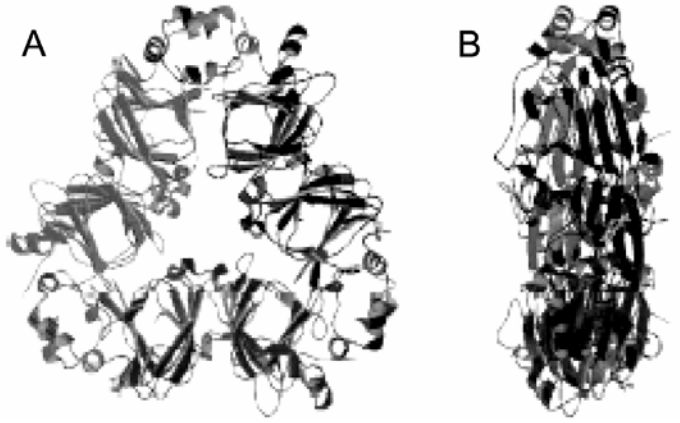


**Fig. 3.** The ribbon diagram of native β-conglycinin.The view in A is depicted as threefold symmetry axis runs perpendicular to the paper and the view depicted in B is related to the view in A by rotation of 90°(15)

Due to the different structure, there are a number of physicochemical differences in the 7S and 11S protein components. For example, 11S protein is insoluble at pH 6.4 and 2-5°C, whereas β-conglycinin is insoluble at pH 4.8. The literature contains numerous references that describe the physicochemical and functional properties of these proteins and their subunits.

## SOY PROTEIN MODIFICATION

The nutritive, physicochemical and functional properties of soy and soy-based protein product can be changed by physical, chemical and enzymatic treatment. These treatments include heating, pH adjustment, hydrolysis and covalent attachment of other constituents.

### *Thermal modification*

Thermal treatment is the oldest and most frequently used method for modification soy proteins. The purposes of the thermal modifying are different. Thermal treatments reduce protease inhibitor activity, eliminate lipoxygenase and volatile compounds that induce undesirable flavor, improve specific functional properties. Also, heating increases digestibility of soy proteins.

The effects of heating on pure glycinin and β–conglycinin solutions, their mixtures and soy flour extracts are well known. Heating soy proteins above 70°C causes dissociation of their quaternary structures, denatures their subunits, and promotes the formation of protein aggregates via electrostatic, hydrophobic and disulfide interchange mechanisms. 7S and 11S globulins have different thermal susceptibility. Glycinin has higher thermal transition point (92°C) than β–conglycinin (72°C) (16). This relationship is consistent with the observation that 11S globulin has a more compact and stable structure.

Also, different thermal stability of glycinin subunits was registered. Damodoran and Kinsella (17) reported that the insoluble aggregates formed in pure heat treated glycinin solutions consisted of basic 11S subunits, only. Whatanabe and Hashizume (18) detected stabilizing effect of 7S protein on thermally induced glycinin aggregation. Damodoran and Kinsella (17) proposed that acid 7S protein subunits interact with and stabilize the 11S basic subunits to prevent protein aggregation when both 7S and 11S soy proteins are present during heating. These subunits interact via non-specific electrostatic interactions.

The level of the changes of soybean storage proteins is significantly affected by the thermal treatment mode (19-21). Veličković et al. (19) showed that the main reserved proteins were more stable during heat treatment of cracked soybean than in solutions. Even after 45 minutes of moist steaming at over pressure of 2.0 bars, these authors detected relatively high content of soluble glycinin, while $B_0$-conglycinin represented the residual component of 7S fraction. Furthermore, they reported that the most labile were lipoxygenase, and basic 7S globulin. In opposite, soy flour prepared from cracked beans treated for 1.5 minutes by microwave were characterized with the polypeptide compositions similar to the raw soybean flour (20). Longer microwave treatment caused a significant increase of low molecular weight (<20 kDa) polypeptide products.

In past twenty years, many workers have studied the effect of heat treatment under different conditions (temperature, time, protein concentration, pH, ionic strength) on functi-

onal properties such as solubility, water absorption, gelation, emulsification and foaming ( 22-26). Most of these investigations were conducted on soy protein isolates. It has been demonstrated that the isolate functionality depends basically on the degree of dissociation, denaturation and aggregation of 7S and 11S globulins (27). It is also known that soy protein isolates show varying percentages of soluble and insoluble proteins. Sorgentini et al. (28) showed that some functional properties (such as water imbibing capacity) are basically determined by the type and content of the insoluble fraction.

Soy protein concentrates are the products which contain more than 65% proteins because soluble carbohydrates are removed by washing with either acid or aqueous alcohol. Aqueous alcohol washing, most commonly used method, reduces solubility to less than 10%. Wang and Johnson (29) suggested the direct steam–infusion treatment as an effective way to improve solubility, foaming and emulsifying properties of concentrate. After 30 seconds of steam jet cooking, the solubility of concentrate increases to 56% while emulsifying properties increase about four times.

**Thermal reduction of protease inhibitor activity.** Inhibitor activity of soybeans is result of two proteins, Kunitz (KTI) and Bowman-Birk (BBI) trypsin inhibitors. Trypsin inhibitors in raw soybeans cause growth inhibition, pancreatic hypertrophy and hyperplasia in experimental animals (30). Depending on cultivar soybeans have not more than three Kunitz isoinhibitors and 5-12 Bowman –Birk inhibitors (31). Kunitz inhibitor consists of 181 amino acid residues with two disulfide bridges; one of these bridges being essential for inhibitor activity. BBI is a low-molecular weight protein (7-8 kDa) consisting of 70-80 amino acid residues with seven disulfide bridges. BBI is a rich source of amino acid residues with sulfur; these amino acids represent 18-20% of all residues. Although both types of inhibitors represent 8-10 % of total soybean proteins they are responsible for 40 % of adverse effect of raw soybean consumption.

Thermal treatment is the most common method used for reducing protease inhibitor activity to the level that will have no adverse effect in nutrition. Different modes of treatment are in use, such as live steam treating (toasting), heating at different steam pressure, cooking, roasting, dielectric and microwave heating. The level of residual activity depends on treatment conditions (treatment mode, level of temperature, time of heating) (32), initial content of moisture (33, 34), pH conditions and the presence of reducing agents (35, 36). It is well known that dry heat have no significant reducing effect on Ti-activity even at 121°C. In opposite, treatments with steam at over a range of 0.5-2.0 bar (37- 40), steam jet cooking (41, 29) and autoclaving (42) were more effective. Further, microwave roasting at 620W- 2450 MHz for only 2.0 minutes reduced inhibitor activity to 13.33% (43). The type of inhibitor responsible for residual activity varied also depending on treatment used. According to our results, both types of inhibitors, KTI and BBI, were responsible for the activity of microwave treated flour. On the other hand, studies performed earlier in our laboratory (37, 44) showed that TI- activity of the moist heat treated flour was result of KTI, only. Today, in opposite to the traditional theory protease inhibitors, especially BBI, are considered as bioactive proteins that exhibit anticancer activity. It is known that most recently discovered polypeptide lunasin is a major component of BBI with cancer preventing activity (45). According to these facts, the level of these compounds will be neceessary to adjust by closely controlling thermal treatment parameters.

Soy protein structure and the protein-protein interactions are sensitive to the static high pressure. Intramolecular hydrophobic and electrostatic interactions are disrupted by the application of high pressure, with important consequences for the tertiary and quaternary structures of proteins (46, 47). Galazka et al. (48) showed that the static high pressure alters the quaternary structures of 11S globulin, increasing both protein-protein interactions and the interactions between the globulins and polysaccharides present in solution. In addition, Molina Ortiz et al. (49) showed that the static high pressure (200-600 MPa) dissociated 7S subunits of soy protein isolates, while the dissociation of 11S led to aggregation by changing its surface hydrophobicity and as a consequence, its solubility.

Today, very little information is available on the effect of dynamic pressure on functional properties of proteins, especially on commercial soy protein ingredients. Roesch and Corredig (50) investigated the effect of high pressure homogenization with or without heat treatment on the behavior of oil-in-water emulsion prepared with soy protein concentrates. They showed that high pressure homogenization caused the disruption of soy proteins and aggregation. A network of protein and flocculated droplets in concentrated emulsion prevented the oil droplets from creaming. They suggested that utilization of high pressure homogenization of soy protein concentrates may lead to development of new soy-based products.

### *Chemical modification*

In the last twenty years, chemical modifications of soy protein products with the aim of changing their properties have become increasingly popular as a research area. The purpose of these modifications is quite different and they include the reduction of protease activity, the reduction of phytic acid content, the increase or decrease of protein solubility to obtain better solubility-dependent functional properties, to increase nutritive value by covalent binding of amino acids with sulfur, and to eliminate undesirable odor and flavor.

**Acid-alkali denaturation.** The effect of extremely high or low pH values on pure glycinin and β–conglycinin solutions, as well as on soy flour extracts is well known. These are globular proteins with minimum solubility in the range of pH 4.0-5.0. Acidification to pH 4.0-4.2 insolubilizes about 90% of soy flour proteins (51). The low solubility of soy proteins in acid media complicates their utilization in foodstuffs of moderate acidity (citric beverages, dressings, etc.), especially when the required functional properties depend on solubility, e.g. foaming and emulsifying properties (52). Adjustment of pH is an effective method to modify the properties of soy protein products, either alone or in conjunction with heating. Solubility of soybean proteins is substantially increased by adjustment of the pH at 11-12. The solution then may be formulated into desired products by acidification of the solutions in the bulk or via extrusion. However, toxic products such as lysinoalanine could be created at these pH values. To avoid their formation, mild alkaline modification (pH 8.0) at slightly increased temperatures (50-60°C) may be used to produce better solubility of soy protein products. Under these conditions solubility of traditional soy protein concentrates increased to 45.35-56.16% (53).

Acid hydrolysis of defatted soy proteins were conducted by Watanabe et al. (54). To obtain the so-called hydrolyzed vegetable protein, they proposed a method based on hy-

drolysis in 18% HCl solutions followed by vacuum distillation and neutralization. To increase the solubility of soybean proteins, Matsudomi et al. (55) suggested the use of acid treatment (pH 2-3) in combination with thermal treatment. The increase of the solubility is due to the partial deamidation and mild hydrolysis. However, a significant increase in protein solubility is only achieved at considerably low pH, high temperatures and long incubation times.

**Chemical inactivation of protease inhibitors.** Kunitz and Bowman-Birk inhibitors of soybean can be inactivated by chemical treatments. These inhibitors can be inactivated by cleavage of two disulfide bridges in Kunitz and four of the seven disulfide bonds in Bowman-Birk inhibitors (56). Sulfiting agents, such as sodium metabisulfite (0.05M-1.00 M) at increased temperature (75°C) reduced TI activity to 75-94% within 1 hour, while in combination with glutaraldehyde under same conditions inactivated up to 99% inhibitors. Sodium metabisulfite cleave disulfide bonds and destroy TI activity. Glutaraldehyde, a protein cross-linking reagent interacts with reactive groups of active site of inhibitors and inactivates them. Friedman et al. (57, 58) suggested the use of l-cysteine and N-acetyl-l-cysteine in conjunction with increased temperature to inactivate inhibitors and to increase nutritive value of soybean flour.

Inhibitor activity may be reduced by amino-carbonyl interactions between glucose and arginyl or lysine residues near reactive center of inhibitor. Kato and Matsuda (59) reported that after 5 days of reaction at 50°C inhibitor activity decreased to above 20%. Furthermore, linear relationship between decrease of BBI-activity and the content of free amino acids residues was detected.

**Reduction of phytic acid concentration.** For a long time, phytic acid (PA) has been considered as an antinutrient. At high concentrations it can reduce the bioavailability of minerals (60). Further, phytic acid interacts with proteins and decreases their digestibility (61). In opposite, today it is well known that low concentration of PA has some beneficial effects; these include controlling dental caries; improving oxygen-providing ability of red blood cells; cancer preventing activity (62- 64).

The phytic acid content of soy protein products is highly influenced by the conditions employed for its preparation. PA accounts for about 2.86% of isolates and 4.81-4.95% of concentrates prepared under laboratory conditions (53, 65). Commercial products contain between 1.4 to 3% of phytic acid (66). The major portion of the phytate is in the 7S protein, and 11S protein contains only about 0.07% phytate (67).

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 (61). Changes in these parameters decrease these interactions. Johnson and Kikuchi (66) proposed several methods for phytic acid removal from soy protein isolates. These methods involved the decrease of pH of mother liquor solution to 2.5-4.0 in the presence of $Ca^{2+}$- or $Mg^{2+}$ ions and ultrafiltration, or the increase of pH to 7.0-11.0 in the presence of EDTA.

### *Enzyme modification*

The use of hydrolysis of 7S and 11S proteins and soy protein products to change the undesirable flavor, to improve nutritive and functional properties is well known. Earlier investigations of enzyme-induced modification were conducted to remove undesirable flavor and/or their precursors as well as to mask the flavor. A major problem concerning un-

desirable soy flavor is the green, beany character caused largely by medium-chain aldehydes such as hexanal (68). Most of these works were performed with proteases (to break the bonds between proteins and volatile components) and some oxidases (to irreversibly oxidize aldehydes which could be easily removed). Abdo and King (69) accomplished the earliest reported work have been the pioneers in this area. They concluded that the extraction of defatted soy flakes with water containing a mixture of enzymes obtained from *Pestalotiopis weterdijkii* yielded a product with improved flavor. Fujimaki et al. (70) showed that 12 individual proteolytic enzymes increased beany flavor significantly, but in some cases the intensity of bitterness, too. The increased bitterness was attributed to the formation of low molecular peptides. In their series of papers, Takahashi and co-workers (71-73) have shown that it is possible to prepare soy protein isolates with no green beany flavor by treatment with different oxidases. These enzymes irreversibly oxidize free or bonded aldehydes, which can be removed by protein precipitation or filtration.

**Limited proteolysis.** From the standpoint of safety the most appropriate way of modifying is limited proteolysis. The peptides produced by partial proteolysis have smaller molecular size and less quaternary structure than the original proteins. They contribute to increased functional properties such as solubility, foaming, gelling and emulsifying when compared with those of the native proteins. Several workers have studied limited proteolysis of soy proteins (74-77). Most of these studies have been conducted on pure 7S, 11S fraction and soy protein isolates. Commercial proteases, such as trypsin, alcalase and papain have been used in the majority of studies reported in the literature. Different results have been obtained with respect to the improvement of the functional properties of the modified proteins. 7S and 11S protein expressed different susceptibility to the enzyme-induced hydrolysis. Proteases preferentially hydrolyze ß–conglycinin over glycinin (76, 77). This is due to their different structures; the compact structure of glycinin makes it difficult protease to act.

Additional pretreatment such as heat treatment, mild–alkali hydrolysis, facilitates the enzyme-induced degradation of glycinin. Wu et al. (78) reported that subunits of 11S globulin of alkali-pretreated isolates were more susceptible than those of 7S globulin. Barać (53) obtained similar results in the case of traditional protein concentrates modification.

Shutov et al. (79) and Kamata et al. (80) characterized the effect of trypsin on pure 7S and 11S proteins. Two mechanisms, so-called zipper and one-by-one mechanism participate in glycinin hydrolysis. As a result, stable intermediate was formed. This product, named glycinin T, had lower molecular weight than the native protein. In the case of β–conglycinin it was registered five stable products; two originated from the hydrolysis of -α- and -α`- subunits and three from the -β-subunits.

To obtain desirable functional properties of soy protein hydrolysates, hydrolysis must be carried out under strictly controlled conditions to a specified degree of hydrolysis. A limited degree of hydrolysis usually improves solubility, emulsifying and foaming capacities, whereas excessive hydrolysis often causes loss of some of these functionalities. In some cases it has been possible to correlate the degree of hydrolysis with the changes in solubility (75). Furthermore, Molina Ortiz and Anon (77) reported that the solubility of hydrolysates (obtained with five proteases) and their ability to form and stabilize foams correlated well with the structural properties. Several authors (81-84) showed the existence of strong correlation between surface hydrophobicity and emulsifying activity of modified protein isolates. In contrast to this, Wu et al. (78) detected high correlation between

these properties for unmodified isolates, but for papain-obtained hydrolysates the correlation was low. They suggested that solubility and molecular size, rather than surface hydrophobicity, might be the major factors for the high emulsifying activity of the small polypeptides. Molina Ortiz and Wagner (85) investigated the effect of bromelain hydrolysis on protein solubility at pH 4.5, surface aromatic hydrophobicity and foaming properties of native and thermally denatured isolates. Bromelain treatment increased solubility of both types of isolates. The soluble fraction of these hydrolysates showed differences in molecular mass and surface aromatic hydrophobicity.

Limited proteolysis increases digestibility of soy protein products, also. It is believed that partial protein hydrolysates or oligopeptide mixtures are nutritionally superior to the corresponding amino acid mixtures. Arai and Kimura (86) showed that papain-modified isolate containing peptide-bound methionine at 3% was significantly more effective than the corresponding amino acid mixtures, and even the soy protein itself.

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## МОДИФИКОВАЊЕ ПРОТЕИНА СОЈЕ – ПРЕГЛЕД

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Висока нутритивна вредност, погодне функционалне особине и ниска цена чини протеинске производе од соје погодним за примену у прехрамбеној индустрији. Протеини соје најчешће се користе у форми брашна, концентрата и изолата. Да би се постигле оптималне нутритивне, функционалне особине и погодне сензорне карактеристике примењују се различити поступци. Протеини соје могу се модификовати физичким, хемијским и ензимским методама. Примењени поступци узрокују физичке и хемијске промене, а тиме и функционалне карактеристике протеинских производа од соје. У овом раду разматрају се поступци који се најчешће користе за модификовање протеина соје, њихов утицај на особине доминантних протеина и неке биолошки активне компоненте.

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