Modification Of Soy Protein For The Production Of Bioactive Peptides And Their Utilization

Edy Subroto, Muhammad Abdillah Hasan Qonit

Abstract: Soybean is a source of vegetable protein that is often consumed to meet the needs of protein in the body since it is rich in various essential amino acids. However, soy protein has various advantages if it is consumed in a simpler form of protein in the form of bioactive peptides. This review discusses the use of soy protein mainly in the form of bioactive peptides through various modifications, so that it has a good impact on health. Modification can be conducted by hydrolysis, fermentation, and crosslink. Hydrolysis of soy protein, either enzymatically or fermented, can release bioactive peptides that can be used as antioxidant, antihypertensive, anti-allergen, and for the food and beverage industry as a functional food.

Index Terms: Soybean, protein modification, bioactive peptides, functional food

1 INTRODUCTION

Soybean (Glycine max) is a type of legume (Leguminosae) which contains high amounts of vegetable protein. Soybean is consumed almost every day, especially for Asians in the form of processed soybeans such as tofu, tempeh, sprouts, and others [1]. Soybean is a highly nutritious food, including 40% of protein, 23% of carbohydrate, and 20% of fat. Soybeans also contain various vitamins and minerals, fiber, antioxidants, and other useful compounds such as phytosterols and lecithin [2]. The main components of soy protein are glycinin (11S globulin) and β-conglycinin (7S globulin) [3]. Soy protein is widely used in various food industries, especially in the form of soy protein isolates. Soy protein isolate is obtained through the process of extracting and isolating or purifying of soy protein. Soy protein from soybeans contains about 18 types of amino acids, including 23% amino acids that are acidic such as glutamic acid and aspartic acid; 25% basic amino acids such as threonine, tyrosine, lysine, arginine, serine, tryptophan; 30% neutral amino acids such as proline, isoleucine, alanine, leucine, valine, phenylalanine, histidine, and glycine; as well as some essential amino acids containing sulfur such as cysteine (1%) and methionine (0.35%) [4]. Amino acids in soy protein are needed primarily to meet the needs of essential amino acids for humans because they cannot be synthesized endogenously, such as tryptophan, threonine, methionine, phenylalanine, isoleucine, leucine, lysine, histidine, cysteine, and valine [5]. Soy protein also contains isoflavones such as daidzein, genistein, and glycitein, which are generally conjugated as glycoside compounds [6], [7]. However, some types of isoflavones can have adverse effects on human health if consumed in excessive amounts [8], [9]. Soy protein provides good properties for health because of its high nutritional content. The consumption of soybean is often intended for diet programs by taking advantage of the high isoflavone content in soybean in the form of aglycones [10]. However, consuming an excess of soy protein or raw soybean can have a bad effect on the body.

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The isoflavone-genistein content that is too high due to consuming excessive soy protein or soybean has the potential to damage the organs such as adverse effects on the endocrine glands, the effects of toxins on the kidneys and liver, carcinogenic effects on the pancreas, breast, and thyroid gland [9]. Several studies on the risk of consumption of isoflavones in soybean show that phytoestrogens can have a bad impact in the form of accelerated puberty, estrogen cycle disorders, ovarian hormonal disorders, pituitary changes, and hypothalamic dysfunction [8]. To get beneficial effects of consuming soy protein or soybean is to consume based on a daily dose. The daily dose of consuming isoflavones that have been considered safe by the Japan Food Safety Commission is 70 mg/day [11]. Meanwhile, according to Yan et al. [12] based on observations in various studies showed that there is no relationship between consuming isoflavones in soybeans with the risk of coronary heart disease, stroke, and cardiovascular disease so that it is safe to consume in moderation. Another alternative to obtain the goodness of soy protein is through the modification of soy protein into simple peptides in the form of bioactive peptides. Various studies on bioactive peptides continue to grow along with the benefits of bioactive peptides that are good for health. Bioactive peptides are proven to be able to provide various beneficial health effects [13], [14].

2 BIOACTIVE PEPTIDES PRODUCTION

The bioactive peptide is a sequence of short-chain peptides in protein that has a good effect on human health [15]. The peptide has various bioactive activities such as antimicrobial, antithrombotic, immunomodulatory, antihypertensive, and antioxidant [16]. In addition, the breakdown of proteins into simple peptides can improve the functional properties such as foam stability, foam height, and gelatinization properties [17].

The bioactive activity of peptides is determined by the order in which amino acids are arranged (generally consisting of 3 to 20 amino acids), which will interact with other proteins that will trigger metabolic processes [18]. The production of bioactive peptides generally uses raw materials derived from an animal such as eggs, milk, fish, meat [14], and from vegetables rich in protein such as soybeans, peas, mushrooms, rice, pumpkin, and wheat [19]. Bioactive peptides can be synthesized or produced by several methods, including the breakdown of proteins through fermentation and hydrolysis, or by adding active groups through the formation of cross-links. Simplification of peptides due to protein hydrolysis in soybeans can activate the bioactive properties of peptides [20]. The approach commonly used to release peptides that have bioactive properties to proteins is the use of proteolytic enzymes to hydrolyze proteins and release large amounts of peptide fragments in hydrolysate [21]. In addition, another way that can be conducted to separate bioactive peptides is through the breakdown or hydrolysis by using enzymes in microbes and in vitro [19].

2.1 Fermentation

Soybean fermentation is a way to modify the protein in soybean. Fermentation increases the quality of nutrition and improves the sensory quality of legume and cereal products. Fermentation can also reduce antinutrient factors such as tannin, phytic acid, and protease inhibitors [22], [23]. Protease inhibitors, such as trypsin inhibitors, which reach 30-50%, can inhibit human growth [24]. The presence of a trypsin inhibitor impedes the performance of the trypsin enzyme so that protein digestion will be inhibited [25]. The fermentation process in soybeans can reduce the content of trypsin inhibitor compounds [26]. Fermentation in soybeans can also eliminate the allergen compounds in it. Another advantage of fermented protein production is that it can reduce the bitter taste produced by the release of low molecular peptides and other properties such as hydrophobicity, spatial structure, and diversity of peptides [27]. Fermentation to modify proteins is carried out by using peptidase-producing microbes that are able to hydrolyze polypeptides in soy protein and produce free amino acids and simpler peptides with lower molecular weight [28], [29]. The production of protein hydrolysate by microbes is affected by several factors such as temperature, pH, enzyme and substrate ratio, and types of microbes and substrates used [30]. Simple proteins produced from the hydrolysis process by microbes can increase protein digestibility and increase antioxidant activity [31], [32]. Microorganisms used as fermentation agents can produce different nutritional qualities and functional properties due to different strains. Microbes produce different enzyme systems and different degradation abilities [23]. The compilation of various studies regarding the use of fermentation in improving the quality of protein in various products can be seen as in Table 1.

***Table 1**. Fermentation using various microbes on various protein-rich substrates*

Substrates	*Microorganisms*	*Fermentation results*
Soybean flour	*Lactobacillus plantarum Lp6 with the addition of proteases of 0.01 g/g*	*Increased nutritional characteristics and functional properties such as solubility, emulsifying activity, foaming capacity and stability, and protein digestibility [33].*
Soy Protein Hydrolysate	*Lactobacillus perolens, Rhizopus oryzae, and Actinomucor elegans*	*Degradates allergen peptides such as β-conglycinin, glycinin. Reduces bitter taste in the protein hydrolysates produced by degrading hydrophobic amino acids [34].*
Peas	*Lactobacillus plantarum*	*Reducing the content of non-nutritional compounds and being able to increase protein digestibility by inhibiting digestive enzymes such as trypsin and chymotrypsin inhibitors, but the fermentation carried out causes a decrease in sulfuric amino acid content [35].*
Lupine seeds	*Candida utilis*	*Increased protein and amino acid content, decreased anti-nutrient compounds, increased protein digestibility, and increased concentration of high-density lipoprotein (HDL) [36].*
Tempe flour	*Rhizopus sp.*	*Tempe flour (soybean fermentation) shows lower levels of trypsin inhibitors and phytic acid (anti-nutrient compounds) than soybean flour. Protein digestibility levels are higher in tempe flour compared to soy flour, but tempe flour has a lower protein content, higher capacity and stability of emulsifying ability [29].*
Soybean Flour	*Bacillus subtilis*	*An increase in the concentration of a single protein such as glycine, alanine, aspartic acid, threonine, arginine, and serine, while the proline content has decreased [37].*

2.2 Enzymatic Modification

Modification of protein in soybeans can be done by using the protease enzyme. These enzymes can be obtained from plants or microorganisms [28]. The advantage of enzymatic hydrolysis compared to fermentation is that the reaction time required is shorter, predictable, and easy to develop [38]. In addition, enzymatic modification of proteins can be conducted in combination using either two types of hydrolytic enzymes or a combination of hydrolytic enzymes with microbial fermentation for the production of higher and more stable bioactive peptides [39]. This combination was carried out by Daliri et al. [21] to produce soy protein isolate products that have higher angiotensin-converting-enzyme inhibitors (ACE-inhibitors). Temperature and pH in enzymatic hydrolysis will affect the level of hydration and enzyme penetration in proteins [40]. In addition, the ability of hydrolysis is affected by enzyme activity. The activity of each enzyme in hydrolyzing proteins varies depending on the characteristics of the enzyme with respect to the temperature, pH, and substrate used [41]. Each protease enzyme used will produce a different peptide sequence and biological activity [42]. The research conducted by Sbroggio et al. [43] showed that the application of the enzyme Alcalase (endopeptidase) in okara has a higher hydrolysis ability and increased antioxidant activity compared to the enzyme Flavourzyme (exopeptidase). The hydrolysis ability of various protease enzymes in the production of a soy protein hydrolysate is shown in Table 2 [44].

Table 2. *The degree of hydrolysis of soy protein hydrolysate in various types of proteases.*

Protease	Degree of Hydrolysis (%)	Reaction condition
Alcalase® 2,4L FG	13	50°C; 120 min; pH 8; enzyme concentration 0.5%
Papain	10.6	80°C; 120 min; pH 7; enzyme concentration 0.5%
Flavouryme® 1000L	8.5	50°C; 120 min; pH 6; enzyme concentration 0.5%
Corolase® 2TS	7.8	70°C; 120 min; pH 7; enzyme concentration 0.5%
Corolase® 7089	6.8	55°C; 120 min; pH 7; enzyme concentration 0.5%
Neutrase® 0.8 L	6.3	50°C; 120 min; pH 6.5; enzyme concentration 0.5%
PTN® 6.0 S	2.8	50°C; 120 min; pH 9; enzyme concentration 0.5%

2.3 Protein Crosslinking
Alternative modification of protein content in soybeans can be conducted by the Protein Crosslinking method. The Crosslinking Protein Method is a combination of protein molecules with intermolecular covalent bonds to produce physicochemical and functional properties that are different from previous single protein molecules [45]. This method has been applied to the research of Yue et al. [46] to obtain wood adhesives with non-toxic, environmentally friendly, and recyclable properties using soy protein with denaturation agents in the form of urea, sodium dodecyl sulfate, and sodium hydrogen sulfate. Crosslinking proteins can be done non-enzymatically as in Maillard reactions or enzymatically, such as using transglutaminase, tyrosinase, and peroxidase [47]. Crosslinking between soy protein isolates and gelatin using the transglutaminase enzyme gives an effect in the form of increased rheological properties, water holding capacity, and better emulsifying stability compared to soy protein isolates without crosslinking [48]. Crosslinking can be utilized in producing biodegradable coatings by using soy protein cross-linked with 4% pentaerythritol glycidyl ether (PEGE) and the addition of surfactants in the form of sodium dodecylbenzene sulfonate (SDBS) [49].

3 UTILIZATION OF BIOACTIVE PEPTIDES

3.1 Anti-allergens
Protein modification can be used to reduce allergens in soybeans. Soy protein, such as glycinin (Gly m 6) and β-conglycinin (Gly m 5) is an allergen contained in 70% of the total protein [50]. In addition, allergen proteins that have been reported in general are Gly m Bd 28K, Gly m Bd 30K, and Gly m Bd 60K [51]. This method has been applied as an option to reduce allergen compounds in soybeans. Other methods are treatments such as microwave waves, high-pressure processes, ultrafiltration, UV light exposure, irradiation, electric field waves, and genetic or chemical modification of soybeans [52], [53]. Various protein modification methods can be applied to reduce allergen compounds in soybeans. Based on research conducted by Wang et al. [54] showed that the use of Alcalase enzymes with a 10-minute hydrolysis process was able to reduce the allergen β-conglycinin and glycinin allergens to reach hypoallergenic status (low level of possibility of allergic reaction). The use of microbes to reduce allergen compounds is shown in the research of Song et al. [55], who reported that the fermentation treatment of soybeans using Saccharomyces cerevisiae was able to reduce the level of immunoreactivity to reach 89% and increase the content of essential and non-essential amino acids.

3.2 Anti-hypertension
Anti-hypertension or high blood pressure is a degenerative disease that can cause other chronic diseases such as kidney disease and cardiovascular disease [56]. One alternative to prevent this disease is to consume soy. Consuming 25 g of soybean every day can potentially prevent heart disease by lowering blood pressure [57]. Antihypertensive properties in soy are caused by the bioactive nature of peptides that contain Angiotensin-Converting Enzyme (ACE) inhibitors. ACE inhibitors are compounds that have the ability to reduce blood pressure by inhibiting the performance of the ACE enzyme in converting angiotensin I to angiotensin II [58], [59]. Protein modification through the breakdown of proteins into simpler peptides will release bioactive peptides that act as ACE inhibitors that can inhibit the conversion of angiotensin I to angiotensin II by inhibiting the performance of the Renin Angiotensin System (RAS) which plays a role in regulating blood pressure [60]. Peptides that have ACE-inhibitors from soy flour are Leu-Val-Tyr, Leu-Gly-Pro, and Leu-Lys-Tyr [61], [62]. The role of protein modification in anti-hypertension is through increasing the concentration of peptides, which have bioactive properties as ACE-inhibitors by breaking the protein bonds more simply. Fermentation for 24 hours in soybean in the process of making tempe has been proven to be able to increase the protein content that has bioactive properties as ACE-inhibitors. The fermentation also increases 16 essential and non-essential amino acids with a protein level that is 6 times greater compared to soy without fermentation [63]. Based on research conducted by Daliri et al. [21] showed that the production of soy protein hydrolysate using prozyme® was able to increase the activity of ACE-inhibitors. ACE-inhibitors were further enhanced by fermentation treatment by utilizing the microbial of Lactobacillus rhamnosus EBD1. The level of ACE-inhibitors activity on soy protein hydrolysate by 88.24% was able to approach the synthetic drug in the form of captopril, which has inhibitory activity reaching 94.20%. Peptides that have bioactive properties as ACE-inhibitors in soy protein hydrolysate are IAKKLVLP, PDIGGFGC, PPNNNPASPSFSSSS, GPKALPII, and IIRCTGC with the highest ACE inhibiting ability found in IIRCTGC peptides.

3.3 Antioxidant
Bioactive peptides which have antioxidant properties can play a role in radical binding, inhibition of lipid oxidation, and metal ion binding [64]. The use of bioactive peptides as antioxidants is considered safer and can be used as an alternative to the use of commercially available synthetic antioxidants [65]. The antioxidant activity of peptides is affected by the specificity of the protease, which determines the amount, size, composition, and sequence of amino acid composition [66]. Increased antioxidant activity in proteins can be improved through the release of bioactive peptides, which have antioxidant properties. This has been shown in the study of Esfandi et al. [67], who reported that peptides resulting from protein hydrolysis in several types of cereals produce bioactive peptides that have antioxidant activity. Silva et al. [68] also showed that protein hydrolysis in flaxseeds using Alcalase® as a catalyst produces bioactive peptides that have antioxidant

properties such as QGRGGQGGQGQ, NGSGYPGSDLDSSPPGAKVP, GREEIGNVMRSLM, and GVKVEGDGGLVRRDEI. Whereas in the research conducted by Mahdi et al. [69], it was found that the fermentation treatment in goat milk is able to produce bioactive peptides that have antioxidant properties such as LYQEPVLGPVRGPFPI, YQEPVLGPVRGPFPIL, and VQSWMHQPPQPLSPT. Besides being beneficial for health, bioactive peptides that have antioxidant properties can be used in the food industry to increase shelf life and quality of food products [66]. Oliveira et al. [70] utilizing proteases from Chryseobacterium sp. to produce the bioactive peptides that able to delay the occurrence of lipid oxidation, which causes a rancid odor in pork and salmon.

3.4 Functional Food Ingredients

Protein is an important source of nitrogen for the body to build and repair tissues, cell signaling, energy supply, and play a role in the function of hormones and enzymes [71]. The results of protein modification, such as hydrolyzed protein or simple peptides, can be utilized to meet daily protein needs. The results of the hydrolysis of soy protein, such as soy protein hydrolysate, can also be used as functional food ingredients, flavor enhancers, protein additives, and as ingredients in the medical field [72]. Short-chain peptides produced by hydrolysis have high nutritional value and are able to eliminate allergen compounds [73]. In addition, the functional properties of a soy protein hydrolysate, such as solubility, emulsifying properties, foam formation and stability, and gel formation, can improve the quality of food products [71]. The utilization of modified protein has been carried out to produce low-fat ice cream. Soy protein isolate has been used as a substitute for skimmed milk powder in ice cream that produces ice cream that is harder, thicker, and does not melt quickly [74]. Another use of soy protein hydrolysate is used as a substitute for dairy products for people who are allergic to lactose and to prevent diseases caused by high blood pressure. Soymilk, with the addition of protease PROTIN SD-NY10, can trigger the release of bioactive peptides LEPP, VNP, FFYY, IAV, FVP, WHP, and WNPR which have properties that can reduce blood pressure [75]. Soy protein can be used in meat products to improve taste, color, texture (increased ability to retain water), shelf life, and reduce fat content in meat [76]. The use of soy protein hydrolysate about 20% in the dough proved to be able to improve the quality of the dough as well as the physical and sensory characteristics of the sponge cake [77]. Soy protein hydrolysate can also be used to increase microbial growth. Hydrophilic and electropositive amino acid residues such as histidine, arginine, and lysine in soy protein hydrolysate can increase the growth of Saccharomyces pastorianus strain FBY0095 in beer fermentation [78].

4 CONCLUSION

Modification of soy protein can be used to increase compounds that are good for health and improve product quality. Various methods can be utilized for protein modification such as fermentation, utilization of protease (hydrolysis), and crosslink. The product produced from protein modification in the form of soy protein hydrolysate can be utilized in improving health, such as anti-allergens, anti-hypertension, and antioxidants. In addition, bioactive peptides can be used to improve the quality and safety of food as a functional food.

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