Review: Emulsification properties of soy bean protein

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Abstract. Chen W, Li X, Rahman MRT, Al-Hajj NQM, Dey KC, Raqib SM. 2014. Emulsification properties of soy bean protein. Nusantara Bioscience 6: 196-202. Emulsion stability and emulsifying ability are two important factors in food industry. Soy protein has the great of interest because of its amphilic structure. β-Conglycinin and glycinin are main components in soy protein which can be used as emulsifiers in food processing. However, due to its size and molecular weight, the emulsifying ability of soy protein is limited. By chemical, physical and enzymatic modification, the emulsifying ability of soy protein can be improved. The addition of polysaccharides in emulsion is common. The interaction of polysaccharides and proteins are being discussed in this review. In some complex food emulsion, the function of soy protein molecules and emulsifier at the interface need to be investigated in the future study.

Key words: Emulsion, soy bean, protein


INTRODUCTION

Soy and soy products have been consumed by millions of Asian people for centuries as the main source of protein source. In west society, soy protein has been processed as foods since about the late 1950s because of its nutritional and functional properties. From 1997, the consumption of soy protein in USA has been increased sharply. The realization of the physiological properties of soy protein is the main reason for this increase. There is 38-44% protein in soybean seeds on moisture-free basis (Foroud et al. 1993). Glycinin and β-conglycinin are the largest mass in the seed protein as the storage protein. Compared with the adequate content protein and unbalanced amino acid composition in cereal crops, soy protein products are an ideal source of the essential amino acids with good profile for human consumption (Liu et al. 2014). World Health Organization (WHO) adopted the soy protein digestibility corrected amino acids (PDCAAD) is 1.0, which show soy protein is able to meet the Children growth need (Kärjä et al. 2010). Anderson and Wolf (1995) indicated that the consumption of soy protein can lead to the significant reduction in total cholesterol (9.3%), LDL cholesterol (12.9%) and triglycerides (10.5%), with a small increase in HDL cholesterol based on 743 subjects’ research. According to Kahlon and Woodruff (2002), the hydrophobic peptides in the soy protein can bind well to bile acids which were found from the stool. The stimulation of bile acid synthesis results in the reduction of serum cholesterol.

The active peptide fragments in soy bean protein have hypocholesterolemic, anticarcinogenic, hypotensive, immunostimulating and antioxidant effects. The molecular weight peptide-Met-Leu-Pro-Ser-Tyr-Ser-Pro-Tyr in soy protein has anti-carcinogenic properties. The peptide -Gln-Arg-Pro-Arg and His-Cys-Gln-Arg-Pro-Arg from soy bean glycinnin A1b subunit can stimulate phagocytic activity of human polymorphonuclear leukocytes (Kim et al. 2000). Singh et al. (2014) found six antioxidative peptides from soy bean. β-conglycinin have been identified to have against peroxidation of linoleic acid. Some minor components coexist in soy protein such as isoflavones, saponins and so on are considered to have preventative effects on cancer (Messina and Barnes 1991). Besides those physiological functions of soybean, soy protein has excellent processing ability in food manufacture such as emulsifying ability, gelling and water holding ability (Liu et al. 1999; Jambrak et al. 2009; Jung et al. 2005; Kasran et al. 2012; Chen et al. 2013) which will be discussed in the review.

The problem of soy protein which limits its expanded use is the strong off-flavors for the consumers in the western countries. Grassy and beany flavors are associated with the activities of lipoxygenases. Bitter flavors are caused by isoflavones. Some scientists try to apply conventional breeding and genetic engineering to overcome this problem.

THE MAIN COMPONENT OF SOY PROTEIN

Major protein in soybean is storage protein which takes 40% of total dry matter base of soy bean. Approximately
90% of the proteins in soybean are storage proteins. β-conglycinin and glycinin are main components in storage proteins. β-conglycinin is a glycoprotein and a trimer which consists of three major subunits (α, σ, β) and a minor subunit (γ). The molecule weight of those subunits is 72, 68 and 52 kDa respectively (Johnson et al. 2008). α, σ -subunit has one cysteine residue near the N-terminal and β subunit does not have cysteine residue (Utsumi et al. 1997). In the different pH and ionic strength of the solution, those subunits in β-conglycinin can cause association or dissociation (Johnson et al. 2008). The molecule mass of Glycinin is about 300-380 kDa. There are two parts polypeptide (acidic and basic) in glycinin. They are linked together by a disulfide bond (Staswick et al. 1984). Glycinin shows molecular heterogeneity because of a different subunit composition. Whey protein is a minor part in the soy protein which makes up 9-15.3% of soybean protein (Smith et al. 1966). According to Iwabuchi and Yamauchi (1987), whey protein contains lipoxigenase (102 KDa), α-amylase (61.7kDA), lectin (33kDa) and KTI (20kDa). At different gravitational force, soy protein isolate can be classified into four protein categories according to their sedimentation coefficients 2S, 7S, 11S and 15S. 7s fraction the separation of those ingredients depends on the different molecule weight. 7S fraction is around 30% of the total protein and 11s fraction is about 41% of the total protein. Glycoprotein comprise one half of 7s fraction. Three subunits α (ca. 67 kDa), α (ca. 71kDa) and β (ca. 50 kDa) make up of 7S globulin. 11s is making up of 12 different subunits with quaternary structure. Its molecule weight is about 360 kDa (Murphy 2008).

THE SOY PROTEIN STRUCTURE AND ITS EMULSIFYING PROPERTIES

The three dimensional structure of the protein molecule decides the physicochemical functions of the proteins. The lipophilic and hydrophilic groups in the soy protein polymer chains make its facilitating with fat and water Figure 1.

Figure 1. Three-dimensional structure of a peptide bond between an alanine and an adjacent amino acid (top). Chemical structure of the peptide bond (bottom) (Dobson 2000).

The emulsion of fats and water are thermodynamically unstable because of the positive free energy caused by interfacial tension. The formation of a charged layer around fat globules will lead to mutual repulsion to prevent collision. In the food industry, emulsifying function is important in food production and storage such as some soft drink and cream liqueurs in the short time. The emulsifying ability can be measured by determining the maximum oil quality with a fixed amount of the protein. The emulsion stability can be determined by measuring the velocity of phase separation into water and oil in the storage of emulsion. When protein applies into emulsion system, it will be adsorbed on the surface of oil and form a layer to prevent the oil globular from coalescing. The protein on the globular surface can lower down the interfacial tension between oil and water. This is a process which protein will migrate to the surface of oil globules. Protein will rearrange its structure at the interface.

Normally, in the aqueous solution, a thermo dynamical state of protein was formed. The polar segment was exposed to aqueous phase. However at the water/oil interface, the hydrophobic segments in protein were exposed to lipid phase and the polar segments was exposed to aqueous phase. Protein denaturation was involved in this process. The composition-conformation of the protein viscosity, dispersion, pH, ions and temperature will impact the emulsifying ability. The diffusion ability of molecules, mobility of protein, surface charge, ease of unfolding and facility for packing at the interface will impact the equilibrium surface tension. 11S globulin consists of two identical hexamers. Three subunits in the hexamer are acidic and the other three in the hexamer are basic in the nature. They are linked by disulfide bond. This model is the most accepted model. The most hydrophobic subunits are located in the interior of molecules and the hydrophilic subunit stay at the surface of the molecule (Lakemond et al. 2000). Because of close packed globular and larger molecular size conformation in the native protein, the foaming properties and emulsifying ability are limited.

Large size of soy protein molecules make it diffuse relative slowly at the interface. Salt may reduce the charge repulsion between the proteins and increase hydrophilic association at the interface. The migration speed may speed up. In the isoelectic point, the protein maximum adsorption at the oil-water interface leads to the maximum emulsifying ability. High energy input to food emulsion will lead to the stable food emulsion system. High solubility of soy protein leads to high emulsifying properties because of high soy protein availability. Higher surface hydrophobicity of 7s soy protein leads to stability of emulsion. Wagner and Gueguen (1999) indicate a decrease in molecular size of Glycinin lead to increasing hydrophobicity and emulsifying function. Glycinin has low surface hydrophobicity, less molecular flexibility and big molecules size which prevent its migration to fat globule surface (Liu et al. 1999). The modification of soy protein to improve the emulsifying ability will be discussed in the last part of this article. Solubility, surface hydrophobicity and molecular flexibility are important factors which impact the emulsifying activity. Due to different structures of the tertiary and
quaternary of 7S and 11S decides the different emulsifying ability.

THE METHODS TO IMPROVE THE SOY PROTEIN EMULSIFYING ABILITY

Many methods have been applied to improve the emulsifying ability. Those methods include physical, chemical and enzymatic methods. The physical methods are heat treatment, high pressure and filtration. Those treatments changed the soy protein molecule structure or select effect subunits in soy protein in emulsifying ability (Figure 2).

According to Keerati-u-rai and Corredig (2009), applying heat to soy protein solution will lead to the change of interaction of protein. The protein subunits were disassociated and molecular changed. Heating the solutions before the emulsification will lead to smaller fat droplet size which stabilized by heated soy protein. Heating make the change of soy protein structure and contributes to improvement of emulsifying ability. More buried hydrophobic amino acids in soy protein are exposed to the surface. Higher surface hydrophobicity means increasing ability for protein to adsorb to the oil side at the interface so that emulsion capacities will be increased (Kim et al. 2005). The protein segments such as "loop" or "tail" which radiate from the interface will form steric stabilization to restricting droplet coming together.

The viscosity of continuous phase will be increased with addition of the protein, which will reduce the diffusion of oil droplet in the emulsion. The coalescence of smaller fat globular becomes less. High pressure treatment is another method to change the soy protein capacity. With the 200 MPa treatment, the partial denaturation of 7s leads to the expose of hydrophobic groups. The surface hydrophobicity increases. Solubility was not greatly influenced by high pressure at this pH value. Molina et al. (2011) found, the 7s emulsion treated by the combination of 60℃ and 400 Mpa pressure indicated higher emulsifying ability. About 11s emulsion with the same treatment are not

Figure 2. Some physical change of soybean protein (Nishinari et al. 2014).

Figure 3. The effect of soy protein structural modification on emulsion properties (Zhang et al. 2014a).
Figure 4. Producing novel SPI from SPC (Yang et al. 2014).

Figure 5. Schematic representation of the possible mode of interaction between polysaccharides and proteins (Ghosh and Bandyopadhyay 2012).
modified. Fractionation of soy protein can separate soy protein isolate into retentates and permeates by microfiltration. Chove et al. (2002) indicated emulsions stabilized by the retentate fractions has high value of emulsion stability index and emulsifying activity index than those stabilized by the fraction from the permeates. Soy protein fractions rich in molecules with isoelectric point above 5.1 exhibited good emulsion stability and emulsifying activity. Jambrak et al. (2009) found, ultrasound treatment (20 kHz probe and 500 kHz bath) can improve the soy protein emulsion emulsifying and foaming ability.

There are a lot of researches about chemical medication of soy protein to improve the emulsification. Introducing some groups in the soy protein by chemical reaction can increase the performance of soy protein in emulsion (Figure 3). Saturated fatty acids were introduced into 7s and 11s by acylation reaction. Covalent attachment of fatty acid in 7s and 11s lead to 1.4-2.2 and 1.1-1.8 fold increase in the oil binding ability so that emulsifying activity and emulsion stability increase (Matemu et al. 2011). Glucose group are joined to SPI by Maillard reaction. Soy protein modified by glucose shows high solubility at a wide range of pH value and heat stability. Glycosylated SPI was effective in enhancing in emulsifying ability and can be a promising new ingredient for the food industry. SWPI are modified by introducing fenugreek gum by forming conjugate. Maillard reaction occurred in a controlled dry state condition (60°C, 75% relative humidity for 3 days). SWPI-fenugreek gum conjugate has better emulsifying properties near the isoelectric pH of protein (Kasran et al. 2012). Modification of soy protein isolate can be achieved by oxidation. Chen et al. (2013) applied peroxy radical derived from 2, 2-azobis, 2-aminodipropyl dihydrochloride (AAPH) to modify the soy protein molecules. The results indicated moderate oxidation can generate soluble protein aggregates with more flexible structure and emulsion stability. Zhang et al. (2014b) found soy protein isolate are conjugated to maltodextrin by maillard reaction (high temperature 140°C and short time 2 hours). Higher glycosylation degree of soy protein-maltodextrin generates excellent storage stability of emulsion and emulsifying stability in oil-water emulsions because of steric effect in the solution. Yuan et al. (2013) found soy protein isolate reacted with chitosan at 121°C at low pH. The complex formed show higher emulsifying ability.

Limited hydrolysis of soy protein has been studied by many scientists. Soy protein hydrolysis by enzyme partially reveals hidden hydrophobic group and increase surface hydrophobicity (Figure 4). Reduction in the molecular weight and size will allow for better adherence to the oil-water interface (Tsumura et al. 2005). The quaternary structure of sop protein is the main reason of less desired surface activity of soy protein although soy protein has good lipophilicity. Degree of hydrolysis, temperature and enzyme selected will decide its functionality and characteristic. The functional properties are close related to the degree of subunit dissociation, denaturation and aggregation. According to Liu et al. (1999) the acidic subunits of glycinin have a good emulsifying ability compared with unfraccionated soy glycinin. Jung et al. (2005) found that endo-protease treatment to soy protein isolate can significantly decrease apparent viscosity of the solubilized hydrolysates and improve the emulsification capacity of soy protein isolate solutions. Phoon et al. (2014) make use of trypsinization to conduct the limit hydrolysis of 7S. Hydrolysis product can improve oxidative stability at pH 7 under low ionic strength. Tsumura et al. (2005) applied enzyme to modify soy protein and get reduced glycinin hydrolyse. Compared to unmodified soy protein, reduced β-conglycinin hydrolysat and reduced-glycinin hydrolysat indicates high emulsifying ability at acid pH. Extrusion pre-treatment and controlled enzymatic hydrolysis in soy protein attribute to increased protein solubility and decreased molecular weight. Reduced soy protein molecules can migrate to the interface faster and lead to higher emulsifying ability (Chen et al. 2013).

SOY PROTEIN INTERACTS WITH POLYSACCHARIDES IN FOOD EMULSION SYSTEM

Food system always contains polysaccharides except the protein and fat such as stabilizers in ice cream. The addition of polysaccharides in the emulsion always leads to a positive or negative effect on emulsion stability. The solvent properties, the properties of polysaccharides and soy protein will contribute to emulsions. Emulsion preparation in mixed system can greatly influence the composition, structure and dynamic behavior of biopolymers at the interface (Figure 5).

Soy protein stabilized emulsion droplets were resistant to coalescence because of their globular structure and their adsorption at the interface. Because of the larger size of soy protein molecules, some small oil droplet may be bridged via soy protein molecule to form large oil droplet. Once the pH of solution is near to isoelectric point, the emulsion will be unstable and phase separation may occur. The application of polysaccharides to protein-based emulsion has been reported to increase the emulsion stability. Depletion flocculation may occur when a negatively charged polysaccharide to the negatively charged SPI covered oil emulsion. The emulsion made with mixture of protein and polysaccharides were less susceptible to destabilization by flocculation (Jourdain et al. 2008).

The factors such as the polysaccharides and protein properties, concentration, ratio and solvent condition contribute to the formation of coacervate. Tran and Rousseau (2013) reported that soy soluble polysaccharides were applied into soy protein isolate stabilized solution to improve the emulsion stability because polysaccharides can coat the protein to prevent protein-protein interaction between two fat droplets. Yin et al. (2012) found electrostatic and hydrophobic interaction between soy protein soy polysaccharides form dispersible complex at pH 3.25 so that the polysaccharides are fixed on the droplet surface. The nano sized emulsions exhibited long term stability. This system can be used in food grade delivery system in encapsulating lipophilic bioactive compounds.
The mixture of denatured soy whey protein (dSWP) and soluble soybean polysaccharides shows greater the ability against coalescence and phase separation (Ray and Rousseau 2013). Soy soluble polysaccharides prevent the soy protein isolate base oil in water emulsion under acidic condition (Tran and Rousseau 2013). In fact, the structure of soluble soy bean polysaccharides has larger branch neutral chains (galactan and arabinan). SSPS structure and a peptide fraction in the molecules exert a key role in the surface behavior. The depletion does not occur because SSPS interact with soy bean protein isolate at the interface at neutral pH. The branched portion of SSPS molecules may play more effective role in stabilizing oil droplets compared to that of steric stabilization.

**FUTURE RESEARCH OF THE INTERACTION OF SOY PROTEIN AND EMULSIFIER AT THE INTERFACE**

Protein /surfactant mixture are common in food system such as ice cream. It is important to conduct the research on adsorption behavior and its dynamics of mix protein and emulsifier system. The mechanism of the molecule interaction between proteins and other surface-active components present at the interface of emulsion droplets is important to understand the emulsion stability. The addition of a protein to oil-water interface, protein will reorient and realign to minimize the number of thermodynamically unfavorable interaction and reduce the interfacial tension. Interfacial intension can be defined as the free energy needed to increase the area of an interface by unit amount (N/m) (Bos and Van Vliet 2001). Emulsifiers are surface -active lipids, low molecule weigh surfactant and phospholipids. Surfactants are water soluble in monomeric and micellar form. Surface tension measurement is the tool to study protein-emulsifier interaction. The protein and lipid interaction is the effect on the stability of the protein in solution as well as on its behavior at interfaces. Nonionic surfactants such as Tween 20 lead to a significant decrease in surface tension. There are no definite prediction can be achieved to concern the surfactants and soy protein on the adsorption dynamics. There are few studies of the adsorption of mixture of soy protein with different ionic and non ionic surfactant. In order to understand the function of soy protein at the interface, the adsorption equilibrium qualitatively is required. The adsorption kinetics of soy protein should be clear before the further steps. Currently, many standard proteins such as BSA, HSA, β-casein and β-lactoglobulin were measured by the many researchers. For the specific binding of protein and emulsifier, the ionic and hydrophobic interactions mediate the binding. The interaction between soy protein and surfactant are required to be invested in the future study.

For the interaction between protein and emulsifier at the interface, this topic has been investigated in those years because the consequence of competitive adsorption of these two species at the interface can impact the emulsion stability against coalescence. Soy protein size is larger compared to low molecular weight surfactants however the protein indicate the high affinity for interface in the low concentration. In the high concentration of emulsifier, the saturation coverage with emulsifier at the interface will reduce the interfacial tension. It is possible to replace soy protein and emulsifiers. Soy protein molecules and emulsifiers in the interfacial layer is great interest. Normally, the hydrophobic group in the soy protein will be anchored at multiple sites at the interface. The emulsifiers can reduce more interfacial tension compared to protein by forming a fluid-adsorbed layer at the temperature above transition temperature. It is possible that competitive adsorption of emulsifiers could weaken or interfere with the formation of protein in the adsorption layer and destroy the integrity. In the future research, the surface activity of molecule will be determined between soy protein molecules and emulsifier.

**CONCLUSION**

Better understanding of soy protein’s structure and emulsifying ability mechanism will help to improving soy protein application in food products and boarding the use of soy protein by the food industry as an ingredient. By controlling processing condition, solvent and biopolymer condition, the performance of soy protein at the interface will be controlled and tailored for more high-value controlled delivery application in food manufacture. Modification soy protein by the methods discussed in the article will lead to greater advances in protein-based emulsifiers.

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