Effects of Emulsifier on Emulsification, Physical and Chemical Properties of Soybean Protein

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Abstract. Emulsification is an important functional characteristic of soybean protein, but the emulsifying ability and stability of natural soybean protein are not ideal, which limits its application in food. This paper mainly studies the application of other emulsifiers in the production of soybean protein, and studies the properties of the final product, in order to optimize the preparation technology of soybean protein, to provide new ideas for the development of high emulsifying soybean protein products and expand the scope of soybean protein application. The paper points out that soybean protein products with high emulsification can be obtained by the complex reaction of sucrose ester with the modified soybean protein as the main raw material.

1. Introduction

Soybean has a very high nutritional value. It is rich in protein, oil, carbohydrate, oligosaccharide, mineral and phytochemical components. It has the reputation of "king of beans". It has more than 3000 years of cultivation history in China. It is rich in resources and is an important natural material. Soybean products such as tofu and soybean milk are also loved by the public. Soybean contains 40% protein and 20% oil. Among them, 86% - 88% of the protein is water-soluble protein, minerals and vitamins are also more complete. Soybean is also a cheap source of plant protein. The soybean meal after oil extraction is the raw material for extracting high-quality plant protein[1]. In recent years, soybean is widely used in dairy products processing. As an important functional substance and nutrient of organism, it can also supplement nitrogen in food and provide important support for the growth, metabolism and immunity of the body. Therefore, the development, processing and sales of soybean milk increased rapidly.[2]

Soybean protein is the protein of soybean products. Its amino acid composition is similar to that of milk protein. Except for methionine, other essential amino acids are rich. It is a complete plant protein. In terms of nutritional value, it can be equivalent to animal protein, and in terms of gene structure, it is closest to human amino acid, so it is the most nutritious plant protein[3]. Soybean protein has incomparable advantages compared with animal protein. Although soybean protein has very little methionine, it does not contain cholesterol. Soybean protein is not a single component. SPI accounts for 90% of soybean protein. At present, there are two classification methods which are generally accepted by the academia. The first is to divide soybean protein into 2S, 7S, 11S and 15s under the condition of pH 7.6 and ionic strength 0.5; The other is to divide soy protein into soy globulin, soy protein and soy protein according to immune response α- Conglycinin β- Conglycinin and γ- Conglycinin. The components of 2S are trypsin inhibitor and cytochrome c. The main components of 7S are hemagglutinin, lipoxygenase, and β- Amylase, β- Conglycinin, γ- Conglycinin. Soy globulin is the main component of 11S, β- Conglycinin is the main component of 7S. Soy globulin and β- The content of conglycinin was the highest, accounting for about 70% of the soybean protein composition, mainly 7S and 11S. The properties of these two storage proteins determined the functional characteristics of SPI[4].

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The functional properties of soybean protein are related to its structure, i.e. amino acid composition, arrangement order, conformation, molecular shape and size, charge distribution, and the role of intramolecular and intermolecular bonds[5]. High proportion of polar residues affect the interaction between peptide chains, hydration, solubility and surface activity, hydrophobic interaction is important in protein tertiary folding, it affects emulsification, foaming and flavor binding ability. Charged amino acids can enhance static interaction, play a role in stabilizing globulin, binding water, hydration, solubility, gelation and surface activity. Sulphydryl group can be oxidized to form disulfide bond. The conversion of mercaptan and disulfide will affect the rheological properties. The properties and quantity of covalent and noncovalent bonds determine the size, shape and surface charge of proteins. All these properties are affected by pH, temperature and other environmental factors as well as processing. [6]The solubility and stability of soybean protein in different dissolving systems are the most important problems in processing, and the function of soybean protein should be based on the dissolving operation. The solubility of soybean protein, also known as the dispersion characteristics of soybean protein, refers to the percentage of soluble protein in soybean protein in a specific environment. The main factors affecting the solubility of soybean protein are temperature, pH value of medium and ionic strength, etc. Meanwhile, the solubility of protein is closely related to other functional properties[7]

2. Preparation of soybean protein

2.1 Materials and reagents
The frozen defatted soybean meal was purchased from Shandong Yuwang Co., Ltd; BSA, SDS, edta-2na, Na2HPO4 · 12H2O, NaH2PO4 · 2H2O, CuSO4 · 5H2O, potassium sodium tartrate, sodium hydroxide, 95% ethanol, acetic acid, bromphenol blue (BPB, 5,5'- disulfide bis (2-nitrobenzoic acid) (DNTB), copper sulfate (CuSO4 · 5H2O), potassium sodium tartrate, potassium sodium hydroxide, sodium bisphenol blue (BPB, sodium bisphenol blue (2-nitrobenzoic acid) (dntb), sodium dodecyl sulfate (SDS), sodium edta-2na, sodium dodecyl phosphate (Na2HPO4 · 12H2O) Urea, Coomassie brilliant blue R250, glycine, glycerol, Tris, dialysis bag (molecular weight 14000) ± 8-anilino-1-naphthalenesulfonic acid and other reagents were purchased from Guangzhou Congyuan science and Technology Instrument Co., Ltd. the reagents were analytical pure; Electrophoretic preformed gel (4% concentrated gel, 12% separated gel), molecular weight standard (PM2510, 10 kDa – 180 kDa) and sample buffer (reduced) were purchased from biyuntian Biotechnology Co., Ltd.[8]

2.2 Instruments and equipment

| Table 1. Experimental instruments and equipment |
|-----------------------------------------------|
| Multifunctional pulverizer | SL 2000A | DeZhou Xian HuiHang Mechanical Equipment Co. Ltd. |
| Precision electronic balance | EL 204/EL 3002 | Changzhou Huada Kejie Opto-Electro Instrument Co., Ltd. |
| High speed freezing centrifuge | CR 22N | Hitachi in Japan |
| Visible spectrophotometer | UV-1800 | Hitachi in Japan |
| pH meter | pHS-3E | Changzhou Huada Kejie Opto-Electro Instrument Co., Ltd. |
| Electrophoresis apparatus | Mimi PROTEAN 3 Cell | Bio-Rad Laboratories company in America |
| Magnetic stirring rod | JB-1 | Shanghai Leici Xinjing Instrument Co.,Ltd. |
| Freeze dryer | ALPHA1-4/2-4 | Christ Co.,Ltd. in Germany |
2.3 Experimental methods

Soybean protein isolate was extracted by traditional alkali solution and acid precipitation method. Defatted soybean meal was dissolved in 8 times water, then adjusted to pH 7.80 with 3mol / L sodium hydroxide, and stirred at 50 °C for 45min. The solution was centrifuged at 3300g centrifugal force for 10min, and the supernatant was taken. The bottom precipitate was redissolved with 5 times water, stirred at 50 °C for 10 min, centrifuged at 3300 g for 10 min, and the supernatant was taken. The supernatant obtained from two centrifugations was mixed at 40 °C and adjusted to pH 4.50 with 3mol / L hydrochloric acid. Hydrochloric acid was added while stirring. After reaching the isoelectric point, the stirring was stopped immediately, standing for 10min, centrifuging at 3300g for 10min, and the bottom precipitated. Add a certain amount of water to dissolve the precipitate, adjust the pH to 7.20 with sodium hydroxide, and prepare 10% soy protein isolate solution. The solution was homogenized by high-pressure homogenizer 20MPa and spray dried. The inlet air temperature was 170 C and the outlet temperature was 80 C. Soybean protein concentrate was extracted by dilute acid extraction. Defatted soybean powder was dissolved in 10 times water, then adjusted to pH 4.50 with 3mol / L hydrochloric acid, stirred at 50 °C for 20min, and centrifuged at 3300g for 10min. After repeating the above steps for 2 times, the precipitate was redissolved with water and adjusted to pH 7.20 with 3mol / L sodium hydroxide to prepare 10% soybean protein concentrate solution. The solution was homogenized by high-pressure homogenizer 20MPa and spray dried. The inlet air temperature was 170 C and the outlet temperature was 80 C. The modification process of soybean protein is based on the traditional extraction process of alkali soluble acid precipitation and dilute acid extraction, adding acid-base modification, heat treatment and enzyme modification.

3. Effect of emulsifier on Emulsification of soybean protein

Emulsifying property refers to the property that oil and water are mixed together to form emulsion. Emulsification is the main functional property of soybean protein. The protein has the characteristic structure of emulsifier, the two affinity structure, which contains hydrophilic groups and affinity groups in protein molecules. It can aggregate on the oil water interface, reduce interfacial tension and promote emulsion formation. Soy protein isolate can not only reduce the surface tension of water and oil, but also reduce the surface tension of water and air, so it is easy to form a stable emulsion. The evaluation indexes of emulsifying properties include emulsifying capacity (EC), emulsifying stability (ES) and emulsifying activity (EA). Emulsifying ability refers to the ability of protein to dissolve or emulsify oil. The emulsifying activity refers to the ability of protein to participate in the formation of emulsion and the ability of emulsion to stabilize. Emulsion stability refers to the ability of emulsion to remain stable, and there is no ability to float, flocculate or aggregate within a certain period of time.

On the basis of previous experiments, we selected the unmodified SPI prepared by traditional alkali soluble acid precipitation, alkali modified SPI treated at pH11 for 6h, heat modified SPI treated at 80 °C for 5min and enzyme modified SPI hydrolyzed by trypsin for 30min to study the addition of different kinds of emulsifiers (lecithin, monoglyceride, sucrose ester The effect of Tween on the emulsification of protein was studied. The addition amount was 3G emulsifier / 100g protein[9].
Figure 1. The influence of different emulsifiers on emulsifying activity of soy proteins

Figure 2. The influence of different emulsifiers on emulsifying stability of soy proteins

Figure 2 shows the effect of different emulsifiers on the emulsifying activity of protein. It can be seen from the figure that the emulsifying activity of all proteins except SPI decreased after adding tween. The emulsifying activity of the products hydrolyzed by trypsin for 30 min and then added with Tween was lower than that of unmodified SPI; When tween was added to SPI, lecithin was added to alkali modified protein, lecithin or monoglyceride was added to heat-treated protein, and lecithin or sucrose ester was added to enzyme modified protein, the emulsifying activity of each protein was significantly
improved. Figure 3 shows the effect of different emulsifiers on the stability of protein emulsification[10]. It can be seen from the figure that the emulsification stability of alkali modified protein and enzymatic hydrolyzed protein decreased significantly after adding tween, which was lower than that of unmodified SPI; The stability of SPI and protein hydrolysate emulsification was significantly improved by adding monoglyceride; The emulsifying stability of alkali modified protein and heat-treated protein was significantly improved by adding sucrose ester. After heat treatment at 80 °C for 5 min and adding sucrose ester, the product has the highest emulsifying stability. Through the previous analysis, we can find that different kinds of emulsifiers have different effects in improving the emulsifying activity and emulsifying stability of different kinds of soybean protein. After adding tween, the emulsification of alkali modified protein and enzyme hydrolyzed protein decreased; After adding emulsifier to SPI, the improvement of emulsification effect is not obvious; Lecithin played a good role in improving the emulsification of alkali modified protein; Monoglyceride has a good effect on improving the emulsification of protein hydrolysate; After heat treatment at 80 °C for 5 min and adding sucrose ester, the product has good emulsifying effect.

4. Effects of emulsifier on physical and chemical properties of soybean protein

4.1 Effects of emulsifier on protein solubility

Solubility is the main index that affects the functional properties of protein, and it is the key physical and chemical properties that determine the application of protein. The solubility of protein is affected by some factors, such as pH, ionic strength, temperature and solvent type[11]. When the pH value is higher or lower than the isoelectric point, the net charge of protein is negative or positive, and its solubility increases. Although the solubility of protein is the lowest at pH, there are some differences for different proteins. Some proteins, such as casein and soybean protein, are almost insoluble at isoelectric point, while whey protein is still very soluble at isoelectric point. It is very convenient to extract and separate proteins whose solubility changes greatly with pH value by changing the pH value of the medium; For the protein whose solubility changes little with pH value, other methods are needed to achieve the purpose of separation and purification. Figure 3 shows the effect of different emulsifiers on protein solubility. It can be seen from the figure that after adding lecithin, the solubility of each protein is significantly improved; After adding tween, the solubility of alkali modified protein and enzyme hydrolyzed protein decreased; In addition, other kinds of emulsifiers can improve the solubility of protein, but the effect is not very obvious.

Figure 3. The influence of different emulsifiers on solubility of soy proteins
4.2 Effects of emulsifier on protein particle size distribution

The particle size distribution of SPI was determined by PALS laser particle size analyzer. SPI sample was prepared into 0.2% protein solution with 50 mmol/L phosphate buffer (pH 7.0) and 0.45% protein solution. The measurement was carried out at room temperature. The average particle size is the equivalent diameter of the largest particle with 50% cumulative distribution in the particle size distribution curve. For an actual particle swarm composed of particles of different sizes and shapes, compared with a hypothetical particle swarm composed of uniform spherical particles, if the full length of the particles is the same, the diameter of the spherical particles is called the average diameter of the actual particle swarm. Micro particulate matter is widely used in daily life and industrial production. Its size and distribution are directly related to the industrial process, product quality, energy consumption and safety of production process[12]. Therefore, it is very meaningful to measure the size and diameter of micro particles accurately and conveniently and get the particle size distribution function. After adding emulsifier, the shape of particle size distribution of each product was similar, although the peak and average particle size increased slightly, and the dispersion index decreased slightly, but the changes were not significant. The reason may be due to the interaction between the hydrophilic and hydrophobic groups of emulsifier and protein, the emulsifier attached to the protein and changed the protein particle size, but the addition of small molecular emulsifier had no significant effect on the existing state of soybean protein, so the protein particle size changed little.

Table 2. Particle size analysis of proteins mixed with different emulsifiers

|                  | Peak value (nm) | Peak width (nm) | Strength (%) | Average particle size (nm) | Dispersion index |
|------------------|-----------------|-----------------|--------------|-----------------------------|------------------|
| Control group    | 262.9           | 122.5           | 100%         | 204.6                       | 0.239            |
| Lecithin         | 264.2           | 120.6           | 100%         | 203.8                       | 0.234            |
| Monoglyceride    | 269.8           | 125.8           | 100%         | 207.4                       | 0.235            |
| Sucrose ester    | 275.1           | 114.2           | 100%         | 210.5                       | 0.233            |
| Tween            | 263.5           | 118.1           | 100%         | 204.9                       | 0.231            |

4.3 Effects of emulsifier on protein surface tension

Surface tension refers to the increment of surface potential energy when the unit surface area is increased. When the protein denatured at the interface, hydrophobic group would be inserted into oil phase, and the hydrophobic amino acids in oil phase had lower activation energy and spontaneous. As emulsifier protein, it must be able to adsorb to new oil water interface quickly and then reduce the interfacial tension to a low level. The decrease of surface tension is the primary condition for emulsification and foaming, and the degree of surface tension decrease can reflect the ability of protein to expand rapidly, rearrange and expose hydrophobic groups on the interface, which is an important factor of emulsifying force and foaming force. Generally speaking, the stronger the hydrophobicity of proteins, the higher the concentration of proteins adsorbed at the interface; The lower the interfacial tension, the more stable the emulsion[13]. The decrease of surface tension is the first condition of foaming, and the degree of surface tension decrease is an important factor of foaming capacity. Surface tension refers to the work needed to increase the unit surface area of the material on the interface. The surface tension can reflect the ability of the material to expand and rearrange rapidly on the interface, and it is an important factor affecting the emulsification of protein. Figure 4 shows the change of surface tension of the modified protein and the complex of the modified protein and emulsifier. It can be seen from the figure that the surface tension of the modified products has different degrees of decrease compared with SPI, among which the surface tension of the heat modified protein decreases the largest; The surface tension of the complex decreased after adding emulsifier to the modified protein, and the surface tension decreased significantly after SPI was added with Tween and other modified proteins were added with lecithin; The
surface tension of the product was the lowest after heat treatment at 80 ℃ for 5min and then lecithin was added.

Figure 4. The influence of different emulsifiers on surface tension of soy proteins

4.4 Effects of emulsifier on protein surface hydrophobicity
Surface hydrophobicity is a key indicator of the number of hydrophobic groups on the surface of proteins in polar water environment. Hydrophobic interaction is the main force to maintain the tertiary structure of proteins, which plays an important role in the stable conformation and functional properties of proteins. The surface hydrophobicity has more influence on the structural and functional properties of proteins than the overall hydrophobicity because of its intermolecular interaction. ANS fluorescence probe is a classical method to explore the hydrophobicity of protein surface, which can reflect the changes of protein tertiary structure in aqueous solution. The principle is that ANS, as a fluorescent probe, does not emit light in the water environment, but can emit relatively strong fluorescence after binding to protein[14]. Therefore, it can be used as a fluorescent probe to reflect the change of the polarity of the environment where the binding site is located, so as to know the change of protein tertiary structure. Surface hydrophobicity is one of the surface structural properties of protein molecules, which reflects the changes of hydrophobic groups on the surface of protein molecules. It is of great significance to the stability, conformation and functionality of proteins. The dissociation of protein subunits and the unfolding of polypeptide chains expose the hydrophobic groups hidden in protein molecules, resulting in the increase of hydrophobicity of protein surface, while the aggregation of protein molecules may cause the decrease of hydrophobicity. Figure 5 shows the change of surface hydrophobicity of different protein molecules. It can be seen from the figure that after alkali modification, heat treatment and enzyme modification, the surface hydrophobicity index of protein is significantly improved, which is consistent with previous studies. The reason for the increase of hydrophobicity is probably due to the change of conformation, the expansion of protein structure and the exposure of hydrophobic groups embedded in the molecule. Small molecular emulsifiers can combine with protein molecules through hydrophobic interaction[15], thus changing the surface hydrophobicity of protein. It can be seen from the figure that the surface hydrophobicity of each modified protein increased after adding tween; After adding lecithin, monoglyceride and sucrose ester, the surface hydrophobicity of SPI, alkali modified
protein and enzymatic hydrolyzed protein decreased in varying degrees, but the surface hydrophobicity of heat-treated protein did not change significantly.

Figure 5 The influence of different emulsifiers on surface hydrophobicity of soy proteins

5. Conclusions
Different kinds of emulsifiers have different effects on the emulsification of soybean protein. Among them, lecithin, monoglyceride and sucrose ester can improve the emulsifying activity and stability of soy protein in varying degrees[16], while tween only plays a limited role in promoting the emulsifying activity of SPI and the emulsifying stability of heat modified protein. After adding emulsifier, the surface tension of protein molecules decreased in varying degrees; except for tween, the surface hydrophobicity index of the complexes formed by other kinds of emulsifiers with SPI, alkali modified protein and enzymatic hydrolyzed protein decreased, but the thermal modified protein had no significant change; the addition of emulsifier had no significant effect on the protein particle size distribution.

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