Functional Properties of Sesame Seed Protein Prepared by Two Different Methods

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Summary: The functional properties of sesame seed protein was investigated using reverse micelles system compared to the traditional procedure (alkali-solution and isoelectric precipitation). The solubility, emulsifying capacity and emulsion stability, foaming capacity and foam stability of sesame seed protein prepared by the two above methods were analyzed. The results indicated that the functional properties including solubility, emulsifying capacity and emulsion stability of sesame seeds protein extracted by reverse micelles were superior to those by the traditional method, which was helpful to maximize the use of sesame seed protein resources.

Introduction

Sesame (*sesamum indicum*) is one of Chinese four major oil seeds and the output of sesame reached 546,000 tons in China in 2008 [1]. On the other hand, sesame is also a good resource of protein. Recent studies indicate sesame protein is an excellent quality protein (nearly 80% α-globulin and 20% β-globulin) with high nutritional and biological values, i.e., high net protein utilization and digestibility [2, 3]. But the defatted sesame meal, which contains 60% sesame seed protein [3], has traditionally been used as animal feed or fertilizer due to limited information of its functional properties, leading to waste of resources. Therefore, it is necessary to study comprehensively the properties of sesame seed protein in order to expand the use of protein resources.

Currently, protein is traditionally prepared by alkaline extraction and isoelectric precipitation. The sesame protein is prepared by alkaline extraction after the sesame oil extraction, the supernatant is centrifuged, and pH of the solution is adjusted to the isoelectric point to obtain protein precipitation. One disadvantage of this approach is that high acid and alkali consumptions, resulting in serious water pollution and the limited capacity of raw material treatment leads to low extraction efficiency of the protein. Moreover, it is easily to cause protein denaturation [4].

Reversed micelle extraction method is that the surfactant is dissolved in an organic solvent and a certain amount of water is then added to the above solution to form reverse micelle solution as the extraction medium. This method can be applied in extraction oil and protein compound from oilseeds. Oil is extracted into organic solvents and protein is dissolved in the polar core of reverse micelles [5]. After that, the protein is recovered with strong ionic of the salt solution by a backward extraction. The advantages of this technique include mild extraction condition, maintaining high activity of protein extracted and economic efficiency of the technology.

The aim of this research is to exploit the utility of sesame seed protein in food products, main functional properties such as solubility, oil absorption, and foaming, emulsification and emulsion stability were studied.

Results and Discussion

Table-1 shows that the content of the protein prepared by reversed micelles extraction was higher than that by alkali-solution with acid-isolation. Moreover, the color of the former was better. There were two possible reasons for the result. On one hand, the micro-environment of reversed micelles extraction is conducive to the dissolution of the protein, and vice versa for small molecular impurities. Therefore the quality of the protein with reversed micelles extraction was better. On the other hand, the protein extracted by alkali solution and acid-isolation may contain other water-soluble molecules (physic acid, etc.) in solution, leading to lower purity and worse color.

Table-1: The color and content of proteins prepared by Reversed micelles extraction and alkali-solution with acid-isolation, respectively.

Sesame protein	Protein content (%)	Color
Reversed micelles extraction	86.2	Milky
Alkali-solution and acid-isolation	85.4	Buff

As can be seen in Fig. 1, the solubility of

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sesame protein varied at different pH levels. The isoelectric point of sesame protein was approximately pH 4.5 and the solubility of sesame protein was good both in acidic and alkaline conditions. There were some reasonable explanations. First, sesame protein molecule was amphipathic considering it contains both amino and carboxyl groups. In acid medium, protein molecules exist mainly in the positive ion state, and negative ions that in alkaline medium. Thus sesame protein showed good solubility both in acidic and alkaline pH regions. At the isoelectric point, sesame protein was in zwitterionic state and the solubility became the lowest. The solubility decreased with the pH value increasing when the pH value was lower than the isoelectric point, and the solubility increased when the pH value was higher than the isoelectric point. It should be noted that the solubility was larger than 90% when pH value was 2.0 or 11. Additionally, the solubility of sesame protein by reversed micelles extraction was slightly higher compared to that by alkali-solution extract and small molecular weight proteins which had better dispersion and higher solubility [6].

In the emulsification process, small molecular weight molecules could easily spread to the oil-water interface and reduce the interfacial tension rapidly while large weight molecular was easy to form the emulsion layer. As for the emulsifying capacity of sesame protein, on the whole, it was high in alkaline solution (for example, 150 mL/g at pH 11.0) and low (≤ 5.5 mL/g) in acid solution. In addition, there was little difference of the emulsifying capacity of sesame proteins at the same pH value by the two extraction methods (Fig. 1). It can be seen from Fig. 2 that the emulsion stability of sesame protein increased sharply with the increasing of pH in the alkaline pH region, ranging from 5.2 to 140 mL/g when pH value increased from 7 to 11, while there was no significant variation in the acidic pH region, varying between 2.3 and 5.2 mL/g in pH value range of 2 ~ 7. The trend of pH versus emulsifying capacity was similar to that of pH versus emulsion stability (Fig. 1 and Fig. 2), suggesting that emulsifying capacity and solubility both depended on the structure and charged state of protein surface [7]. Interestingly, the variation of water holding capacity of protein at different pH levels was also similar to that of the solubility (Fig. 1 and Fig. 2). In brief, the water holding capacity of was minimum at pH 4.5 (isoelectric point), and then increased with the pH value increasing when pH value was higher than 4.5. The reason was that net electrostatic charge carried by the protein was zero and the hydration ability was minimum at the isoelectric point. Nonetheless, water holding capacity of protein extracted using reverse micelles was smaller than that using alkali-solution and acid-isolation, for reverse micelles under influence of the size of "water pool" had relatively weak ability of extraction large molecular weight protein, which had higher water holding capacity relative to small molecular weight protein.

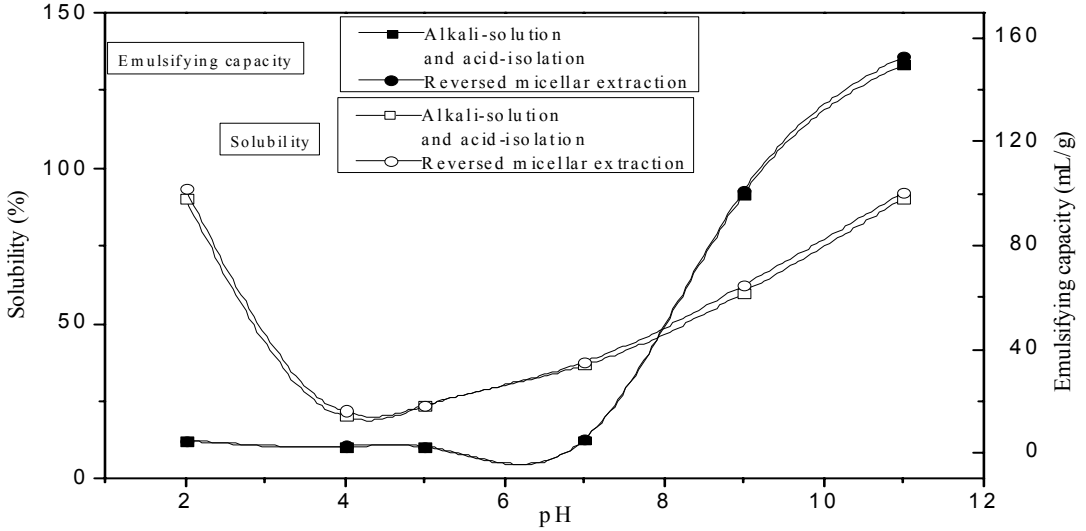

Fig. 1: Effects of pH on the solubility and emulsifying capacity of the proteins prepared by reversed micelles extraction and alkali-solution with acid-isolation, respectively.

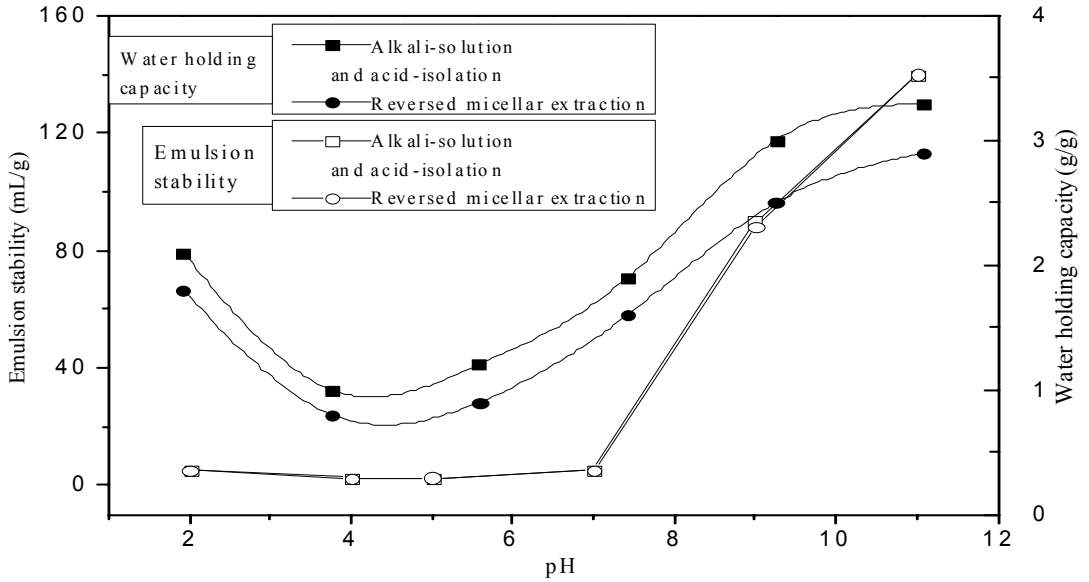

Fig. 2: Effects of pH on emulsion stability and water holding capacity of the proteins prepared by reversed micelles extraction and alkali-solution with acid-isolation, respectively.

The foaming capacity of protein extracted using reverse micelles was higher than that using alkali-solution and acid-isolation (Fig. 3). On the one hand, high protein solubility was a prerequisite to achieve better foaming capacity and foam stability because the foaming capacity of proteins was closely related to the solubility. If protein solubility was high, the ability of diffusion and being adsorption in the air and water interface would be strong and the interfacial tension would reduce, which promoted the formation of foam [8]. On the other hand, there was a few residual AOT in the protein extracted by reverse micelle method, so the protein extracted with reverse micelle method had higher foaming capacity.

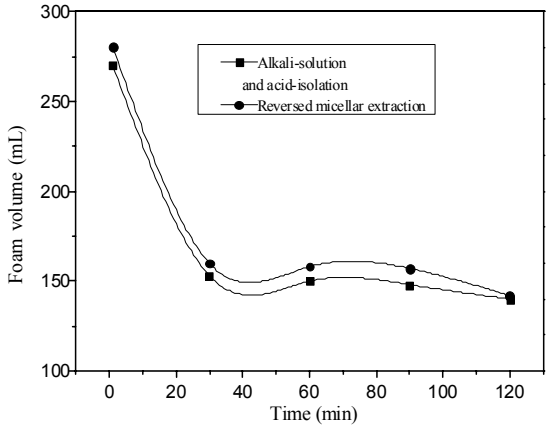

Fig. 3: Foaming capacity and foam stability of the proteins prepared by reversed micelles extraction and alkali-solution with acid-isolation, respectively.

Oil absorption capacity is expressed in the amount of oil adsorbed by per gram of protein products, which is usually determined by centrifugation method. The oil absorption capacity is depended on the main factors such as the type and source of protein products, particle size of protein, operating temperature and processing methods and so on. As shown in Fig. 4, oil absorption capacity of sesame protein extracted using alkali-solution and acid-isolation was 2.7 g/g and slightly higher than that by reverse micelles (2.3 g/g).

The viscosity of protein systems is an important property. The flow properties of protein dispersions have practical importance in the optimal operating conditions, such as conveying, mixing, heating, cooling, and spray drying. The viscosity of protein dispersions will affect the process of mass and heat transfer. The viscosity of sesame protein mainly depends on the properties of protein (such as molecular size, shape, surface charge and concentration), pH and temperature. The viscosity of

sesame protein solution prepared by reverse micelles was similar to that by alkali-solution and acid-isolation, both increased with increasing of protein concentration (Fig. 5). While protein extracted by alkali-solution and acid-isolation had slightly higher viscosity compared to the protein extracted by reverse micelles. With the increasing of protein concentration, intermolecular interactions among protein enhanced, thus the viscosity of protein solution increased. Most of protein extracted using reverse micelles had small molecular weight, and the hydrated protein had small apparent hydrodynamic diameter. Therefore, the viscosity of the solution was low.

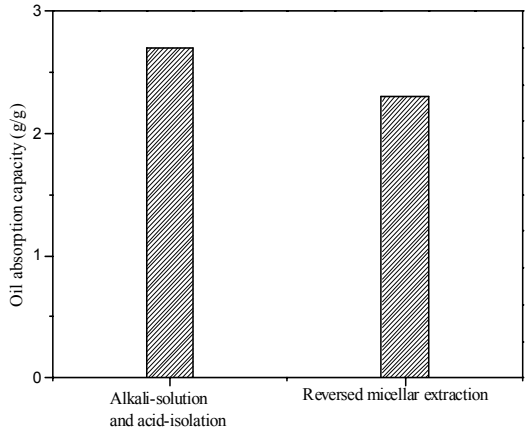

Fig. 4: Oil absorption capacity of the proteins prepared by reversed micelles extraction and alkali-solution with acid- isolation, respectively.

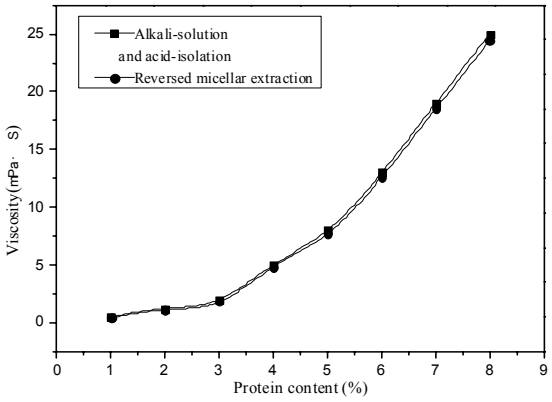

Fig. 5: Comparisons of the viscosity of the protein solution prepared by reversed micelles extraction with that by alkali-solution and acid-isolation.

Experimental

Protein was prepared by the method of alkali-solution and acid-isolation: The sesame seeds were crushed and defatted by Soxhlet extractor with petroleum ether for 24 h. The sesame meal was air-dried at room temperature, ground again to pass through a 60 mesh sieve and dispersed in distilled water (1:10, w/v), and then adjusted to pH 11.0 using 1 M NaOH. The suspension was oscillated for 1h in a shaker at 50~55℃, and then centrifuged at 3000 rpm for 15 min. The extract was acidified to pH 4.5, stirred for 15 min and centrifuged at 3000 rpm for 10 min. The precipitates were neutralized by 1.0 M NaOH to pH 7.0, dialyzed in distilled water for 48 h and freeze-dried.

The reverse micellar systems were formed by anionic sulphosuccinic acid bis -(2-ethylhexyl) ester sodium salt (AOT), isooctane and KCl solution. There were forward and backward extractions for the procedure of protein preparation using reverse micelles. Forward extraction (proteins from aqueous solutions to micellar solutions) was achieved by adjusting experimental conditions to let the partition favor the transfer of proteins into the organic micellar phase; and vice versa for backward extraction (proteins from micellar solutions back to aqueous solutions) [9]. The sesame seeds were crushed and extracted with reverse micelles solution. The pH of the aqueous solution was adjusted to being lower than the isoelectric point of the protein in order to make protein molecules carry positive charge and tend to enter the micelles composed of anionic surfactant through mixing, and centrifuged at 3000 rpm for 20 min. Then the buffer solution with a pH usually higher than the isoelectric point was added to the protein micellar phase to achieve the backward extraction and centrifuged at 3000 rpm for 20 min.

Detailed determination procedures of the solubility of sesame protein were described elsewhere [10]. In brief, 250 mg sesame protein samples were dispersed in 9.5 mL distilled water, and the pH value was adjusted using 0.1 M HCl or NaOH at room temperature, and then the dispersion was centrifuged at 4000 rpm for 15 min. At last, the protein content of supernatant and sesame protein samples were determined according to Kjeldahl method.

The method to determine the water holding capacity of sesame protein was detailed in a previous study [11]. Briefly, 1.0 g sesame protein sample was

transferred into a pre-weighed centrifuge tube, distilled water was added drop by drop, and stirred until the sample became paste, and the dispersion was centrifuged at 2000 rpm for 10 min. Supernatant was discarded and the residues was weighed. If no supernatant, more water would be added until a small amount of supernatant appeared. Water holding capacity (g water/g sample) = [(centrifuge tube weight + precipitate weight)-(centrifuge tube weight + sample weight)]/sample weight.

Foaming capacity and foam stability of sesame protein were determined on the basis of the method in a previous reference [12]. Approximately 100 mL 1% protein solution product was adjusted to pH 7.0 and mixed at 8000 rpm for 2 min with a DS-1 high-speed organization pounding machine. The emulsion was rapidly transferred into a 500 mL graduated cylinder, and foam volume in different standing time and the cease time of mixing were recorded before and after stirring. Then foaming capacity and stability of samples were compared with those of the different standings.

Oil absorption of sesame protein was analyzed according to the program in a reference [13]. Accurate 0.5 g protein sample was transferred into 10 mL centrifuge tube and 3 mL pure soybean oil was then added. The mixture was stirred for 1 min, placed at room temperature for 30 min, and then centrifuged at 1000 rpm for 25 min. The upper unabsorbed soybean oil was adsorbed and weighed. Oil absorption was defined as: oil absorption = (weight of sample absorbed oil- weight of sample)/weight of sample.

Emulsifying capacity and emulsion stability of sesame protein were obtained by the methods in previous studies [14, 15]. Protein sample (1.0 g) was dissolved in 100 mL phosphate buffer solution, and then a certain volume of soybean salad oil was added. The solution was stirred until forming a uniform emulsion. The new prepared emulsion was diluted 100 times by adding distilled water. Then 1 mL of the new diluted emulsion was diluted 40 times by adding sodium dodecyl sulfonate solution (1 g/kg). The final dilution time was 4000. The absorbance of this solution was determined at 500 nm (n=9). Emulsifying capacity (EAI) and emulsion stability (ESI) were calculated using the following formula.

$$EAI = 2 \times T \times \frac{A_0 \times \text{Dilution times}}{C \times \phi \times 1000}$$

$$ESI = \frac{A_0 \cdot t}{A_0 - A_{10}}$$

where *EAI* was emulsifying capacity, *T* was set at 2.303, dilution times was 4000, *C* was the concentration of protein in aqueous solution before emulsification (g/mL),φ was the volume fraction of oil in emulsion (0.025); *ESI* was emulsion stability (min), A_0 was the absorbance of diluted emulsion, A_{10} was the absorbance of diluted emulsion standing for 10 min after dilution; t was standing time (10 min).

In addition, viscosity of sesame protein solution was also measured by Ubbelohde viscometer.

Conclusions

The protein extracted by reverse micelles had some better properties, i.e., water holding capacity solubility, emulsifying capacity and emulsion stability , foaming capacity and foam stability and viscosity, compared to the protein extracted using alkali-solution and acid-isolation, which would be helpful to further make good use of sesame.

Acknowledgement

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