Effects of homogenization on the molecular flexibility and emulsifying properties of soy protein isolate

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Abstract The sensitivity of soy protein isolate (SPI) to trypsin was characterized by its flexibility. The effects of different homogenization conditions on soy protein isolate flexibility and emulsifying properties were investigated. Set the homogenization pressure was 120 MPa (megapascal) and the homogenous number of times is 0–4 times, the flexibility increases with the increase of the homogenization times (0–3 times), the change trend of flexibility is not obvious (3–4 times). When the homogenization times was 0–3 times, the emulsifying activity increases, and the emulsifying activity was the strongest at 3 times, after homogenization 3 times, the change trend of emulsifying activity is not obvious, the trend of emulsification stability and emulsification activity were similar. The surface hydrophobicity increases with the increase of homogenization times, while the turbidity decreases. The other structural indicators such as Ultraviolet scanning and endogenous tryptophan fluorescence spectroscopy suggest that the structure of SPI becomes more stretch as the flexibility increases.

Keywords Soy protein isolate · High pressure homogenization · Flexibility · Emulsifying properties

Introduction

Soy protein isolate has been in use in many food applications due to its good functionality, nutritional value and potential health benefits [1, 2]. Because of their amphiphilic characteristics (with both hydrophobic and hydrophilic properties), SPI demonstrated an excellent emulsifying property [3]. The relations between soy protein emulsifying and structure have been extensively studied. The previous data on the structural role in emulsification mainly focusing on the general comprise, for instance the superficial degree of hydrophobic [4]. Although Damodaran et al. [5] recommended that a conformational flexibility (capability to experience conformational restructuring) at the boundary might act as a more important role in emulsifying characteristics of the proteins, than an external degree of hydrophobic.

As we know, the structure flexibility plays an important role in emulsification [6, 7], the suppleness of the protein structure distinguished by the protease absorption can be the fundamental characteristics regarding an emulsification [8]. Recently, Tang et al. [9] inspected the conformational flexibility of bovine serum albumin by S–S cleavage on the emulsifying characteristics, they found that the conformational flexibility of the protein (in solution) act as an essential role in various phase of its emulsifying features. Few studies provided solid evidence to show a direct relationship between emulsifying properties and the interface-digestion on soy protein, and other spherical proteins [10–14].

High pressure homogenization is a non-thermal technology that has been widely used in the pharmaceutical, food and cosmetic applications to produce stable emulsion. The high pressure shearing treatment is indeed necessary to create a stable emulsions by decreasing the drop size to

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submicron ranges via various concurrent forces such as cavitation, friction, trim, turbulence, contraction, promotion, hasty pressure reduction and collision [15, 16]. Puppo et al. [17] had suggested that elevated pressure homogenization have a significant consequence on the tertiary and quaternary structure of soy protein, but still little effect on the secondary structure when the homogenization pressure exceeds 100 MPa. Bouaouina et al. [18] had suggested that high pressure homogenization processing resulting in exposure of hydrophobic groups protein molecules, thus improving the protein foaming ability and stability. Nevertheless, very few research has been focusing on structure flexibility of SPI on emulsifications.

However, the impact of high pressure homogenization, especially homogenization times on SPI flexibility and emulsifiability is seldom reported.

In this paper, the concept of flexibility is introduced into high-pressure homogeneous modification. The main work is to study the influence of homogenization times on SPI flexibility, emulsification properties and other structural properties, in order to provide theoretical support for actual production.

Materials and methods

Materials

Soybean was attained from the Northeast agricultural university Soybeans Research Institute (Heilongjiang, China). Soy oil was gifted by Jiusan Company (Heilongjiang, China). Sodium dodecyl sulphate (SDS) was purchased from Sigma (St. Louis, MO, USA), all other reagents were of analytical grade.

Preparation of soy protein isolate

According to the method adapted from Sorgentini et al. [19]. Soybean was grounded and sifted through a 60 mesh sieve. The resulting soybean powder was defatted by extracting with ether according to the soxhlet method. The defatted soy flour and deionized water 1:10 mixing 2 h, with 2 mol/L NaOH adjusted to 8.5, 4000 g centrifuged for 20 min. The supernatant was adjusted to pH 4.5 using 2.0 mol/L HCl, then refrigerated overnight at 4 °C. The resulting solution was subsequently centrifuged at 4000 g for 5 min. The precipitation was rinsed twice using deionized water. The precipitation was solubilized by neutralized using 2.0 mol/L NaOH to pH 7.0, and then freeze-dried. The SPI protein content determined by Kjeldahl methods was $89.69 \pm 0.44\%$ W/W ($N \times 6.25$).

High pressure processing

SPI (5 mg/mL) was rehydrated in a phosphate buffer solution (0.2 mol/L, pH 7.0), constantly being mixed and stirred at room temperature for 2 h using the T18 fundamental speedy admixture (IKA, Staufen, Germany) at 10,800 rpm. The solution was then refrigerated overnight at 4 °C. Then, the dispersions were stirred until reaching room temperature, homogenization pressure is 120 Mpa, homogenized at homogenized once, twice, thrice and four times respectively. The protein solution without homogenization was used as control.

Determination of flexibility

According to Kato, protein flexibility was established with the chymotrypsin. An enzyme reaction was conducted at 37 °C in the beginning with an enzyme to protein ratio at 1:16. Four mL of 1 mg/mL protein solution was added to 0.05 mol/L Tris-HCl buffer solution (pH 8.0) mixed with 250 μ L of 1 mg/mL chymotrypsin solution. The proteolysis was monitored by trichloroacetic acid (TCA) precipitating. 4 mL of 5.0% TCA was added to terminate the enzyme reaction and precipitate the excessive protein. The composition was rested for 10 min and filtered using No 5b filter paper (Toyo Roshi Ltd), and the absorbance of TCA-soluble peptides in the filtrate was measured at 280 nm on a spectrophotometer to estimate the amount of digest in the filtrate [8], Characterization of flexibility with absorbance A.

Determination of protein surface hydrophobicity

The surface hydrophobicity was measured via hydrophobic fluorescence probes According to Schmal method [20]. The protein sample was in sequence diluted by 0.01 mol/L phosphate buffer (pH 7.0) to get 2 mg/mL protein concentration. Then, the resulting sample (2 mg/mL) were diluted to 0.1, 0.05, 0.0025 and 0.00125 mg/mL using 0.01 mol/L phosphate buffer (pH 7.0). Then 20 μ L of 1-aniline-8-naphthalenesulfonate (ANS) (0.008 mol/L in 0.1 mol/L phosphate buffer, and pH 7.0) solution was added. Intensity of fluorescence (FI) was determined using an Aminco Bowman spectrophotofluorometer (Hitachi, F-4500) at the induced wavelength of 391 nm, the emitted wavelength of 471 nm, and the slit length of 5 nm. The net FI on every protein concentration was obtained by reducing the FI of each solution. The first slope (S_0) of the FI versus concentration of protein (%) plotted was used as the index' protein hydrophobicity.

Determination of turbidity

The SPI (5 mg/mL) solution was diluted to 3 mg/mL using phosphate buffer solution (0.2 mol/L, pH 7.0), and an absorbance was determined at a stated wavelength of 400 nm, measured by F-4500 fluorophotometer (Hitachi, Japan), the OD value was used to represent the turbidity. All the results were averaged on triplicates.

Particle size distribution

Treated models were diluted by using deionized water to a protein concentration that was 1 mg/mL, and a particle size was calculated on a particle size analyzer (American Bruker Hay Instruments Inc), and each protein sample was tested three times.

Second-derivative ultraviolet (UV) spectroscopy

According to Liang [21], Baseline corrected UV spectra were recorded between 250 and 310 nm and the scanning rate was 100 nm/min, The data interval was 0.2 nm. The second-derivative treatment of UV spectra was performed by Origin 9 software (Origin-Lab Corp, Northampton, MA).

Emulsifying activity index (EAI) and emulsifying stability index (ESI)

According Tang, a SPI solution (5 mg/mL) was diluted to 2 mg/mL. Mix with oil at the rate of 3:1 using a T18 Basic Speedy Disperser/Homogenizer (IKA company, Germany) to mixed for 1 min (10,800 rpm/min) to create the emulsions. Immediately pipet 50 μ L of bottom emulsion into 5 mL of 0.1% SDS (w/v) solution and mix well on a vortex mixer, the absorbances of 0 and 10 min were measured [22], and the emulsification activity was expressed by the absorbance A_0 . The formula for calculating the emulsion stability was as follows:

$$ESI = \frac{A_0 \times 10}{A_0 - A_{10}}$$

where A_0 and A_{10} , the absorption rate of the diluted emulsions at 0–10 min, respectively. Measurements were performed in triplicates.

Statistical analysis

All researches were conducted in triplicates. Means were weighing against using one-way ANOVA (Analysis of Variance) of SPSS 19 Statistical software allowed by Duncan's Multiple Range Test ($p < 0.05$). Origin 9 was applied to create forms.

Results and discussion

Effect of different homoenization pressures on the SPI flexibility

As shown in Fig. 1, a significant increase of the molecular flexibility was detected between the samples with no homogenization and homogenized, and the flexibility of protein molecules was detected when the homogenization times increased from 0 to 3 times, This is because the high pressure treatment had a disruptive effects on the internal arrangement of proteins [23, 24]. That was basically obtained by high shear stirring, cavitation, turbulence, friction, heat, compression, acceleration, rapid pressure drop, high pressure homogenization has a significant effect on the tertiary and quaternary structures of the SPI and disrupts the SPI through non-covalent bond cleavage [25, 26], thus destroying the rigid structure inside the protein and leading to an increase in flexibility, and the greater the breaking strength as the number of homogenizations increases. While only slightly change was observed while homogenized thrice and four times, it is because the SPI through non-covalent bond cleavage has reached saturation (Fig. 2).

Effect of homogenization pressures on the protein surface hydrophobicity

Hydrophobic interactions within the SPI molecule are the main forces that maintain their tertiary structure and are critical to the stability of the protein structure. It has been proposed that pressure leading to the alterations in the molecular structure of protein due to the cleavage between

Fig. 1 Flexibility of SPI under different homogeneous times treatment conditions. Different lowercase letters indicate significant differences among different homogeneous treatment conditions in flexibility ($p < 0.05$)

Fig. 2 Surface hydrophobicity of SPI under different homogeneous times treatment conditions. Different lowercase letters indicate significant differences among different homogeneous treatment conditions in surface hydrophobicity ($p < 0.05$)

the weak hydrogen bonds and intermolecular forces [27], probably with a molecular unfolding of the protein with the exposure of the hydrophobic groups to the medium. The hydrophobic groups that were originally hidden inside the SPI were gradually exposed to the outside as the homogeneous times increased, and the structure of the SPI became more stretched. The curves for the flexibility of proteins closely corresponded to the surface hydrophobicity curves, respectively. Since the surface hydrophobicity of protein increases with denaturation at the air–water interface when the proteins undergo high pressure homogenization treatment, so the emulsifying may be enhanced. High pressure treatment also improved the ability of soy proteins, particularly b-subunit of b-conglycinin and acidic subunit of glycinin, to be adsorbed at the oil/water interface of oiling–water (30/70) emulsions [17], therefore, the increased hydrophobicity of the SPI surface means that the emulsification activity also increases, and the importance of the surface hydrophobicity was consistent with that of flexibility as structural factors of the properties of proteins.

Effect of homogenization pressures on turbidity

As depicted in Fig. 3, the turbidity of the protein decreases along with the homogenization times increased, when the homogenized once, the turbidity drops rapidly compared with the no homogenized samples. This is because that the unhomogenized protein has a lot of macromolecule polymer protein deposited at the bottom, the protein solution was not uniform solution and the larger particles can be processed into the fine grain of stable emulsion or suspension under the high pressure homogenization treatment,

Fig. 3 Turbidity of SPI under different homogeneous times treatment conditions. Different lowercase letters indicate significant differences among different homogeneous treatment conditions in turbidity ($p < 0.05$)

and results in a decrease in turbidity of the solution. Cromwell considers turbidity to be related to the size and amount of aggregates in the solution [28]. Larger SPI particles can be processed into stable fine particles of emulsion or suspension as the turbidity of the solution decreases as the larger SPI particles are treated under high pressure and homogenization conditions. İbanoğlu [29] in the whey protein isolates also found similar results, the authors speculate that the protein molecules become smaller, easier to faster under high pressure in the interface adsorption, form a uniform and stable emulsion, namely the turbidity is reduced.

Effect of homogenization pressures on particle size distribution

In theory, the particle size distribution can comprehensively reflect the size and uniformity of the particles in the sample solution. Fernándezávila [30] found that ultra-high-pressure emulsions (100 and 200 MPa) exhibited low particle size and polydispersity, and Hebishy [31] also found that the increase of homogeneous pressure can generate more tiny droplets. As depicted in Fig. 4, the mean particle size of unhomogenized sample 948.5 nm, Under the condition of homogenization pressure of 120 MPa, when the number of homogenization times is 0–3, the size distribution curves were shifted to more minute sizes as the homogeneous times raised, indicating that the SPI protein molecule is dissociated into smaller molecules, which may be one of the reasons for the increase in flexibility, these results were in agreement with those obtained by determination of turbidity. When homogenized four times, the particle size shows an

Fig. 4 Particle size of SPI solution under different homogeneous times treatment conditions. Before measurements the SPI solution (5 mg/mL) were diluted to 2 mg/mL with phosphate buffer (0.2 mol/L, PH 7.0)

increasing trend and the increase is not obvious. This may be because the dissociated SPI molecules are partially re-aggregated. The aggregation may be due to high pressure causing protein unfolding and/or aggregation. By forming new disulfide bonds, complex aggregates are formed, resulting in aggregation of protein subunits [32], which is consistent with the changing trend of flexibility.

Effect of homogenization pressures on second-derivative ultraviolet (UV) spectroscopy

UV absorption of protein is mainly due to the absorption of ultraviolet light by the chromophore in the protein. The chromophore includes side chain groups of tryptophan and tyrosine residues. Based on the absorption of ultraviolet spectrum, it can be inferred Conformational changes in protein molecules [33], decrease in UV absorbance, indicating reduced UV-absorbing amino acid residues within the SPI molecule, as shown in Fig. 5, the UV absorption decreased as the increasing homogenization times, which indicated that the UV-absorbing amino acid residues in SPI (such as tyrosine acid residues, etc.) exposed to the surface of protein molecules due to homogenization. Chromophore group in which the environment happened from non-polar to polar changed, homogenization treatment promote the part of the peptide chain of protein molecules at the air–water surface, the conformational flexibility was enhanced. This was consistent with the trend of turbidity. This confirms the results of endogenous tryptophan fluorescence spectroscopy, and as the protein becomes more flexible, the SPI structure becomes more stretch.

Fig. 5 UV Spectra of SPI under different homogeneous times treatment conditions

Effect of homogenization pressure on emulsifying activity index (EAI) and emulsifying stability index (ESI)

As shown in Fig. 6, when the number of homogenization is 0–3 times, the emulsification activity increases with the increase of the number of homogenization, and then decreases with it; when the number of homogenization is 0–2 times, the emulsion stability The increase in the number of homogenizations increased, and then showed a downward trend. This is because the high-pressure homogenization allows the protein molecules to further expand at the air–water interface, the flexibility of the protein is enhanced, the original hydrophobic groups are exposed, increases the lipophilicity of SPI. The

Fig. 6 Emulsion properties of SPI under different homogeneous times treatment conditions. Different uppercase letters indicate significant differences among different homogeneous times in emulsifying stability. Different lowercase letters indicate significant differences of emulsifying activity in different homogeneous times ($p < 0.05$)

lipophilicity improves the emulsifying power of the protein. Wang et al. [34] found that high pressure treatment led to the unfolding of the globulin structure, along with the dissociation and repolymerization of the subunits, thereby significantly improving the processing properties such as emulsifying properties of globulin. Subirade et al. [35] found that the high-pressure treatment resulted in a significant change in the tertiary structure of β -lactoglobulin, and the emulsification properties of the protein were significantly improved, in which the flexibility and surface hydrophobicity of the protein were related to the trend of high pressure homogenization and emulsification. While in this study the flexibility and the surface hydrophobicity of protein increases with denaturation at the air–water interface when the proteins undergo high pressure homogenization treatment and results in good emulsifying. The conformational flexibility rather than the surface hydrophobicity of the protein was a crucial parameter determining their emulsifying Properties [36], so it is clear that the relationship between emulsifying activity and flexibility is more close than surface hydrophobicity when the SPI undergo high pressure homogenization treatment.

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