Effect of Homogenization at a Lower Pressure on Structural and Functional Properties of Soy Protein Isolate

Chaoyang Li¹, Zhongyou Dou¹, Ping Ma, Changyuan Wang^{*}, and Lianzhou Jiang^{*}

Heilongjiang Bayi Agricultural University, Daqing 163000, CHINA

¹ These authors contributed equally to this work.

Abstract: In this paper, the effects of homogenization at low pressure (1~40 MPa) on structural and functional properties of soy protein isolates (SPI) are investigated. Homogenization at low pressure increase solubility, surface hydrophobicity, emulsification activity and foaming capacity of SPIs, these all functional properties increases and then decreases with the homogenization pressure. Whereas, emulsion stability and foaming stability of SPIs treated by homogenization initially decrease and then increase with homogenization pressure. There is a dramatic decrease in hardness, springiness and cohesiveness of homogenized SPI gels. Generally, homogenization at low pressure do not change the subunit composition of SPIs. It is observed that, when the homogenization pressure is lower than 10 MPa than there is no significant impact on structural change. The content of β -sheet decreased, while unordered structure significantly increased, when the homogenization pressure increased from 10 MPa to 20 MPa. Furthermore, the content of β -sheet increases, when the content of the other structures decreases with the increasing homogenization pressure. The maximum emission wavelength ($\lambda_{\max}$) for SPIs increases with homogenization pressure increases from 10 Mpa to 20 Mpa, which is attributed to the gradual structural unfolding exposing more hydrophobic residues in protein surface. While, the decreased $\lambda_{\max}$ of SPIs treated with 20 Mpa to 40 Mpa homogenization corresponds to the protein aggregation. It can be deduced that appropriate selection of homogenization pressure is important for improving the functional properties of SPIs.

Key words: homogenization, low pressure, soy protein isolate, functional properties, structure

1 Introduction

Soy proteins are composed of two main storage globulins, β -conglycinin(7S) and glycinin(11S), and a small amount of soy whey proteins which constitutes about 9-15.3 g/100 g of soy seed protein¹. Soybean protein is an important kind of oilseed legume protein which has been widely used in food industry due to its excellent functional property and physiological activity, high nutritional value and low cost. The soybean protein is the powdered soybean protein frequently used as a meat substitute. To gain better properties, physicochemical and enzymatic modification, Great opportunities are offered in this field using physical methods to modify physicochemical properties of soy protein isolate. High-pressure homogenization is an important unit operation based on cavitation, which can improve extraction yields from soybean processing materials². Im-

provements in yields were found as a result of disruption of all intact storage cells, with a maximum total protein yield up to 94% reported for okara solution treatment with a single pass through a homogeniser at 100 MPa².

During high-pressure homogenization process, the protein undergoes high pressure, shear stress, and cavity explosion force, which leads to significant changes in protein structural and functional properties². High-pressure homogenization can change the three-dimensional structure of the protein in solution and even cause protein denaturation, aggregation, or gelation depending on the protein concentration, homogenization pressure, processing temperature and treated time. The intramolecular non-covalent bonds in proteins are destroyed by high-pressure homogenization, and then protein structure rearranged are determined by intramolecular and intermolecular forces³.

* Correspondence to: Changyuan Wang, Lianzhou Jiang; Changyuan Wang, College of Food Science, Heilongjiang Bayi Agricultural University, Daqing 163000, CHINA

E-mail: byndwcy@163.com (CW), jlz0109@neau.edu.cn (LJ)

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High-pressure homogenization treatment is safe and efficient and it is an important processing program for food processing. It can reduce the defects such as, nutrient loss and flavor change in traditional processing methods. It can be used for continuous industrial operation and more conducive to industrial food production. It is considered as one of the most potential and future development prospects in food processing methods.

To achieve required properties, many physical, chemical and enzymatic modifications have been applied to soy proteins. Recently, the application of high-pressure (HP) to modify the properties of food proteins has become an area of considerable research interests. Masson *et al.*⁴⁾ found that the high strength mechanical effect of high pressure homogeneity induced the structure recombination and protein aggregation and even partial precipitation. Molina *et al.*⁵⁾ found that high pressure homogenization improves the functional properties of proteins through inducing denaturation of protein molecule by mechanical energy and then changing the network conformation. Flourey *et al.*³⁾ found that high-pressure homogenization increases the denaturation temperature of protein, which indicates that homogenization also increase the stability of protein structure. Subirade *et al.*⁶⁾ assumed that high-pressure homogenization significantly changes the tertiary structure of β -lactoglobulin, and then affects its functional properties, however homogenization did not significantly influence its secondary structure. Torrezan *et al.*⁷⁾ found that the acidity and alkalinity of the solution had a significant/substantial effect on the solubility of the SPI treated by homogenization, the solubility of soy protein isolate (SPI) in acidic pH was lower than that in neutral pH. The modification of high pressure homogenization on structural and functional properties of SPI has been widely studied, still, homogenization at low pressure is always used in preparation of SPI. No detailed report revealed about the effects of homogenization at low pressure on structural and functional properties of SPI.

Thus, the objective of this study is to investigate the impact of homogenization at low pressure on structural and functional properties of SPI.

2 Materials and Methods

2.1 Materials

Defatted soybean seed flour, commercially produced during the production of soy oil by low-temperature technology, provided by XIANGCHI Cereal and Oil Co. Ltd. (Shandong Province, China). Analytical chemicals and reagents purchased from Sigma Chemical Corp. (MO, USA).

2.2 Preparation of SPI

50 g of soybean flour defatted by using hexane (1:3 w/v)

for 3-times and then dispersed in 1000 mL deionized water. The pH of the dispersion then adjusted to 8.0 with 0.5 M NaOH, followed by precipitation at pH 4.5 with 0.5 M HCl. The precipitate adjusted to pH 7.0 with 0.5 M NaOH and lyophilized. Protein content of SPI determined by performing the Kjeldahl method ($N \times 6.25$).

2.3 Protein solubility

Protein solubility of SPI is determined according to the method proposed by Ortiz *et al.*⁸⁾, with minor modification. Protein solubility expressed by nitrogen solubility index (NSI). 100 mg of SPI are dispersed and stirred in 10 mL distilled water at room temperature for about 1 hour. Then, the dispersions centrifuged at 10,000 g for about 30 min at 20°C. The protein content in the supernatant was measured by micro-Kjeldahl method ($N \times 6.25$).

2.4 Particle size distribution and ξ -potential

Particle size and ξ -potential of SPIs were measured by using a back-scattered quasi-elastic light scattering device (Malvern Mastersizer 2000 equipped with a 100 mm lens, Malvern Instruments Limited, Malvern-Worcestershire, UK with Hydro MU sample dispersion unit). Particle size distribution (PSD) determined from the velocity distribution of particles suspended in a dispersing medium, using the principles of dynamic light scattering. The power spectrum of the interference signal calculated by using a high-speed fast Fourier transform digital signal processor and inverted to give PSD. The fact that, the signal is taken at an angle of 180° from the original beam to make it possible to analyze turbidity with sizes ranging from 0.8 to 6.5 microns. The ξ -potential by measuring the response of charged particles in an electric field. In a constant electric field, particles drift at a constant velocity. Through the velocity, the charge and zeta potential can be determined.

2.5 Surface hydrophobicity

Surface hydrophobicity (H_0) of SPI determined through the hydrophobicity fluorescence probe 1-anilino-8-naphthalenesulfonate (ANS). SPI samples were dispersed into buffer solution (0.01 M phosphate buffer, pH 7.0) to reach different level of concentrations (0.004-0.02%, w/v), followed by stirring at 4°C overnight. After centrifugation (12,000 g, 20 min, 20°C), the supernatants are collected. Then, 20 mL ANS (8 mM in the same buffer) added to 4 mL of sample. Fluorescence intensity (FI) measured at wavelengths 390 nm (excitation) and 470 nm (emission) using a RF-5301 PC spectro-fluorometer (Shimadzu Corp., Kyoto, Japan) at 26°C, with a constant excitation and emission slit of 5 nm. The initial slope of fluorescence intensity versus protein concentration (mg/mL) is calculated through linear regression analysis and used as an index of H_0 ⁹⁾.

2.6 Emulsifying properties

The emulsion properties are determined from the method presented by Pearce & Kinsella¹⁰. The absorbance of the resulting dispersion measured at 500 nm. The emulsion activity index (EAI) and the emulsion stability index (ESI) calculation is performed by using the formula given below. At 30 minutes, the emulsion stability of the sample is taken as the absorbance. The percentage EAI and ESI calculated, according to the following equation:

$$EAI(m^2/g) = 2T \frac{A_0 \times N}{C \times \phi \times 10000}$$

$$ESI(\text{min}) = \frac{A_0}{A_0 - A_{30}} \times 30$$

where, T , A_0 , C , N and ϕ represent turbidity, absorbance immediately after emulsion formation ($t = 0$ min), initial concentration of protein 1 g/100 mL, dilution factor, and oil fraction, respectively.

2.7 Foaming properties

Foaming capacity and stability of SPI are determined according to the method proposed by Sathe and Salunkhe¹¹. Twenty milliliters of 0.5% sample solution, adjusted to pH 2, 4, 6, 8 and 10, followed by homogenization at a speed of 16,000 rpm, using a homogenizer (IKA Labortechnik, Selangor, Malaysia) to incorporate the air for 2 min at room temperature. The whipped sample, immediately transferred to a 25 mL cylinder and the total volume was noted after 30 s. The foaming capacity calculation is performed according to the following equation:

$$\text{Foaming capacity (\%)} = \frac{A - B}{B} \times 100$$

Where A is the volume after whipping (mL); B is the volume before whipping (mL).

The whipped sample could stand at 20°C for 3 min and the volume of whipped sample was then recorded. Foam stability was calculated as follow:

$$\text{Foaming stability (\%)} = \frac{A - B}{B} \times 100$$

Where A = volume after standing (mL), B = volume before whipping (mL).

2.8 Textural properties

The textural characteristic of SPI gels in a compression mode was analyzed according to texture profile analysis (TPA; Bourne, 1978) using a TA-XT2 Texture Analyzer (Stable Micro Systems Ltd., England). The gel samples (5 mL) were directly formed from the mixtures of protein solutions and enzyme in sealed cylinders (20 × 25 mm), by incubation at 37°C for 4 hours. Before performing the measurements, the gel samples were pre-cooled in an ice bath to room temperature. The samples were compressed twice with a cylinder probe up to 50% of their original height at a constant cross-head speed of 1 mm/s. From the TPA curves, the following texture parameters were obtained:

hardness at 50% of deformation, springiness, cohesiveness, adhesiveness, gumminess and chewiness. Hardness is defined as a peak force (g) during the first compression cycle. Fracture ability is defined as the force (g) required to produce the first fracture during the first compression. Springiness is defined as a ratio of the time recorded between the start of the second area and the second probe reversal to the time recorded between the start of the first area and the first probe reversal. Cohesiveness is calculated as the ratio of the area under the second curve to the area under the first curve (dimensionless). Gumminess is obtained by multiplying hardness and cohesiveness. Chewiness is defined as the direct multiplication product of hardness, cohesiveness and springiness.

2.9 Fourier transform infrared spectroscopy

The soybean protein samples, were analyzed by Fourier transform infrared spectroscopy (FT-IR) using a Perkin-Elmer Spectrum 100 FTIR spectrometer (PerkinElmer, Waltham, MA, USA). The FT-IR spectra recorded with 64 scans at 4 cm⁻¹ resolution from 1700 to 1600 cm⁻¹. The parameters of deconvolution are set with a gamma value of 2.5 and a smoothing length of 70%.

2.10 Fluorescence spectroscopy

SPI samples (500 mg) suspended in 100 mL 10 mM sodium phosphate buffer (pH 7.0) and these samples magnetically stirred thoroughly at room temperature. Then, the SPI suspension centrifuged at 10,000 g for 15 min and collected the supernatant. And, the protein concentration in the supernatant determined, by using a BCA protein assay kit. Then, protein concentration was separately adjusted to 0.1 mg/mL and 0.2 mg/mL for the measurement of tryptophan. The excitation wavelength set at 285 nm, and the emission spectrum was recorded from 300 to 400 nm. The slit width set at 5 nm, and data were collected at 240 nm/min.

2.11 Statistical analysis

All the experiments conducted in triplicate and results are presented as mean ± standard deviation. Means are then compared using ANOVA followed by Duncan's test ($p < 0.05$). Statistical and chart analysis is performed using Origin 9.1 software.

3 Results and Discussion

3.1 Effect of homogenization at low pressure on solubility of SPI

Solubility is one of the most important functional properties of proteins, since it has impacts on other functional properties¹². In many protein-based formulations, for instance emulsions, solubility of the protein is usually re-

Fig. 1 Effect of homogenization at a low pressure on the solubility of SPI.

quired¹³⁾.

The results presented in Fig. 1 indicates that NSI of native SPI is 66.86%, which increases and then decreases with the homogenization pressure, while homogenization at low pressure generally increase the solubility of SPI. The decreased particle size and increased enacted area with water through hydrogen bonds and electrostatic interactions, results in increasing solubility of SPI due to the shearing and impact force of homogenization¹⁴⁾. In addition, more charged residues exposed increasing the electrostatic repulsion, preventing the aggregation of protein particle. Unfolding of protein structure increases the formation of protein aggregation, and then decreasing the solubility of SPI treated with a homogenization higher than 20 MPa. At pressures up to 8 MPa, NSI monotonously increases

while surface hydrophobicity, emulsifying activity, foaming properties are constant. But it is consistent with the trend of random coil and α -helix. It may be that homogenization changes the flexible range of protein, which leads to the increase of NSI.

3.2 Effect of homogenization at low pressure on particle size of SPI

Table 1 shows the particle size distributions of SPIs treated by homogenization at low pressure. The Dx(10) is representative of the smaller particle in SPI, while the Dx(90) is related to the larger particle in SPI, especially the protein aggregation. The average particle size of SPI treated by homogenization initially decreased, and then significantly increased with the homogenization pressure ($p < 0.05$). The Dx(10) and Dx(90) of SPIs treated by homogenization both initially decreased, and then increased with the homogenization pressure. It can be deduced that homogenization force decreases the particle size of SPI due to the impact force of homogenization shearing larger particle aggregation into smaller particle. The increase in particle size with homogenization pressure increment could be explained that a rise in the number of soy protein macromolecules increases the probability when they stay together, promoting aggregation phenomenon¹⁵⁾.

3.3 Effect of homogenization at low pressure on ξ -potential of SPI

The ξ -potential of SPIs with homogenization treatment are listed in Fig. 2. The absolute ξ -potential value of SPIs treated by homogenization are higher than native SPI, while the absolute ξ -potential value of SPIs initially increases and then decreases with homogenization pressure.

Table 1 Effect of homogenization at a low pressure on particle size distribution of SPI.

Homogenization pressure	Dx (10)	Dx (50)	Dx (90)	D _[4,3]
0 MPa	75.80 $\pm$ 1.02a	303.43 $\pm$ 0.94a	599.42 $\pm$ 0.78a	325.53 $\pm$ 0.66a
1 MPa	68.52 $\pm$ 2.43b	273.22 $\pm$ 2.34b	577.56 $\pm$ 2.19b	300.46 $\pm$ 2.76b
2 MPa	63.01 $\pm$ 2.23c	257.32 $\pm$ 3.02c	508.89 $\pm$ 1.98c	277.52 $\pm$ 3.20c
5 MPa	62.52 $\pm$ 1.30d	251.23 $\pm$ 0.87d	498.23 $\pm$ 0.97d	270.89 $\pm$ 2.01d
8 MPa	53.23 $\pm$ 1.48f	238.35 $\pm$ 2.20e	473.32 $\pm$ 2.43e	256.98 $\pm$ 2.56e
10 MPa	52.91 $\pm$ 2.39g	233.10 $\pm$ 3.02f	452.56 $\pm$ 3.30f	249.03 $\pm$ 1.54f
15 MPa	52.83 $\pm$ 3.31g	215.24 $\pm$ 2.09g	416.89 $\pm$ 2.67g	229.46 $\pm$ 3.05g
20 MPa	38.44 $\pm$ 2.46h	140.21 $\pm$ 1.88j	272.77 $\pm$ 2.08j	150.33 $\pm$ 0.87ia
30 MPa	57.27 $\pm$ 3.42e	191.87 $\pm$ 2.99h	374.67 $\pm$ 3.07i	207.02 $\pm$ 2.40h
40 MPa	62.28 $\pm$ 2.57d	189.78 $\pm$ 2.19i	380.98 $\pm$ 1.39h	209.32 $\pm$ 2.57h

Note : Comparisons were carried out between values of the same column; values with different letter(s) indicate a significant difference at $p \leq 0.05$. Dx (10): 10% of the particles fell below specified diameter; Dx (50): 50% of the particles fell below specified diameter; Dx (90): 90% of the particles fell below specified diameter. D_[4,3]: Volume weighted mean.

Fig. 2 Effect of homogenization at a low pressure on ξ -potential of SPI.

It has been previously reported that the ξ -potential value of protein is related to the disaggregation¹⁶⁾. The increased absolute ξ -potential value of SPIs treated with lower homogenization are related to subunit dissociation, while the decreased absolute ξ -potential value of SPIs treated with higher homogenization are due to the protein aggregation.

3.4 Effect of homogenization at low pressure on surface hydrophobicity of SPI

Surface hydrophobicity is an index number of hydrophobic groups on the surface of a protein molecule which are in contact with the polar aqueous environment and is closely related to its functional properties. As depicted shown in Fig. 3, It can be observed that no significant change in surface hydrophobicity of SPIs, when treated with 0-8 MPa homogenization. Meanwhile, the increase in surface hydrophobicity of SPI, when treated with 8-20 MPa homogenization is related to the unfolding in protein structure. Molina *et al.*¹⁷⁾ found that the exposure of surface residues are related to the increased surface hydrophobicity under high pressure. Surface hydrophobicity of SPIs decreases with homogenization pressure when it is up to 20 MPa. Wang *et al.*¹⁸⁾ reported that exposed protein might be reaggregated under 600 MPa with a decreased surface hydrophobicity. The decreased surface hydrophobicity of SPIs in this research can be related to the reaggregation.

3.5 Effect of homogenization at low pressure on emulsifying activity and stability of SPIs

Proteins are of great interest due to their amphiphilic nature, which allows them to reduce the interfacial tension at the oil-water interface. The amalgamation of proteins at the oil-water interface has allowed scientists to utilize them to form emulsions (O/W or W/O), which can be used in food formulations, drug and nutrient delivery. The sys-

Fig. 3 Effect of homogenization at a low pressure on the surface hydrophobicity of SPI.

Fig. 4 Effects of homogenization at a low pressure on emulsifying activity and emulsification stability of SPI.

tematic study of the proteins at the interface and the factors that affect their stability (i.e., conformation, pH, solvent conditions, and thermal treatment) has allowed for a broader use of these emulsions tailored for various applications¹⁹⁾. Emulsions, from the physicochemical point of view, are thermodynamically unstable systems rapidly or slowly separating into two immiscible phases according to the kinetic stability²⁰⁾.

Emulsifying properties can be evaluated/assessed by the protein's emulsifying stability (ESI) and emulsifying activity (EAI). The ESI is a measure of the stability of the emulsion over a certain time span and EAI is a measurement of how much oil, a protein can emulsify per unit protein²¹⁾. As shown in Fig. 4, the emulsification activity of SPIs treated by homogenization increase with pressure. The slightly increased emulsification activity is related to the increased solubility of SPIs when treated by homogenization at a

pressure lower than 10 MPa. It has been reported that SPI shows great emulsifying properties which is related to its high solubility and high protein content²²⁾. A positive correlation between the solubility and emulsifying capacity of proteins has been reported²³⁾.

While the increased emulsification activity of SPIs when treated by 10~20 MPa's homogenization is mainly due to the exposure of hydrophobicity residues led by the structure unfolding. When, the homogenization pressure increases from 20 MPa to 40 MPa, the emulsification activity slightly decreases due to the protein aggregation. The emulsion stability of SPIs initially decrease and then increase, when treated by/with homogenization. Emulsification activity is a function of protein hydrophobicity and solubility, while emulsion stability is related to the total concentration of proteins. The homogenization at high pressure can lead to the formation of protein aggregates. Protein aggregation may lead to the decrease of solubility and the change of protein interfacial activity, resulting in the decrease of emulsifying activity and emulsifying stability.

3.6 Effect of homogenization at low pressure on foaming properties and foaming stability of SPIs

The property of proteins to form stable foams is important to produce a variety of foods. Foam can be defined as a two-phase system consisting of air cells that are separated by a thin continuous liquid layer called the lamellar phase. Food foams are usually consisting of very complex systems, including a mixture of gases, liquids, solids, and surfactants. The size distribution of air bubbles in foam influences the foam product's appearance and textural properties; foams with a uniform distribution of small air bubbles imparts body, smoothness, and lightness to the food. Proteins in foams contribute to the uniform distribution of fine air cells in the structure of foods. Body and smoothness of food foams is related to the formation of air bubbles that allow volatilization of flavors with enhanced palatability of the foods²⁴⁾.

As depicted in Fig. 5, the homogenization at a low pressure significantly affects the foaming properties and foaming stability of SPIs. The foaming properties of SPIs increase slightly when treated by 1-8 MPa's homogenization, but increase significantly at 8-20 MPa's homogenization and keeps high foaming properties with 20-40 MPa's homogenization. It has reported that the increase in surface hydrophobicity is considered a decisive factor in the improved foam ability and hydrophobic interactions improved the foam stability through the rapid formation of a viscoelastic film²⁵⁾. Thus, in our present research, low pressure homogenization increase foaming properties of SPIs may be related to the increased surface hydrophobicity. It has also reported that the homogenization pressure incited a high shearing force may weaken up hydrophobicity inter-

Fig. 5 Effect of homogenization at a low pressure on foaming capability and foaming stability of SPI.

action, and ionic bond between proteins, increasing the flexibility of protein molecule, leading to fast adsorption onto air/water interface to form a more viscoelastic film at the interface via molecular rearrangement, and then keep a better foaming property²⁶⁾. Homogenization at low pressure, generally decreased the foaming stability of SPI, and the foaming stability initially decrease and then increase with homogenization pressure. Homogenization can improve the interfacial activity by changing the arrangement and hydrophobicity of protein molecules, thus enhancing the foaming activity. At pressures up to 10 MPa, emulsifying stability and foaming stability decreases. It may be that the protein with small particle size is not conducive to the stability of interfacial activity under the condition of constant hydrophobicity.

3.7 Effect of homogenization at low pressure on TPA properties of SPI

The TPA results of finally formed gels of SPI are shown in Fig. 6. The hardness is related to the strength of the gel structure under compression. Springiness (sometimes also called as "elasticity") is a measure of how much the gel structure is broken down by the initial compression. High springiness will results, when the gel structure is broken into few large pieces during the first TPA compression, whereas low springiness results from the gel breaking into many small pieces²⁷⁾. The cohesiveness is a measure of the degree of difficulty in breaking down in the gel's internal structure²⁷⁾. There is a dramatic decrease in hardness, springiness and cohesiveness of homogenized SPI gels, which suggests that homogenization is unfavorable for the gel formation and strength development of SPI gels. It may be that homogenization at very low pressure can only lead to disorder of the ordered structure of protein, but not to the orderly rearrangement. It has been studied that the hardness of protein gels increased with increasing aggre-

Fig. 6 Effect of homogenization at a low pressure on TPA properties of SPI.

Table 2 Effect of homogenization at a low pressure on secondary structure of SPI.

Homogenization pressure	α -helix (%)	β -sheet (%)	β -turn (%)	random coil (%)
0 MPa	13.60 $\pm$ 0.02h	42.50 $\pm$ 0.07a	19.53 $\pm$ 0.09c	24.37 $\pm$ 0.03i
1 MPa	14.34 $\pm$ 0.07g	41.40 $\pm$ 0.08b	19.46 $\pm$ 0.07d	24.80 $\pm$ 0.03g
2 MPa	14.62 $\pm$ 0.11f	41.23 $\pm$ 0.17c	19.58 $\pm$ 0.07c	24.47 $\pm$ 0.04h
5 MPa	15.11 $\pm$ 0.07 e	40.47 $\pm$ 0.09d	19.43 $\pm$ 0.03e	24.99 $\pm$ 0.15f
8 MPa	15.90 $\pm$ 0.03a	40.26 $\pm$ 0.11e	18.55 $\pm$ 0.09h	25.34 $\pm$ 0.09g
10 MPa	15.21 $\pm$ 0.03d	37.52 $\pm$ 0.16g	19.59 $\pm$ 0.11c	27.68 $\pm$ 0.00d
15 MPa	15.81 $\pm$ 0.09b	35.30 $\pm$ 0.12i	20.00 $\pm$ 0.08b	28.89 $\pm$ 0.02b
20 MPa	15.80 $\pm$ 0.11b	33.23 $\pm$ 0.12j	20.90 $\pm$ 0.08a	30.07 $\pm$ 0.02a
30 MPa	15.35 $\pm$ 0.08c	36.91 $\pm$ 0.04h	19.35 $\pm$ 0.06f	28.49 $\pm$ 0.05c
40 MPa	15.21 $\pm$ 0.02d	39.22 $\pm$ 0.03f	18.89 $\pm$ 0.12g	26.78 $\pm$ 0.05e

Note : Comparisons were carried out between values of the same column; values with different letter(s) indicate a significant difference at $p \leq 0.05$.

gate size and degree of protein²⁸). Thus, decreased aggregate size of SPI treated by homogenization account for the decreased hardness. The springiness of SPI gels initially increased and then decreased with homogenization pressure, the more exposed hydrophobic groups account for the increased springiness, while the reformed insoluble aggregate at higher homogenization pressure decreases the springiness of SPI gels.

3.8 Effect of homogenization at low pressure on secondary structure of SPIs

The quantitative analysis of secondary structural components of proteins can be obtained through various experimental methods. The areas of assigned amide-I bands in the second derivative spectra corresponds linearly to the amount of the different types of secondary structures present in the protein. In present study, our second derivative band positions is similar to previous data from the literature that has reported a strong band for α -helix with a

frequency around 1650-1660 cm^{-1} ¹⁸⁾. We also obtained several bands corresponding to β -sheet in the frequency region of 1618-1640 cm^{-1} and 1670-1690 cm^{-1} . A series of bands corresponding to β -turn appeared in the 1660-1700 cm^{-1} range. The random coil structure had a strong band close to 1645 cm^{-1} ²⁹⁾. The percentages of α -helix, β -sheet, unordered and β -turn secondary structures in SPC are shown in Table 2.

The predominant secondary structure of SPI is β -sheet, confirming data reported in earlier studies³⁰⁾. The secondary structure of SPI is remained under a 1-8 MPa's homogenization, meanwhile the content of β -sheet partly decreases, and the content of unordered structure slightly increases. The content of β -sheet decreases and that of unordered structure significantly increases when the homogenization pressure increases from 10 MPa to 20 MPa, while the content of α -helix and β -turn slightly decreases. Stathopoulos *et al.*³¹⁾ has previously reported that the decreased content of β -sheet was related to the more exposed hydrophobic residues, the decreased β -sheet structure content of the protein was often accompanied by the increased surface hydrophobicity. The content of β -sheet then increased with the content of the other structures decrease when the homogenization pressure increased from 20 MPa to 40 MPa. The decreased content of β -sheet is related to the formation of soluble protein aggregation. Lee *et al.*³²⁾ has reported that the increased content of β -sheet was related to the formation of protein aggregation.

3.9 Effect of homogenization at low pressure on tertiary structure of SPIs

Fluorescence spectroscopy is a well-established technique to observe protein conformational changes under different micro-environmental conditions. In soy protein, two aromatic amino acid residues, tryptophan (Trp) and tyrosine (Tyr), makes the major contribution to the ultraviolet fluorescence of protein. The peak position is in between 325–350 nm. The intrinsic fluorescence spectrum measured with an excitation wavelength at 290 nm is chiefly based on the polarity of the environment around Trp residues, thus serving as a sensitive indicator of the conformational changes of protein. The maximum emission wavelength (λ_{max}) moves to a red shift, when the Trp residues are exposed to a more polar environment. A red shift is always used as an indicator of protein structure unfolding.

When using Trp fluorescence λ_{max} information, a Trp is assigned as being buried and in a “nonpolar” environment if λ_{max} is ≤ 330 nm; if λ_{max} is > 330 nm, the Trp is assigned to be in a “polar” environment³³⁾. No significant change is observed in the λ_{max} for SPIs when treated by 1-8 MPa homogenization (Fig. 7), which suggests the tertiary structure is maintained. The λ_{max} for SPIs increased with ho-

Fig. 7 Effect of homogenization at a low pressure on fluorescence spectra of SPI.

mogenization pressure from 10 MPa to 20 MPa, which is attributed to the gradual structural unfolding exposing more hydrophobic residues to protein surface. While, the decreased λ_{max} of SPIs treated with 20 MPa to 40 MPa homogenization corresponds to the protein aggregation.

4 Conclusion

This research concluded that homogenization at low pressure increases solubility, surface hydrophobicity, emulsification activity and foaming capacity of SPI, but decreases the emulsion stability, foaming stability and gels properties. Homogenization, at low pressure did not change the subunit composition of SPIs, while homogenization at the pressure lower than 10 MPa did not change the structure of SPIs. The structural change of SPI confirms that the gradual structural unfolding, when the homogenization pressure increases from 10 MPa to 20 MPa, while SPI tend to aggregate, when the homogenization increases from 20 MPa to 40 MPa. It can be deduced that appropriate selection of homogenization pressure is important to improving the functional properties of SPIs.

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References

- 1) Li, X.; Dong, D.; Hua, Y.; Chen, Y.; Kong, X.; Zhang, C. Soybean whey protein/chitosan complex behavior and

- selective recovery of Kunitz trypsin inhibitor. *J. Agric. Food Chem.* **62**, 7279-7286 (2014).
- 2) Preece, K.E.; Hooshyar, N.; Krijgsman, A.J.; Fryer, P.J.; Zuidam, N.J. Intensification of protein extraction from soybean processing materials using hydrodynamic cavitation. *Innov. Food Sci. Emerg. Technol.* **41**, 47-55 (2017).
- 3) Floury, J.; Desrumaux, A.; Axelos, M.A.; Legrand, J. Degradation of methylcellulose during ultra-high pressure homogenisation. *Food Hydrocoll.* **16**, 47-53 (2002).
- 4) Masson, D.; Tschopp, J. Isolation of a lytic, pore-forming protein (perforin) from cytolytic T-lymphocytes. *J. Biol. Chem.* **260**, 9069-9072 (1985).
- 5) Molina, E.; Defaye, A.B.; Ledward, D.A. Soy protein pressure-induced gels. *Food Hydrocoll.* **16**, 625-632 (2002).
- 6) Subirade, M.; Kelly, I.; Gueguen, J.; Pezolet, M. Molecular basis of film formation from a soybean protein: comparison between the conformation of glycinin in aqueous solution and in films. *Int. J. Biol. Macromol.* **23**, 241-249 (1998).
- 7) Torrezan, R.; Tham, W.P.; Bell, A.E.; Frazier, R.A.; Cristianini, M. Effects of high pressure on functional properties of soy protein. *Food Chem.* **104**, 140-147 (2007).
- 8) Ortiz, S.E.M.; Puppo, M.C.; Wagner, J.R. Relationship between structural changes and functional properties of soy protein isolates-carrageenan systems. *Food Hydrocoll.* **18**, 1045-1053 (2004).
- 9) Hou, D.H.; Chang, S.K.C. Structural characteristics of purified glycinin from soybeans stored under various conditions. *J. Agric. Food Chem.* **52**, 3792-3800 (2004).
- 10) Pearce, K.N.; Kinsella, J.E. Emulsifying properties of proteins: Evaluation of a turbidimetric technique. *J. Agric. Food Chem.* **26**, 716-723 (1978).
- 11) Sathe, S.; Salunkhe, D. Functional properties of the great northern bean (*Phaseolus vulgaris* L.) proteins: Emulsion, foaming, viscosity, and gelation properties. *J. Food Sci.* **46**, 71-81 (1981).
- 12) Sikorski, Z.E.; Kolakowska, A. *Chemical and functional properties of food lipids*. CRC press (2010).
- 13) Hayakawa, S.; Nakai, S. Relationships of hydrophobicity and net charge to the solubility of milk and soy proteins. *J. Food Sci.* **50**, 486-491 (1985).
- 14) Puppo, M.C.; Speroni, F.; Chapleau, N.; de Lamballerie, M.; Anon, M.C.; Anton, M. Effect of high-pressure treatment on emulsifying properties of soybean proteins. *Food Hydrocoll.* **19**, 289-296 (2005).
- 15) Sponton, O.E.; Perez, A.A.; Carrara, C.R.; Santiago, L.G. Impact of environment conditions on physicochemical characteristics of ovalbumin heat-induced nanoparticles and on their ability to bind PUFAs. *Food Hydrocoll.* **48**, 165-173 (2015).
- 16) Song, X.; Zhou, C.; Fu, F.; Chen, Z.; Wu, Q. Effect of high-pressure homogenization on particle size and film properties of soy protein isolate. *Ind. Crops Prod.* **43**, 538-544 (2013).
- 17) Molina, E.; Papadopoulou, A.; Ledward, D.A. Emulsifying properties of high pressure treated soy protein isolate and 7S and 11S globulins. *Food Hydrocoll.* **15**, 263-269 (2001).
- 18) Wang, X.-S.; Tang, C.-H.; Li, B.-S.; Yang, X.-Q.; Li, L.; Ma, C.-Y. Effects of high-pressure treatment on some physicochemical and functional properties of soy protein isolates. *Food Hydrocoll.* **22**, 560-567 (2008).
- 19) Lam, R.S.H.; Nickerson, M.T. Food proteins: A review on their emulsifying properties using a structure-function approach. *Food Chem.* **141**, 975-984 (2013).
- 20) Comas, D.I.; Wagner, J.R.; Tomas, M.C. Creaming stability of oil in water (O/W) emulsions: Influence of pH on soybean protein-lecithin interaction. *Food Hydrocoll.* **20**, 990-996 (2006).
- 21) Boye, J.; Zare, F.; Pletch, A. Pulse proteins: Processing, characterization, functional properties and applications in food and feed. *Food Res. Int.* **43**, 414-431 (2010).
- 22) Gwiazda, S.; Rutkowski, A.; Kocoń, J. Some functional properties of pea and soy bean protein preparations. *Mol. Nutr. Food Res.* **23**, 681-686 (1979).
- 23) Felix, A.; Hill, R.; Diarra, B. *In vitro* and *in vivo* digestibility of soya-bean straw treated with various alkalis. *Anim. Prod.* **51**, 47-61 (1990).
- 24) German, J.; O'Neill, T.; Kinsella, J. Film forming and foaming behavior of food proteins. *J. Am. Oil Chem. Soc.* **62**, 1358 (1985).
- 25) Moro, A.; Báez, G.D.; Busti, P.A.; Ballerini, G.A.; Delorenzi, N.J. Effects of heat-treated β -lactoglobulin and its aggregates on foaming properties. *Food Hydrocoll.* **25**, 1009-1015 (2011).
- 26) Dickinson, E. Protein adsorption at liquid interfaces and the relationship to foam stability. in *Foams: physics, chemistry and structure*. Springer, pp. 39-53 (1989).
- 27) Lau, M.H.; Tang, J.; Paulson, A.T. Texture profile and turbidity of gellan/gelatin mixed gels. *Food Res. Int.* **33**, 665-671 (2000).
- 28) Alting, A.C.; Hamer, R.J.; de Kruif, C.G.; Paques, M.; Visschers, R.W. Number of thiol groups rather than the size of the aggregates determines the hardness of cold set whey protein gels. *Food Hydrocoll.* **17**, 469-479 (2003).
- 29) Boecker, U.; Ofstad, R.; Bertram, H.C.; Egelanddal, B.; Kohler, A. Salt-induced changes in pork myofibrillar tissue investigated by FT-IR microspectroscopy and light microscopy. *J. Agric. Food Chem.* **54**, 6733-6740 (2006).

- 30) Rampon, V.; Robert, P.; Nicolas, N.; Dufour, E. Protein structure and network orientation in edible films prepared by spinning process. *J. Food Sci.* **64**, 313-316 (1999).
 - 31) Stathopoulos, P.B.; Scholz, G.A.; Hwang, Y.M.; Rumfeldt, J.A.O.; Lepock, J.R.; Meiering, E.M. Sonication of proteins causes formation of aggregates that resemble amyloid. *Protein Sci.* **13**, 3017-3027 (2004).
 - 32) Yu, S.N.; Mei, F.C.; Lee, J.C.; Cheng, X.D. Probing cAMP-dependent protein kinase holoenzyme complexes I alpha and II beta by FT-IR and chemical protein footprinting. *Biochemistry* **43**, 1908-1920 (2004).
 - 33) Vivian, J.T.; Callis, P.R. Mechanisms of tryptophan fluorescence shifts in proteins. *Biophys. J.* **80**, 2093-2109 (2001).
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