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Preparation and functional properties of protein from heat-denatured soybean meal assisted by steam flash-explosion with dilute acid soaking

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ABSTRACT

The combined pretreatment of heat-denatured soybean meal using steam flash-explosion (SFE) with sulfuric-acid soaking was investigated to prepare protein from soybean meal. When soybean meal was pretreated by SFE at 1.8 MPa, 2.2 MPa for 8 min and at 2.0 MPa for 8 min and 10 min, combined with 0.9% sulfuric-acid soaking, the extraction yield of protein increased to 67.72%, 70.54%, 69.47% and 71.21% respectively, compared to untreated soybean meal. Scanning electron micrograph of pretreated samples showed the structural disruption of soybean meal. After pretreatment, the protein yield was improved, while protein content of soy protein isolate (SPI) decreased slightly. The functional properties of SPI from pretreated soybean meal were all improved compared to untreated soybean meal and the relationship between functional properties and the changes of surface hydrophobicity of SPI was discussed. The emulsification properties and fat-binding capacity of pretreated SPI were superior to those of SPI prepared from white flakes.

1. Introduction

Soybean meal contains approximately 50% protein. In soybean oil extraction, soybean specially undergoes high-temperature thermal processing for removal of solvent resident and deactivation of anti-nutritional factors (Refstie et al., 2005). Thermal processing denatures and insolubilizes soy protein rendering it poorly functional in foods. If the heat-denatured soybean protein could be refunctionalized, the more benefits of soybean meal can be realized. However, the extraction of high protein yields from high-temperature treated soybean meal is greatly difficult, due to protein denaturation and its location inside the soybean structure. Enzymatic treatment can acquire high protein extraction yield (89–94%) from heat-treated soybean meal (Fischer et al., 2001), but most proteins are converted into peptides and amino acids, resulting in loss of functions for application. Hydrothermal cooking (HTC) and alkali-HTC can increase the extraction of protein from extruded-expelled soybean meal (Wang et al., 2004, 2005). Subcritical water hydrolysis has achieved 46.98% of original protein from soybean meal (Watchararuji et al., 2008). Nevertheless, these methods are based on violent conditions, leading to protein degradation and the loss of processing function.

Steam flash-explosion (SFE) is an innovative method for pretreatment of biomass. This method is based on exposing the biomass to high-temperature pressurized steam and forcing the steam into the tissues and cell of biomass, followed by explosive decompression completed in millisecond (Yu et al., 2012). During the explosion, most of the steam and hot liquid water in the biomass quickly expands and breaks free of the structure. In the previous publication, the results showed that SFE could significantly improve the extraction yield and functional properties of protein from heat-denatured soybean meal (Zhang et al., 2013). However, the particle size of soybean meal must pass through a 20-mesh screen, but not a 80-mesh screen, which limit the application of SFE. It is found that when stem pressure and residence time exceed

Abbreviations: SPI, soy protein isolate; WFs, white flakes; SFE, steam flash-explosion; HTC, hydrothermal cooking; SEM, scanning electron microscopy; ANS, 1-anilino-8-naphthalenesulfonate; SDS, sodium dodecyl sulfate; EAI, emulsifying activity index; ESI, emulsion stability index; FBC, fat-binding capacity; FC, foaming capacity; FS, foaming stability; ANOVA, analysis of variance; LSDs, least significant differences.

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1.8 MPa and 120 s, the protein extraction of soybean meal reached plateau, despite the higher pressure and time. The improvement of mechanical shearing force and tissue damage of soybean can solve the above-mentioned problem.

Dilute sulfuric acid pretreatment is an effective and inexpensive way of hydrolyzing hemicellulose, reducing cellulose crystallinity, and increasing surface area and pore volume of the substrate (Fang et al., 2011). A few available researches demonstrated that the acid treatment influences the structure and function of soy glycinin and SPI (Wagner and Guéguen, 1999a, 1999b). The improvement of functional properties of acid-modified soy proteins was due to their decreased molecular size, and increase in surface hydrophobicity induced by deamidation (Wagner and Guéguen, 1995).

Based on the above viewpoint, a combination of SFE and dilute-acid pretreatment can simultaneously influence the organic tissue and protein structure of soybean meal. The purpose of this work was to define the optimal conditions in which maximum protein extraction yield would be obtained. In addition, the functional properties of protein were also investigated.

2. Materials and methods

2.1. Materials

Soybean meal (protein content 49.49%) was provided by Hangzhou Venus Biological Nutrition Co., Ltd. (Hangzhou, China) with nitrogen solubility index (NSI) of 18.90%. The soybean meal was obtained from dehulled flaked soybeans by extracting oil with hexane and then desolventizing the defatted flakes by means of high-temperature thermal processing. The samples were ground to pass through a 20-mesh screen. The white flakes (WFs) defatted by solvent extracted and desolventized by means of flash- or downdraft-desolventizing to minimize protein denaturation, was purchased from Harbin High Tech (GROUP) Co., Ltd. (Harbin, China) to serve as reference for determination of functional properties. All other reagents and chemicals were of analytical grade.

2.2. Acid soaking optimization

The ground soybean meal was treated with dilute sulfuric acid solution (sample/solution liquid ratio = 1:5) in a stainless steel container immersed in a temperature-controlled water bath maintained at 80 °C for 2 h (Wagner and Guéguen, 1995). The container was equipped with a stirrer to ensure proper mixing of soybean meal with the acid solution. The sulfuric acid concentrations were examined in a range between 0% and 1.2% (w/v). Soaking in deionized water was referred to as a non-acid condition. Following the acid and non-acid treatment, the slurry was filtrated through the eightfold gauze to separate excess sulfuric acid. After filtration, the moisture content of soaked soybean meal was about 70%.

2.3. Steam flash-explosion treatment

About 2 kg of acid soaked and non-acid soaked soybean meal was loaded into a 5-L reactor of the SFE system (Fig. 1). The mechanism of SFE system is different from that of conventional steam explosion machine. In that SFE system adopts a structure in catapult explosion mode that is principally composed of a cylinder and piston. The force of the piston drive system which is composed of a linear actuator and a solenoid valve, comes from compressed air (Yu et al., 2012). The reactor is equipped with a high-pressure autoclave with gas inlet. When the saturated steam was quickly allowed to enter the reactor and steam pressure was maintained for expected time, the steam inlet was shut off and the piston dive device was triggered. The explosion was completed in about millisecond. The samples were carefully recovered, sealed in plastic bags and frozen.

2.4. Description of experimental design

In the acid soaking optimization study, acid soaked and non-acid soaked soybean meal was treated at 1.8 MPa for 10 min. After SFE treatment, the protein extraction yield of soybean meal was examined. When the sulfuric acid concentration was determined, the acid-soaked soybean meals was then treated at 1.4 MPa, 1.6 MPa, 1.8 MPa, 2.0 MPa and 2.2 MPa for 8 min, and the protein extraction yield of SFE-treated soybean meal was examined. In the study of residence time of SFE, the acid-soaked soybean meal was treated at 2.0 MPa for different residence times, and the protein extraction yield soybean meal was examined.

All experimental set points were carried out in triplicate. For every experiment, the materials recovered from receiver were carefully mixed together and constituted a unique batch.

2.5. Protein extraction and soy protein isolate preparation

Pretreated soybean meal slurry samples were dispersed in deionized water in a beaker to maintain a solids-to-water ratio of 1:10 (w/w). The slurry was placed in a 60 °C water bath and stirred for 45 min with pH maintained at 8.5. The samples were then centrifuged at 10,000 g for 20 min at 20 °C. The supernatant was

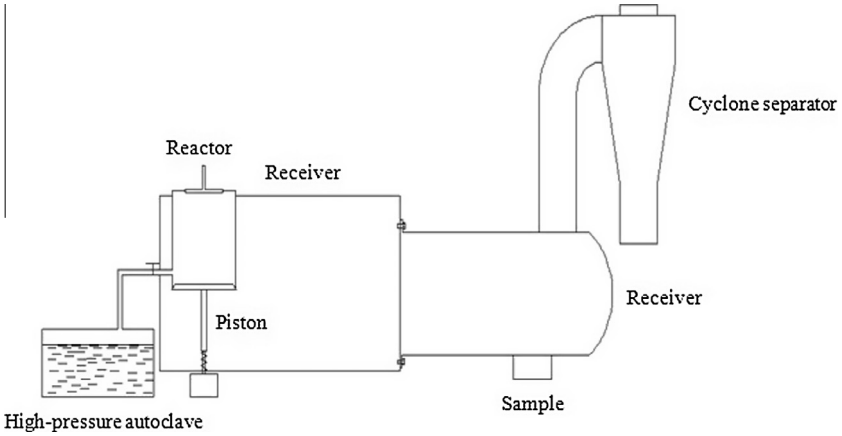

Fig. 1. Steam flash-explosion system.

collected for protein determination. All experiments were performed in triplicate.

The protein extract solution was adjusted to isoelectric point of protein with 2 N HCl at 20 °C and stored for 1 h, followed by centrifugation at 10,000 g for 20 min at 4 °C. Protein curd was obtained as a precipitate and washed with deionized water twice. The curd was redissolved in deionized water at pH 7.0, freeze-dried, sealed in a plastic bag and stored at 4 °C until further use. Controls were similarly prepared from untreated soybean meal and WFs. All experiments were performed in triplicate.

2.6. Protein determination

The protein contents of samples were measured by the micro-Kjeldahl method and a 6.25 conversion factor was used for calculation. The extraction yield of protein was calculated from the protein content in the recovered supernatant relative to the total protein content of the starting soybean meal. The extraction yield of protein was calculated as:

$$\text{Extraction yield of protein } (\%) = \frac{\text{weight of protein in supernatant(g)}}{\text{weight of protein in starting soybean meal(g)}} \times 100\% \quad (1)$$

The protein yield was calculated from the measured protein content in the SPI relative to the total protein content of the starting soybean meal. The protein yield of was calculated as:

$$\text{Protein yield } (\%) = \frac{\text{weight of protein in SPI(g)}}{\text{weight of protein in starting soybean meal(g)}} \times 100\% \quad (2)$$

2.7. Surface morphology observation by scanning electron microscopy (SEM)

The pretreated soybean meals were freeze-dried after centrifugation and FEI Quanta 200 SEM was used to examine surface morphology of pretreated and untreated soybean meal according to a slightly modified version of the method described by Karki et al. (2010). The specimens to be observed were mounted on conductive adhesive tape, sputter coated with gold palladium (SCD-005, BAL-TEC), and observed using a voltage of 5.0 kV. The SEM observations were made at a magnification of 600×.

2.8. Surface hydrophobicity (S_0) measurements

S_0 was determined using 1-anilino-8-naphthalenesulfonate (ANS) as a fluorescence probe according to a method described by Kato and Nakai (1980), in the absence of sodium dodecyl sulfate (SDS). Samples were dispersed into 0.01 M sodium phosphate buffer solution (pH 7.0) and centrifuged at 10,000 g for 20 min. The supernatants were collected, with protein concentration ranging from 0.14 to 0.02 mg/ml and then 30 μl of ANS (8.0 mM in 0.01 M phosphate buffer, pH 7.0, solution) was added to 6 ml of sample solution. Fluorescence intensity was measured using F-7000 PC spectro-fluorescence (HITACHI Corp., Kyoto, Japan) at an excitation wavelength of 390 nm and emission wavelength of 470 nm (both with a slit width 5 nm). The initial slope of fluorescence intensity vs. protein concentration plot calculated by linear regression analysis was used as an index of the protein hydrophobicity. Each sample was calculated from at least three measurements.

2.9. Functional properties of SPI

2.9.1. Emulsifying properties

The emulsifying activity index (EAI) and emulsifying stability index (ESI) of the SPI were determined according to the method described by Pearce and Kinsella (1978) with minor modification. To prepare emulsions, 15 ml of soybean oil and 45 ml of the 0.2% protein solution (w/v in 0.01 M phosphate butter pH 7.0) were homogenized at 10,000 rpm for 1 min with a high-speed ULTRA-TURRAX.T25 homogenizer (IKA, Labortechnik, Germany). Immediately after homogenization, 50 μl of the emulsion were pipetted from the bottom of the container and mixed with 5.0 ml of 0.1% (w/v) sodium dodecyl sulfate (SDS) solution and the absorbance of the diluted emulsion was measured at 500 nm. After 10 min the absorbance of the diluted emulsion was measured again. The EAI and ESI were calculated as follows:

$$\text{EAI } (\text{m}^2/\text{g}) = \frac{2 \times T \times A_0 \times \text{dilution factor}}{C \times (1-\phi) \times 10000} \quad (3)$$

$$\text{ESI } (\text{min}) = \frac{A_0}{A_0 - A_{10}} \times 10 \quad (4)$$

where T = 2.303, dilution factor = 100, C is the weight of the protein per unit volume (g/ml) and ϕ is the oil volume fraction of the emulsion. The experiments were conducted at 25 °C in triplicate.

2.9.2. Foaming properties

A volume of 100 ml protein solution (1%, w/v) was placed in a 250 ml measuring cylinder and whipped for 1 min with a high-speed homogenizer at the speed of 10,000 rpm. The total volume was measured immediately after whipping. The foam forming capacity (FC) was calculated according to the formula of Lawhon et al. (1972) using the following equation:

$$\text{FC } (\%) = \frac{V_0 - 100}{100} \times 100\% \quad (5)$$

The foam volume was recorded at 120 min after whipping to determine foam stability (FS) according to the method described by Ahmed and Schmidt (1979):

$$\text{FS } (\%) = \frac{V_{120} - 100}{V_0 - 100} \times 100\% \quad (6)$$

where V_0 is the total volume directly after whisking and V_{120} is the volume after 120 min. The experiments were conducted in triplicate.

2.9.3. Fat-binding capacity

The fat-binding capacity (FBC) was determined according to a slightly modified version of the method described by Fuhrmeister and Meuser (2003). SPI weighting 0.3 g was mixed thoroughly with 10 ml soybean oil in a tared 10-ml centrifuge tube. After a holding period of 30 min, the protein-oil mixture was centrifuged at 3000 g for 10 min; supernatant was discarded and then the centrifuge tube and its contents were weighed. The FBC was calculated as:

$$\text{FBC } (\%) = \frac{W_2 - W_1}{W_0} \times 100\% \quad (7)$$

where W_1 is the weight of protein and centrifuge tube, W_2 is the weight of protein, oil and centrifuge tube, and W_0 is the weight of protein. The experiments were conducted in triplicate.

2.10. Statistical analysis

All treatments were duplicated and analyses were done in triplicate. Data were analyzed using a one factor analysis of variance (ANOVA) and least significant differences (LSDs) were calculated

at the 5% level to compare group means using the Statistical Product and Service Solutions software package (SPSS, version 13.0).

3. Results and discussion

3.1. Extraction of protein from soybean meal assisted by SFE with dilute acid soaking

In the previous publication (Zhang et al., 2013), the result showed that after SFE treatment the protein extraction yield of soybean meal can be increased to about 65%. However, the particle size of soybean meal limits the application of SFE. It is found that when stem pressure and residence time exceed 1.8 MPa and 120 s, the protein extraction of soybean meal reached plateau, despite the higher pressure and time. The acid soaking can solve the above-mentioned problem.

3.1.1. Effect of acid concentration on protein extraction

The effect of sulfuric acid concentration on protein extraction is showed in Fig. 2. The extraction yield of protein was generally proportional to sulfuric-acid concentration. The extraction yield of protein from soybean meal by conventional alkaline extraction was about 50.50%. Therefore, when the soybean meal was soaked in water and treated at 1.8 MPa for 8 min, no effect on the extraction yield of protein was observed, based on yield of 48.22%. However, after acid soaking and SFE pretreatment, the extraction yield of protein was significantly increased to 60.02%, 66.98%, 71.03% and 77.09%. It is concluded that acid soaking can solubilize the hemicellulosic fraction of the biomass and soften the lignin structure. Therefore the acid soaked material had a loose and porous structure (Wang et al., 2011). More significantly, the loose and porous structure results in an increase in high pressure steam permeation into the cell tissue of soybean meal, which can form more powerful explosive decompression. In addition to the structure damage of cell tissues caused by acid and SFE pretreatment, the changes of protein structure, such as molecular weight and surface behaviors of protein, may have contributed to the increased protein extraction yield as a function of sulfuric acid concentration. High sulfuric acid concentration can raise acid hydrolysis of protein at high degrees of hydrolysis, and the decrease in molecular weight would cause a decrease in functional properties of protein. In addition, high sulfuric-acid concentration is environment-unfriendly and has negative influence on the production equipment. Based on the consideration of the extraction yield of protein, functional properties of protein and sulfuric acid consumption, we suggest using 0.9% sulfuric acid for acid soaking.

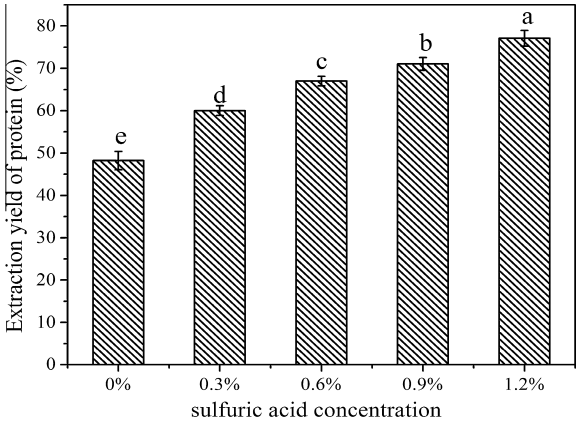

Fig. 2. Extraction yield of protein at various sulfuric-acid concentration soaking combined with SFE treatment. Different letters on the top of a column indicate significant differences ($P < 0.05$).

3.1.2. Effect of parameters of SFE on protein extraction from the acid soaked soybean meal

The effect of SFE treatment on the extraction yield of protein from sulfuric-acid soaked soybean meal was studied. The soybean meal was first soaked in 0.9% H_2SO_4 and then treated by SFE. The results of protein extraction yield are shown in Fig. 3. After SFE combined with acid soaking pretreatment, the protein extraction increased to 62.36%, 63.47%, 67.72%, 69.47% and 70.54% at 1.4 MPa, 1.6 MPa, 1.8 MPa, 2.0 MPa and 2.2 MPa respectively, for 8 min. When the residence time of SFE was extend to 8 min, there were no statistically significant differences in extraction yield of protein between 2.0 MPa and 2.2 MPa ($P > 0.05$). The steam pressure of 2.0 MPa and 2.2 MPa can significantly improve the extraction yield of protein to 70%. According to the above analysis, steam pressure at 2.0 MPa was carried out for different residence time of SFE to examine the extraction yield of protein. As shown in Fig. 3B, when the residence time was 10 min, the extraction yield of protein was improved to 71.21%. Compared with the extraction yield of protein from untreated soybean meal (50.50%), it indicates that after SFE combined with acid-soaking pretreatment, the extraction yield of protein from soybean meal was improved significantly. There were no statistically significant differences in extraction yield of protein between 8 min and 10 min at 2.0 MPa ($P > 0.05$). Scholars suggest that the steam-explosion treatment efficiency is a product of several factors, including steam pressure, residence time, particle size and moisture content (Talebnia et al., 2010). After acid soaking treatment, the soybean meals has the same particle size and moisture content of about 70%, hence the steam pressure and residence time showed significant effect on extraction yield of protein. Based on the data

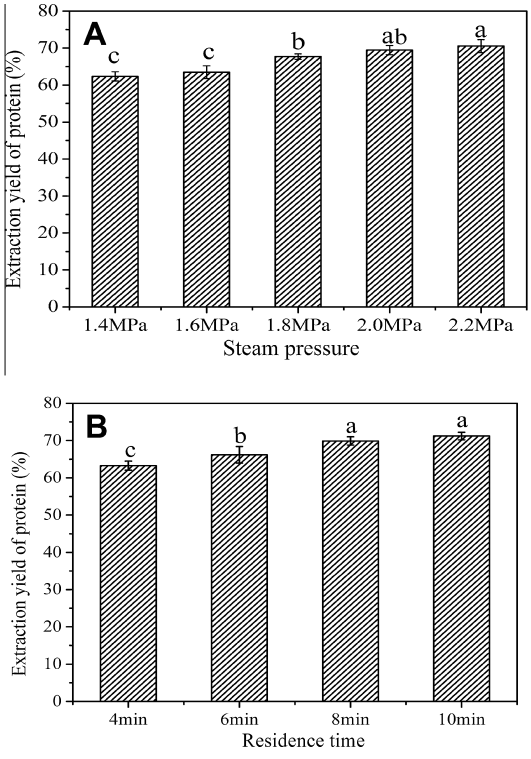

Fig. 3. Extraction yield of protein at various SFE treatment conditions after sulfuric-acid soaking: (A) soybean meal treated by SFE at different steam pressure for 8 min; (B) soybean meal treated by SFE at 2.0 MPa for different residence time. Different letters on the top of a column indicate significant differences ($P < 0.05$).

in Fig. 3, it shows that in a certain range of steam pressure and residence time, the extraction yield of protein reached plateau.

The detailed mechanism how SFE significantly improves the protein extraction is unclear. However, during SFE treatment, the shear stress through thermal explosion decomposition and the flow of treated slurry create tensile forces that breaks the soybean meal and results in further substantial breakdown of the lignocellulosic structure. Meanwhile it can be concluded that soybean meal is subjected to different physicochemical changes, including thermal-induced protein denaturation, Maillard reaction (Karr-Lilienthal et al., 2004) and the shear stress act to break down the large aggregation of protein.

3.1.3. Preparation of SPI from soybean meal assisted by SFE with dilute acid soaking

High solubility is required to obtain maximum soy protein isolate yields. Based on the extraction yield of protein under different pretreatment condition, the SPI prepared from soybean meal pretreated by SFE for 8 min at 1.8 MPa, 2.0 MPa, and 2.2 MPa combined with sulfuric acid (0.9%, w/w) soaking were further analyzed and compared with SPI prepared from non-pretreated soybean meal and WFs.

SFE combined with acid-soaking treatment significantly improved the protein yield as compared to the control (Table 1). The protein yield was increased to 47.18%, 44.38% and 49.73%, after pretreatment. There were no obvious differences in protein yield between soybean meal treated for 8 min at 1.8 MPa, 2.0 MPa and 2.2 MPa. The protein yield followed a trend similar to that of extraction yield of protein, hence it can be concluded that extraction yield of protein accounts for protein yield.

The protein contents in SPI prepared from soybean meal after SFE combined with acid-soaking pretreatment were about 81% without significant differences among them ($P > 0.05$). Although these protein concentrations were significantly lower than for the SPI from soybean meals without pretreatment, the difference was only about 5–6%.

These SPI products do not meet the minimum concentration requirement of 90% protein, they cannot be marketed as "soy isolate". It is supposed that the protein content of SPI was affected by the amount of associated and conjugated non-protein constituents precipitating as impurities with the protein and the non-protein constituent were the carbohydrates in SPI. This finding is similar to the result of Wang et al. (2005).

3.2. Morphology of pretreated soybean meal

SEM examination of pretreated sample was carried out. Based on the extraction yield of protein, the surface morphology of samples treated by SFE at 1.8 MPa, 2.0 MPa, and 2.2 MPa for 8 min were examined. The SEM images of pretreated soybean meal are shown in Fig. 4 along with untreated soybean meal as control sample. The electron micrograph of Fig. 4A reveals that the surface of the untreated soybean meal is intact. After 0.9% sulfuric-acid soaking, the surface of soybean meal was partially disintegrated suggesting that the sulfuric-acid soaking can hydrolyze hemicelluloses and remove pectin, leading to collapsed and distorted cell wall structure. SFE treatment combined with acid-soaking made the cell wall disintegrate and the texture began to loosen, as shown in Fig. 4C–E. There was near complete rupture of the surface of soybean meal with large numbers of fragmented cell matter and there were little perforations appeared on the surface of soybean meal following SFE treatment. The SEM study shows that the kinetic energy of SFE can breaks free of the cell structure of soybean meal. Extensive cellular disruption as shown in SEM examination contributes to release of cell constituents into the aqueous phase resulting in improvement of the extraction yield of protein (Karki et al., 2010).

Table 1
Protein yield and protein content of SPI from soybean meal and acid-soaking soybean meal with or without SFE treatment.

Sample	Protein yield (%)	Protein content (%)
Non-treated soybean meal	29.76 ± 1.51^{d}	87.4 ± 0.7^{a}
0.9% Acid-soaking	40.21 ± 1.04^{c}	85.6 ± 0.2^{a}
0.9% Acid-soaking + 1.8 MPa 8 min	$47.18 \pm 3.59^{a,b}$	81.4 ± 1.1^{b}
0.9% Acid-soaking + 2.0 MPa 8 min	44.38 ± 0.91^{b}	81.5 ± 0.1^{b}
0.9% Acid-soaking + 2.2 MPa 8 min	49.73 ± 0.99^{a}	81.5 ± 0.2^{b}

Different letters in the same column indicate significant differences ($P < 0.05$).

3.3. Physicochemical and functional properties of SPI extracted from soybean meal assisted by steam flash-explosion with dilute acid soaking

3.3.1. Surface hydrophobicity (S_0) of SPI

The S_0 values of the different samples are shown in Fig. 5. Acid treatment can raise the accessibility of some hydrophobic regions through unfolding the polypeptides (Goto et al., 1990). After acid-soaking treatment, the surface hydrophobicity of SPI–ACID–SBM was found to be significantly higher ($P < 0.05$) than that of other SPIs. The result is consistent with the finding of Wagner and Guéguen (1995) who reported that acid treatment increased the surface hydrophobicity of soy glycinin. When the acid-soaked soybean meal were treated by SFE, the surface hydrophobicity significantly was decreased in comparison with the SPI–SBM ($P < 0.05$). There were not significant differences in surface hydrophobicity between different SFE treatment conditions.

During SFE treatment, soybean meals were subjected to different physicochemical changes including Maillard reaction, which occured spontaneously under the heating conditions through the conjugation of a reducing carbohydrate to the amino groups of protein, and thermal denaturation. The thermal treatment may cause more aggregates that bury more hydrophobic side chain of amino group (Sorgentini et al., 1995). Maillard reaction can also significantly affect the surface hydrophobicity. Hiller and Lorenzen (2008) stated that this decrease in S_0 values is related to the Maillard reaction between hydrophilic sugar molecules and hydrophobic amino acid residues mainly on the protein surface and on the formation of heterogeneous protein/saccharide and protein/protein-cross-linked polymers and aggregates. In addition, Maillard conjugates may block the Lys and/or Arg residues by carbohydrate, as ANS may also strongly bind cationic groups of proteins (Gasymov and Glasgow, 2007).

3.3.2. Emulsifying properties of SPI

The effect of acid soaking only and SFE combined with acid soaking pretreatment on emulsifying properties of SPI is shown in Fig. 6. The SPI–SBM, SPI–ACID–SBM and SPI prepared from soybean meal pretreated by SFE combined with acid soaking exhibited higher EAI and ESI than SPI–WFs ($P < 0.05$). After acid soaking, the EAI and ESI obtained for SPI–ACID–SBM were 49.05 $m^2 g^{-1}$ and 65.72 min, which was 49% higher and 4 times more than that obtained with SPI-SBM. The EAI obtained for SPI-1.8-8, SPI-2.0-8 and SPI-2.2-8 were 56.44 $m^2 g^{-1}$, 52.46 $m^2 g^{-1}$, and 53.78 $m^2 g^{-1}$ respectively. There were no significant differences in ESI of SPI between different SFE treatment conditions. However, the ESI of SFE-treated SPI was lower than that of SPI–ACID–SBM. Compared to SPI–SBM, the ESI of SPI from SFE treatment was significantly improved. In brief, acid soaking only and SFE combined with acid soaking have an effect on emulsifying properties of SPI.

Acid modification of protein generally increase the number of polar groups, while it decrease molecular weight, alters the

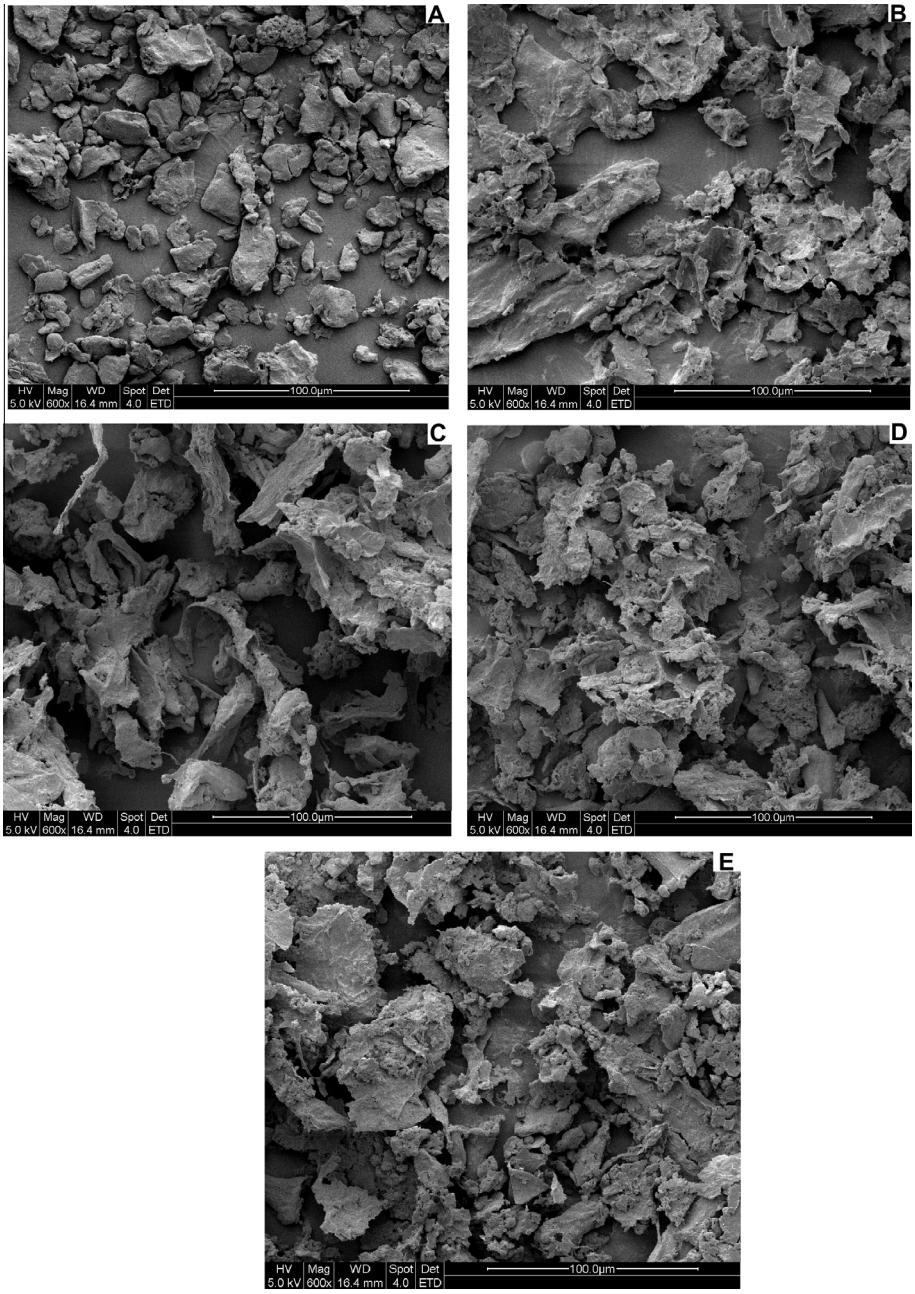

Fig. 4. Scanning electron microcopy images of soybean meal: (A) untreated soybean meal, (B) 0.9% sulfuric-acid soaked soybean meal, (C) soybean meal treated by SFE at 1.8 MPa for 8 min combined with acid soaking, (D) soybean meal treated by SFE at 2.0 MPa for 8 min combined with acid soaking, (E) soybean meal treated by SFE at 2.2 MPa for 8 min combined with acid soaking.

globular structure of the protein to unfold, and exposes previously buried hydrophobic regions (Fig. 5) (Chan and Ma, 1999). The unfolded protein results in flexibility, which facilitate hydrophobic group adsorption behavior in non-aqueous interface (Zhang et al., 2012). Compared with SPI–SBM, the increased surface hydrophobicity of SPI–ACID–SBM contributed to emulsifying properties.

Maillard reaction can significantly affect the emulsifying properties of SPI. Although after SFE treatment the surface hydrophobicity of SPI decreased, some studies showed that soy protein–polysaccharide conjugates could improve emulsifying properties of soy protein. The conjugates adsorbed to the interface can reduce the droplet size and lead to increased droplet surface hydrophilicity and steric interaction enhancement (Diftis and Kiosseoglou, 2003, 2006a, 2006b).

3.3.3. Foaming properties

The effect of acid soaking only and SFE combined with acid soaking pretreatment on foaming properties of the SPIs are presented in Fig. 7. SPI–WFs exhibited higher foaming stability than SPI–SBM, SPI–ACID–SBM and SPI from SFE combined with sulfuric-acid soaking pretreated soybean meal ($P < 0.05$). The results agreed with Lin et al. (1974) who found that native protein gives higher foam stability than the denatured one. SPI–ACID–SBM and SFE-treated SPI however, exhibited significantly better foaming capacity compared with SPI–WSFs and SPI–SBM ($P < 0.05$). The SFE treatment had little effect on FC of SPI compared to SPI–ACID–SBM, similar to the emulsifying properties, because there were no significant differences in FC between SPI–ACID–SBM and SFE-treated SPI. However, the SFE can improve the FS of SPI compared with the SPI–SBM and SPI–ACID–SBM.

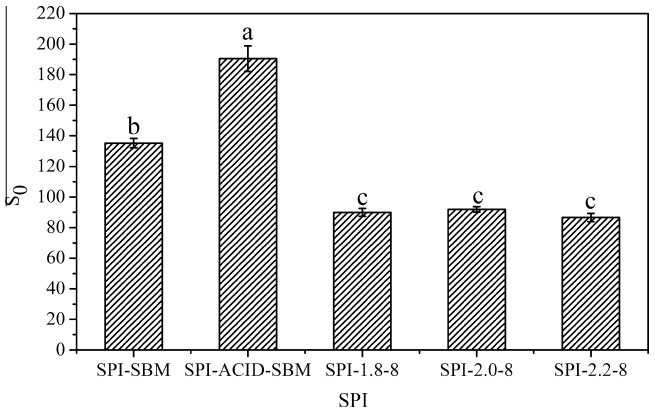

Fig. 5. Surface hydrophobicity (S_0) of SPI. SPI–SBM, SPI prepared from soybean meal; SPI–ACID–SBM, SPI prepared from soybean meal treated by sulfuric-acid soaking; SPI-1.8–8, SPI prepared from soybean meal pretreated by SFE at 1.8 MPa for 8 min combined with sulfuric-acid soaking; SPI-2.0–8, SPI prepared from soybean meal pretreated by SFE at 2.0 MPa for 8 min combined with sulfuric-acid soaking; SPI-2.2–8, SPI prepared from soybean meal pretreated by SFE at 2.2 MPa for 8 min combined with sulfuric-acid soaking. Different letters on the top of a column indicate significant differences ($P < 0.05$).

The soybean meal treated in hot acidic medium can lead to protein modification of unfolding, limited hydrolysis and improved protein flexibility (Wagner and Guéguen, 1995). Therefore, the rearrangement and interactions between protein molecules in the surface film of SPI were improved more rapidly and easily, which was a necessary condition for foam capacity and stabilization.

After SFE treatment, the SPI exhibited higher foaming stability than SPI–SBM and SPI–ACID–SBM, although the surface hydrophobicity of SFE–treated SPI was lower than the SPI–SBM and SPI–ACID–SBM (Fig. 5). It can be concluded that, in addition to surface hydrophobicity, there are other structural properties that enhance the surface behavior of SFE-treated SPI. Similar to the emulsifying properties, Maillard reaction can result in an additional change in the conformation of the protein and contributed to foaming properties.

Corzo-Martínez et al. (2008) observed that a great decrease in surface hydrophobicity of the β-lactoglobulin-galactose conjugate was found. Whereas β-lactoglobulin-galactose conjugate showed a better foaming capacity and higher stability of foams compared to their corresponding controls of protein heated in the absence of galactose (Corzo-Martínez et al., 2012).

3.3.4. Fat-binding capacity (FBC)

The FBC for SPI were compared in Fig. 8. The figure illustrates that the SPI–SBM, SPI–ACID–SBM and SPI from soybean meal pretreated by SFE combined with acid soaking bound significantly more oil than did SPI–WFs. The protein denaturation contributed to the difference in FBC (Nazareth et al., 2009). After SFE combined with acid soaking pretreatment, there were no significant differences in the FBC between SPI–SBM, SPI–ACID and SPI-1.8–8 ($P > 0.05$). When the after acid-soaked soybean meals were treated

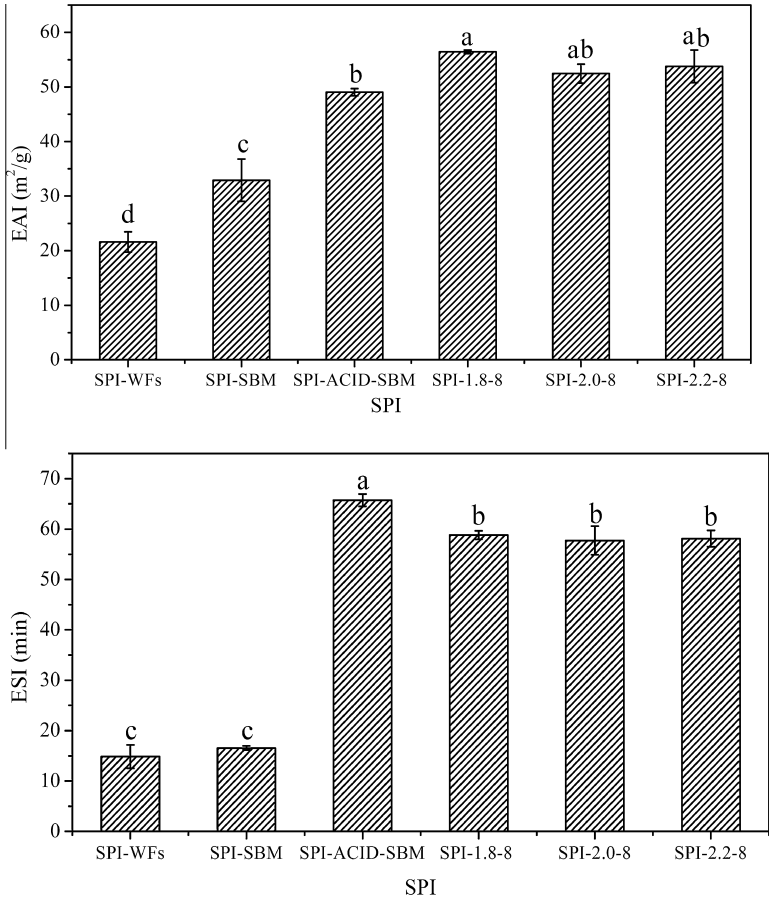

Fig. 6. Emulsifying properties of SPI prepared from different materials. SPI–WFs, SPI prepared from white flakes, for abbreviations see Fig. 5. Different letters on the top of a column indicate significant differences ($P < 0.05$).

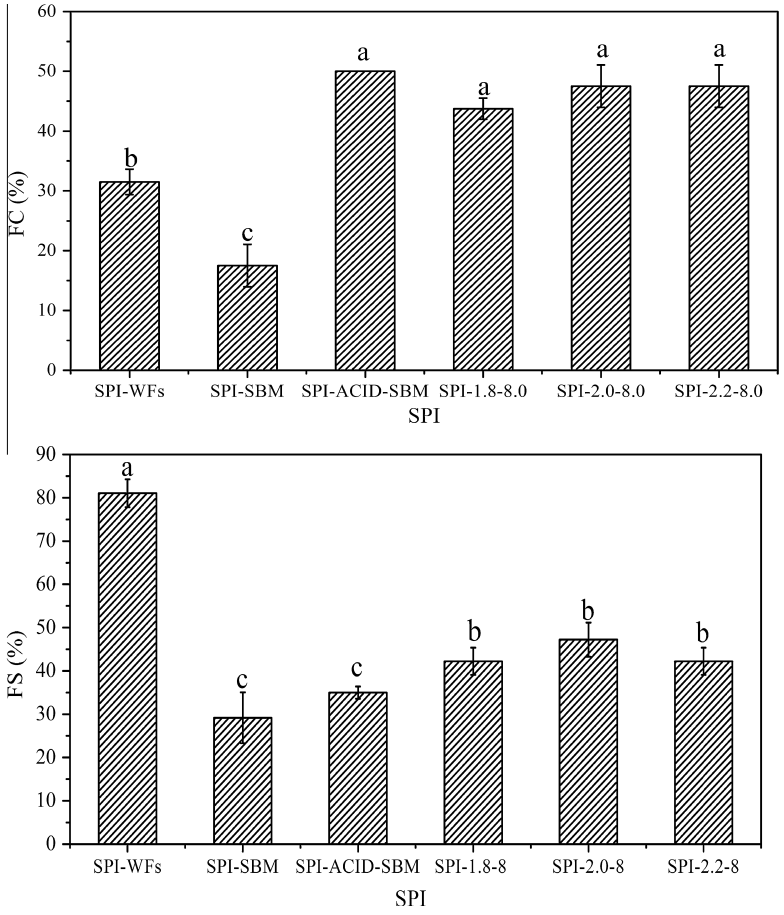

Fig. 7. Foaming properties of SPI prepared from different materials. SPI–WFs, SPI prepared from white flakes, for abbreviations see Fig. 5. Different letters on the top of a column indicate significant differences ($P < 0.05$).

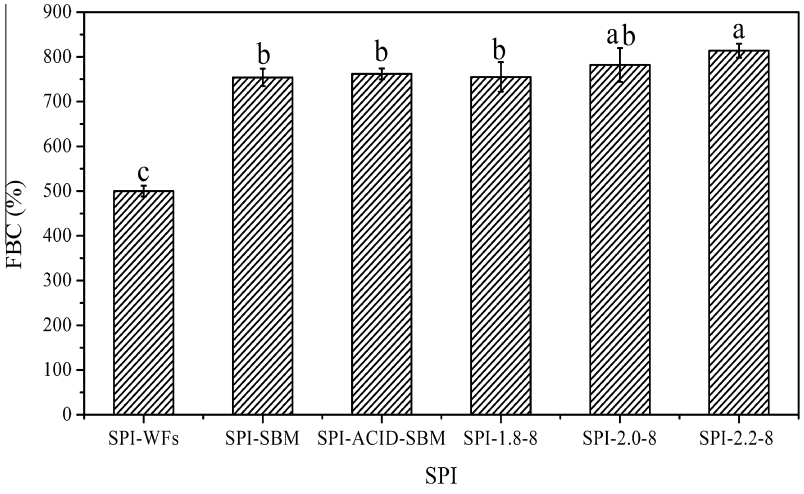

Fig. 8. Fat-binding capacity of SPI prepared from different materials. SPI–WFs, SPI prepared from white flakes, for abbreviations see Fig. 5. Different letters on the top of a column indicate significant differences ($P < 0.05$).

by SFE at 2.0 MPa and 2.2 MPa for 8 min, the FBCs of SPI were improved to 782% and 814% respectively.

Fat-binding capacity is affected by a number of factors including the protein content, surface area, and protein structure. Tomotake et al. (2002) suggested that the ability of protein to bind fat depends on hydrophobicity, degree of denaturation, and the size and flexibility of the protein and Chan and Ma (1999) reported that bulk density of protein affects its fat-binding capacity. In this study, it is hypothesized that the non-polar side chain of protein and flexibility contributes the FBC of SFE-treated SPI.

4. Conclusion

SFE combined with acid soaking pretreatment is an efficient tool for improving the protein yield and functional properties of

protein from denatured soybean meal. After SFE combined with acid soaking pretreatment, the improvement of the extraction yield of protein was partly related to the disruption of the cell structure of soybean meal being evident from SEM images. In addition, the acid soaking only and combined treatment can exert an influence on the surface hydrophobicity of SPI, which has limited effect on functional properties of SPI. The functional properties of SPI from pretreated soybean meal including emulsifying properties, foaming properties and fat-binding capacity were dramatically enhanced compared to SPI from untreated soybean meal. The emulsifying properties and fat-binding capacity of SPI from soybean meal pretreated by SFE combined with sulfuric-acid soaking were higher than the SPI from white flakes. Therefore SPI from soybean meal pretreated by SFE combined with sulfuric-acid soaking can be applied to frozen desserts, sausage and so on. This observation suggests that SFE combined with sulfuric-acid soaking pretreatment has attractive advantages in the utilization of denatured soybean meal to produce value-added protein, which has great potential in various food applications to replace the protein from white flakes.

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