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ORIGINAL PAPER

Destabilization of the Emulsion Formed during Aqueous Extraction of Soybean Oil

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Abstract Characterization and destabilization of the emulsion formed during aqueous extraction of oil from soybean flour were investigated. This emulsion was collected as a cream layer and was subjected to various single and combined treatments, including thermal treatments and enzymatic treatments, aimed at recovery of free oil. The soybean oil emulsion formed during the aqueous extraction processing of full fat flour contains high molecular weight glycinin and β-conglycinin proteins and smaller oleosin proteins, which form a multilayer interface. Heat treatment alone did not modify the free oil recovery but freeze–thaw treatment increased the oil yield from 3 to 22%. After enzymatic treatment of the emulsion, its mean droplet size changed from 5 to 14 μm and the oil recovery increased to 23%. This increase could be attributed to the removal (due to enzymatic hydrolysis) of large molecular weight polypeptides from the emulsion interface, resulting in partial emulsion destabilization. When enzymatic treatment was followed by a freeze–thaw step, the oil recovery increased to 46%. This result can be attributed to the thinner interfacial membrane after enzymatic hydrolysis, partial coalescence during freeze–thaw, and coalescence during centrifugation. Despite the reduction in emulsion stability achieved, additional demulsification approaches need to be pursued to obtain an acceptably high conversion to free oil.

Keywords Aqueous extraction processing · Demulsification · Enzymatic treatment · Soybean flour · Soybean oil emulsion · Soy protein

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Introduction

Soybeans are a major crop in the United States, grown for vegetable oil and protein. Soybean dominance comes from a variety of factors, including favorable agronomic characteristics, competitive price, high-quality edible oil and high-quality protein meal for animal feed. An important factor in soybean success is the amount of protein meal produced, up to 2 MT/ha, a quantity greater for soybeans than for any other commercial oilseed in the United States [1]. Ninety seven percent of the defatted soybean meal is used for feeding livestock, especially poultry and swine, and to a lesser extent dairy and beef cattle; however, an increased variety of food and industrial uses have been developed. Edible protein products are produced from the remaining 3% defatted soybean meal in the United States, but this amount is expected to increase in future years [1, 2]. Hexane extraction is currently the most cost-effective oil recovery method for oilseed processing leading to a meal with residual oil content below 1% [3]. However, hexane is highly flammable, toxic and ends up as fugitive emissions of volatile organic compounds (VOCs). These

safety, environmental and health concerns have generated interest for the replacement of organic solvent extraction [4]. Aqueous extraction processing (AEP) uses water to simultaneously extract oil and protein from oilseeds and therefore is an environmentally clean technology with potential value-added products without the hazards of hexane [5–7]. AEP has been explored to produce oil from soybeans, coconut, peanut, cottonseed, sunflower and rapeseed [5]. When water is used to extract oil from soybean material, the oil extraction yield ranges from 65 to 75% and only a small fraction of the oil is released as free oil whereas the majority is emulsified mainly in a cream layer and also in the aqueous phase (skim milk) [6]. Enzyme-assisted AEP of extruded soy flakes was shown to increase the oil recovery to 88%; however, the formation of a stable oil-rich cream fraction was still observed [6]. Extracted oil has to be recovered from the AEP as free oil for this process to be economically feasible.

Emulsion stability depends on the molecular and chemical properties of the emulsifier at the interface, and environmental and process conditions [8, 9]. These parameters have an effect on creaming, coalescence and flocculation of the emulsion and may be manipulated to modify its stability. Enzymatic, physical and physicochemical treatments have been reported as means to reduce emulsion stability [9]. The majority of the studies have been performed on model emulsions with control of both concentration and composition of the emulsifier(s). Few studies have investigated naturally occurring emulsion stability. In soybean the triacylglycerols (TAG) are at the core of oil bodies stabilized by an outer layer of phospholipids (PL) and basic proteins, termed oleosins [10]. Upon extraction the oil bodies may be disrupted but also further stabilized by coextracted proteins such as the major soy storage proteins glycinin and β-conglycinin.

In this study the approach was twofold. First, the composition of the cream formed during aqueous extraction of soybean flour was determined in order to identify the likely emulsifying agents. Second, enzymatic and thermal treatments applied alone and in combination, were evaluated for their effectiveness in destabilizing this emulsion, thereby achieving a higher yield of free oil.

Experimental Procedures

Starting Material

Soybean flour was prepared in the pilot plant of the Center for Crops Utilization Research at Iowa State University and stored at 4 °C. The flour was prepared from variety IA 1008 soybeans. The soybeans were cracked, the hulls were aspirated and the meats were milled twice with a pin mill. The oil content of the flour, determined by the Goldfisch method [11], was 22% and the crude protein content, determined by the Dumas method [12] with a conversion factor of 6.25, was 48.5% (dry basis).

Oil Extraction and Phase Separation

Extractions were conducted in a 2-L reaction vessel (Model CG-1926-03, ChemGlass Inc., Vineland, NJ). The flour (200 g) was dispersed at 200 rpm in 2 L of distilled water at 50 °C. The pH of the dispersion was adjusted to 8 with 2 N NaOH. After stabilization of the pH, stirring continued for 15 min. The sample was left for 1 h on a laboratory bench to cool at 25 °C. Sodium azide (0.04%, S227I-100, purity $\geq$ 99%, Sigma, Pittsburg, CA) was added to the cooled sample to prevent microbial growth. Insoluble fraction, aqueous phase and cream were separated by centrifugation in 250 mL centrifuge bottles at 3,000*g* for 15 min and 25 °C (Sorvall RC-5B, Newtown, CT) using a HS-4 Swinging Bucket rotor. The cream layer (oil emulsion) was located at the top of the supernatant. The cream was collected on top of a 200-mesh sieve by decanting the supernatant gently through the screen. The yield of cream (%) was calculated as weight of cream (as is) over initial weight of full fat flour (as is) multiplied by 100%.

Enzymatic Treatment

Thirteen grams of soybean cream were mixed with 1.45 g of distilled water in a 250-mL beaker. This small addition of water was necessary for reliable pH determination. The pH was adjusted to 8 and Protex 7 L (bacterial neutral endoprotease kindly provided by Genencor International Inc, Rochester, NY) was added at 1% w/w of cream. The sample was shaken in an incubator shaker (Model C24, New Brunswick Scientific, Edison, NJ) at 115 rpm and 50 °C for 3 h. The enzyme-treated cream was completely transferred to 30-mL glass centrifuge test tubes and the enzyme deactivated by heating in a water bath at 95 °C for 5 min. The tubes were then centrifuged at 3,000*g* for 15 min and 25 °C. The free oil released after centrifugation was removed with a plastic pipette and weighed for oil quantification. Controls were treated identically but water replaced the enzyme added. The complete extraction/demulsification experiment was repeated three times.

Thermal Treatment

An enzyme-treated cream and corresponding control (cream treated as above but without Protex 7 L addition)

were heated at 95 °C for an additional 30-min period after enzyme deactivation and cooled in a chilled water bath before centrifugation, which was performed at the same conditions previously given.

For the freeze–thaw treatment, a fresh set of enzyme-treated cream and corresponding control were held in a freezer (Model Isotemp, Fisher Scientific, Pittsburgh, PA) at −18 °C for 24 h. The samples were then thawed by incubating at 30 °C for 3 h before centrifugation. The complete extraction/demulsification experiment was repeated three times.

Particle Size Distribution

The volume-weighted mean diameters ($D_{4,3}$) of the emulsion obtained after treatments and before centrifugation were measured by a laser light scattering particle size analyzer (Mastersizer 2000 S, Malvern Instruments, Ltd, Chicago, IL). Small portions of the sample were dispersed in 50 mL of distilled water by vortexing for 30 s before the analysis was performed. The refractive index (RI) used for the soybean oil droplets was 1.47 and 1.333 for the dispersant [13]. The absorption value was set up at 0.001. All measurements were carried out at 25 °C.

Cream Characterization

The original cream obtained directly after extraction was prepared for characterization using the method of Hunt et al. [14] and Agboola et al. [15, 16]. The cream was washed by dispersion into four parts of distilled water then recovered by centrifugation (15,000*g* for 30 min at 25 °C; Sorvall RC-5B centrifuge, Newtown, CT) using a fixed angle rotor (Model SLC-1500, Kendro, Ashville, NC) and decanting onto a 200 mesh sieve. The washed cream was then stored at 4 °C before analysis. The crude protein content of the cream was from nitrogen content determination (Rapid NIII combustion analyzer; Elementar Americas, Inc., Mt. Laurel, NJ) using a factor of 6.25. Total carbohydrate content was determined using the method described by Fox et al. [17]. The solids content was determined gravimetrically after drying at 105 °C (Stabil-Therm oven; Blue M Electric Company, Blue Island, IL) with measurements taken over 24 h until constant mass was reached. Crude oil, TAG and PL, were isolated from the cream by the Folch method [18], which includes an extraction step with a chloroform/methanol mixture (2:1 by volume; C606-1 and A452-4, HPLC grade, Fisher Scientific, Pittsburg, PA) followed by a wash step with a methanol/water mixture (1:1 by volume; A 452-4, HPLC grade, Fisher Scientific, Pittsburg, CA). The collected oil extract was evaporated under vacuum in a rotary evaporator for 30 min at 25 °C to remove chloroform, methanol and trace amounts of water. TAG and PL were fractionated by a cross-current extraction method as described by Galanos et al. [19]. The TAG were evaporated in a fume hood to remove hexane (H291-4, certified grade, Fisher Scientific, Pittsburg, PA); the difference between the crude oil and the TAG was considered as the PL yield with no further fractionation of PL pursued.

Proteins of the washed cream were isolated by acetone (A18-4, certified grade, Fisher Scientific, Pittsburg, PA) precipitation [20] where ice-cold acetone was added to the cream in a ratio of 20:1 (v/w). The solution was mixed, incubated at −18 °C for 2 h, and then centrifuged at 14,000*g* for 15 min at 4 °C. The supernatant was removed and the precipitate was washed 4–5 times with cold acetone until no yellow color was seen in the solvent. The protein pellet was air dried at 25 °C to remove residual acetone.

The dried protein pellet (approximately 5 mg) was then dispersed in 1 mL of a solution of 2% sodium dodecyl sulfate (SDS, BP166-100, electrophoresis grade, Fisher Scientific, Pittsburg, PA), 8 M urea (U6504, electrophoresis grade, Sigma, St Louis, MO) and 50 mM dithiothreitol (DTT, D0632, 99%, Sigma, St Louis, MO). Protein samples were combined with an equal volume of sample buffer containing 200 mM Tris–HCl pH 6.8, 2% SDS, 40% glycerol (BP229-1, Fisher Scientific, Pittsburg, PA), 0.04% Coomassie Blue G-250 (161-0406, laboratory grade, Biorad, Hercules, CA) and 350 mM of DTT and heated at 100 °C for 5 min before being loaded to a ready gel Tris–HCl 4–15% acrylamide linear gradient gel (Bio-Rad, Cat # 161-1104, Hercules, CA) and a ready gel Tris–Tricine 16.5% acrylamide resolving gel (BioRad, Cat # 161-1107, Hercules, CA). SDS-PAGE was performed on these protein fractions and run at 200 V on a Mini-PROTEAN® II Electrophoresis Cell (BioRad, Hercules, CA). Soy protein isolate, purified glycinin and β-conglycinin were prepared at a bench-scale according to the procedure of Rickert et al [21]. The loading amount of protein into the gel was 10 μg. After electrophoresis, the gels were stained with Coomassie Brilliant Blue. The protein molecular weight distribution was calculated from the densitometric measurement of protein bands on the gel with the software ImageJ [22].

The surface protein concentration, Γ, was calculated according to Agboola [15, 16]:

$$\Gamma = \frac{M_{P/O}}{SSA} \quad (1)$$

where $M_{P/O}$, the mass ratio of protein to oil, was from the cream composition and SSA, the specific surface area of the oil droplets, was calculated using the following equation:

Table 1 Percentage composition of soybean oil emulsion

	Oil[a]	Water	Proteins	Carbohydrates	Phospholipids
Soy cream	59 ± 5.80	35 ± 6.40	5 ± 0.69	1.3 ± 0.23	0.8 ± 0.15

Mean ± 90% confidence intervals from triplicates

[a] TAG content

$$\mathrm{SSA} = \frac{6}{D_{3,2}} \times \frac{1}{\rho_{\mathrm{oil}}} \quad (2)$$

where $D_{3,2}$, the surface-area averaged particle size was determined with particle size analysis and the soybean oil density was 0.92 g/cm^3.

Characterization of the Proteins Remaining Absorbed at the Emulsion Interface after Treatments

Proteins adsorbed at the oil/water interface were separated following the method of Dickinson and Matsumura [23]. After individual destabilization treatment, each emulsion sample was washed from free unadsorbed protein by centrifugation at 10,000*g* for 15 min at 25 °C. The cream was then dispersed in distilled water and the washing step was repeated. An equal volume of 4% SDS, 20% glycerol, 0.125 M Tris–HCl buffer, pH 6.8, was added to the washed emulsion cream, which was then stirred at room temperature for 24 h to extract proteins adsorbed at the emulsion droplet surface. The resulting emulsion was then centrifuged again. The proteins in the serum phase were mixed at a 1:1 ratio with 1 M Tris–HCl, pH 6.8, containing 20% glycerol, 10% SDS, 0.05 M urea and 0.4% bromophenol blue and boiled for 5 min. Ten micrograms of samples, and 5 μg of molecular weight maker (Sigma M-3913, St Louis, MO) were loaded into 4–20% Tris-HCl gel (Biorad, Cat# 161-1105, Hercules, CA) and run at 200 V on a PROTEAN® Electrophoresis Cell (Biorad, Hercules, CA). After electrophoresis, the gels were stained with Coomassie Brilliant Blue.

Statistical Analysis

A screening experimental design with five factors: enzyme treatment (E), heat treatment (H), freeze–thaw treatment (FT), enzyme + heat treatment (E − H) and enzyme + freeze–thaw treatment (E − FT), was used and analyzed with JMP 6 (SAS Institute Inc., Cary, NC). The responses were the particle size ($D_{4,3}$) and the free oil recovery (%). All the measurements for oil recovery and particle size determination were done in triplicate and analyzed by the one-way analysis of variance statistical method (ANOVA). The means from the cream composition and from each treatment were compared by using Tukey's Honestly Significant Differences (HSD) test.

Results and Discussion

Characterization of Soybean Cream Layer

The yield of cream obtained during aqueous extraction of full fat soybean flour was 18 ± 1.5%. This collected cream layer was stable for four weeks with refrigeration and no noticeable free oil could be observed even after intensive centrifugation (15,000*g*; 30 min).

The two major components of the cream in the emulsion formed during AEP were TAG and water and lesser quantities of proteins, carbohydrates and phospholipids (Table 1).

Both quantity and type of proteins present at the interface might play an important role in the emulsion stability. Surface protein concentration (Γ) is an index that can be used to characterize emulsion stability. Tcholakova et al. [24] has shown that a surface protein concentration of 1–2 mg/m^2 was the smallest coverage of oil droplet to form a monolayer to ensure a stable emulsion. The ratio of protein to oil of our cream was 89.79 mg/g, the specific surface area 6.13 m^2/g and the surface protein concentration of this naturally occurring emulsion, calculated by means of Eq. 1, was 14.65 mg/m^2. This value, which indicates a stable multilayer emulsion [24], was much higher than the value of 3.03 mg/m^2 reported for a 10 g/L pH 8.0 soy protein isolate emulsion [25].

The SDS-PAGE gel of the surface proteins from the untreated-washed cream revealed the presence of the α′, α and β subunits of β-conglycinin and acidic (A) and basic (B) subunits of glycinin, the two major soybean storage proteins (Fig. 1). β-Conglycinin is a trimer with a molecular mass of 150–200 kDa and glycinin is a hexamer of 300–380 kDa. Several bands with molecular weights ranging from 18 to 24 kDa were also identified in the gel and were attributed to oleosin proteins. Oleosins represent the most abundant proteins found at the lipid surface bodies. They are amphipathic proteins composed of amphiphilic N- and C-terminal domains and a hydrophobic

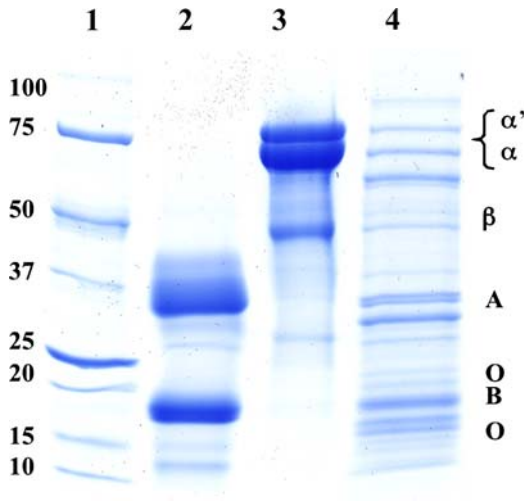

Fig. 1 SDS-PAGE profile of the proteins in the untreated washed cream. *1* M.W. marker (10–100 kDa range), *2* glycinin marker, *3* β-conglycinin marker, *4* polypeptides from untreated cream. *A* acidic subunit of glycinin, *B* basic subunit of glycinin, *O* oleosin

central domain [10, 26]. Polypeptides having a molecular weight higher than 25 kDa, which included mainly the α′, α and β subunits of β-conglycinin and acidic subunit of glycinin, represented 43.6% of the total polypeptide present at the interface. Among the remaining 56.4, 28% was attributed to the basic subunit of glycinin. The fact that glycinin and β-conglycinin in addition to oleosins were at the emulsion interface differed from the result of Guo et al. [27] who reported that in soymilk the oil globules contained mainly oleosin and minor quantities of glycinin and β-conglycinin. Soymilk is obtained through the grinding of soaked soybean with water whereas our aqueous extraction procedure involves the extraction of full fat flakes into water. Therefore, the differences in processing could explain a difference in the proteins appearing at the interface.

Dickinson [28] reported that high M. W. protein emulsifiers form strong viscoelastic interfacial films between droplets and prevent coalescence, which is thought to be one of the major mechanisms stabilizing emulsions [9]. The stability of the soybean oil emulsion formed during AEP might, therefore, be due to the presence of multilayer protein emulsifiers of high molecular weights and the presence of the emulsion-stabilizing oleosin. The presence of phospholipids in the cream would provide additional emulsion stability [10] but their role was not investigated further. Carbohydrates might also play a role in the emulsion stability, but they do not work as emulsifier alone. Rather, carbohydrates interact with proteins to form complex interfacial structures [29].

The focus of this study was on emulsion destabilization through modification of the proteins absorbed at the interface. Accordingly, both thermal and enzymatic treatments were chosen to destabilize the multilayer proteins. First, thermal destabilizations using heating and freezing steps were investigated. It has indeed been established that thermal treatment above the protein denaturation temperature leads to the denaturation and aggregation of the proteins absorbed at the interface and those present in the continuous phase, which reduces the oil droplet size [9, 30]. A thermal treatment of 95 °C treatment applied for 30 min was chosen based on the denaturation temperature of around 75 and 93 °C of the two major soy proteins, β-conglycinin and glycinin, respectively [31]. The effect of this treatment on oleosin protein native state is unknown as its denaturation temperature is not reported in the literature.

Freeze and thaw is thought to promote coalescence due to the concentration of oil droplets between ice crystals that favors droplet–droplet interactions [32, 33] and promotes destabilization of various types of food emulsions. Mechanisms of emulsion destabilization during freezing have been described in many papers and involve a gradual increased contact of the oil droplets in the unfrozen aqueous gaps between ice crystals, enhancement of the droplet–droplet interactions due to decrease of free water available to fully hydrate the droplet surface, concentration of lipids droplets favoring aggregation, and flocculation and/or coalescence at the interface [32–34]. At the same time, freezing to −18 °C causes some of the TAG in the soybean oil droplets to crystallize. This may promote partial coalescence due to penetration of a fat crystal from one droplet through the membrane of another droplet [34].

The addition of protease to the already formed emulsion is expected to cause the hydrolysis of the interfacial proteins, reducing the rigidity of the oil droplet interface and thus permitting oil droplet aggregation/coalescence [35].

Thermal Treatments

The heat treatment (95 °C, 30 min) and the freeze–thaw treatment (−18 and 30 °C) increased the mean droplet diameter $D_{4,3}$ from 5 to 14 μm (Table 2). The particle size distribution of the untreated cream (control) was bimodal and after thermal treatments broader peaks indicating population of larger droplets was observed (Fig. 2a). The increase in the mean droplet size coupled with centrifugation did not modify the oil yield of the heat-treated cream (Fig. 3). On the other hand, with the freeze–thaw treated cream, for which the particle size increase was similar to what was observed with heat-treatment, the oil yield increased from 3 to 22%.

Gel A (Fig. 4) represents the SDS-PAGE profiles of the polypeptides absorbed at the cream interface after individual treatment. Bands at the top of the gel, which are

Table 2 Effect of treatments on the mean droplet diameter ($D_{4,3}$) of the soybean oil emulsion before centrifugation

Treatment	$D_{4,3}$ (μm)
Untreated cream (control)	5 ± 0.27 a
Heat	14 ± 0.48 b
Freeze–thaw	15 ± 0.59 b
Enzyme	14 ± 1.26 b
Enzyme-heat	27 ± 3.00 c
Enzyme-freeze and thaw	25 ± 4.00 c

Values represent mean ± 90% confidence intervals from triplicates. Values with different letters are significantly different from each other. Enzyme treatment was carried out at a 1% (wt) concentration

high molecular weight polypeptides unable to enter the gel, were seen at different intensities depending on the sample. In the untreated cream (Fig. 4, lane 4) the subunits of glycinin and β-conglycinin were not clearly seen which differs with the profile obtained in Fig. 1, lane 4. Because the samples of gel A (Fig. 4) were prepared without use of 2-mercaptoethanol [23], it seems that glycinin and β-conglycinin subunits formed complexes involving disulfide bonds. The band with a ~22 kDa molecular weight was attributed to oleosin protein. After heat treatment, the ~22 kDa band was the most dominant protein remaining as shown in Fig. 4, Lane 6. This result indicated that most larger proteins and subunits dissociated from the interface during heating and/or formed complexes involving disulfide bonds. This increased droplet size (Fig. 2a, Table 2) but did not increase free oil yield (Fig. 3).

After freeze–thaw, the peptide profile of the proteins was unchanged, suggesting that the partial coalescence of droplets (Fig. 2; Table 2) and the increase in oil yield (Fig. 3) were due to droplet–droplet interaction in the frozen cream and centrifugally driven contact of droplets rather than any change in the proteins absorbed at the interface.

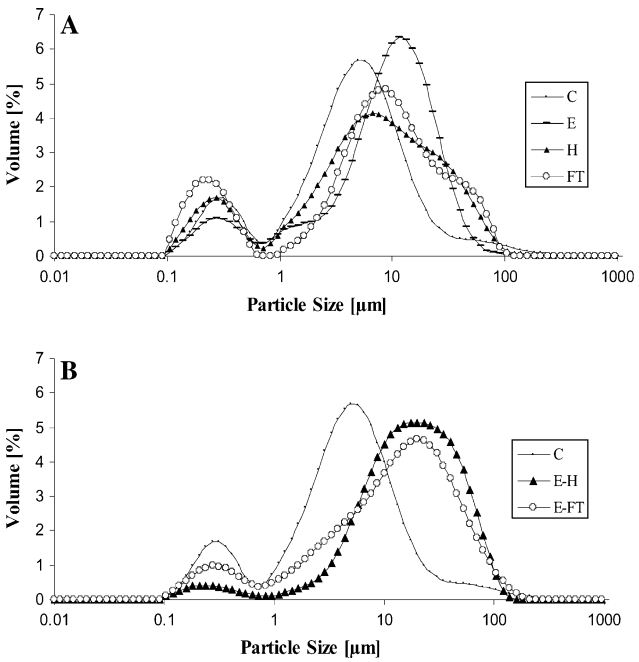

Fig. 2 Particle size distribution profile of cream subjected to different treatments. **a** Single treatment. *C* control, *E* enzymatic treatment, *H* heat treatment, *FT* freeze and thaw treatment, **b** combined treatment. *E-H* enzymatic followed by heat, *E-FT* enzymatic followed by freeze and thaw

Enzymatic Treatment

The enzymatic treatment's effect on the $D_{4,3}$ of the cream was similar to that of the two thermal treatments (Table 2). The particle size distribution of the enzyme-treated cream (Fig. 2a) showed a narrow peak in the 5–90 μm area. This peak was also higher than that represented by the peaks depicted for the other two treatments. On the other hand, it was also noticed that the peak observed in the 0.1–1 μm area was less pronounced. After enzymatic treatment, only polypeptides smaller than 14 kDa remained (Fig. 4a, b), demonstrating that this protease treatment could effectively reduce interfacial protein size. This modification of the polypeptide profile at the emulsion interface did not promote oiling off before centrifugation, but did increase the oil yield to 23% after centrifugation (Fig. 3), a result comparable to that achieved with freeze–thaw. The increase in droplet size and conversion to free oil reflected that protease-modified interface is less stable than the rigid interface provided by the multilayer, large protein interface of the untreated cream.

Combination of Enzymatic and Thermal Treatments

When the cream was subjected to protease treatment followed by heat treatment (E − H) the mean droplet diameter of the soybean oil emulsion increased from 14 to 27 μm (Table 2). The polypeptide profile of the remaining creams obtained by combination of enzymatic and thermal treatment was similar to the profile obtained with enzymatic treatment alone (results not shown). The particle size distribution for this combined treatment showed that an important population of larger droplets was formed; also, the peak observed in the 0.1–1 μm area almost disappeared (Fig. 2b). With this combinative treatment, oil recovery improved to 29% (Fig. 3).

Combination of enzymatic and freeze–thaw (E − FT) treatment increased the particle diameter to 25 μm

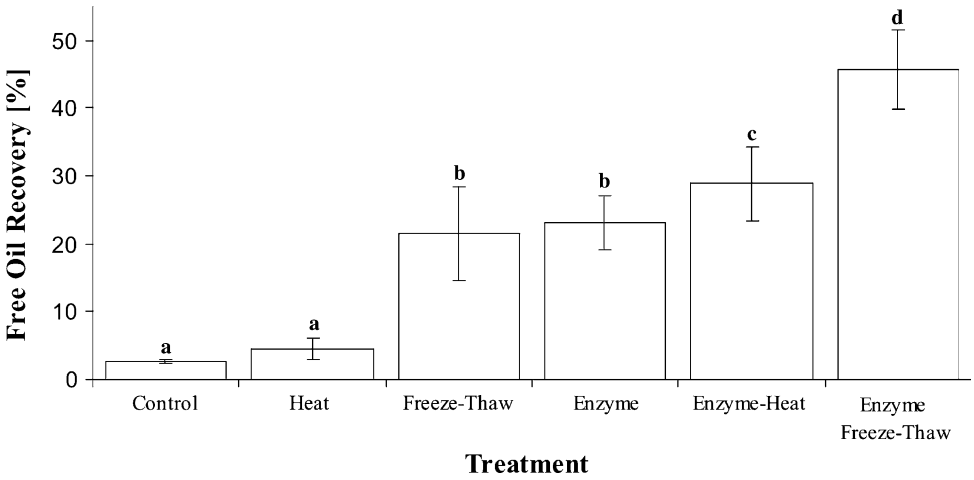

Fig. 3 Effect of treatments on emulsion oil recovery yields. *Bars* with different *letters* are statistically different at $P < 0.05$. *Errors bars* represent 95% confidence interval.*Letters* indicating significance

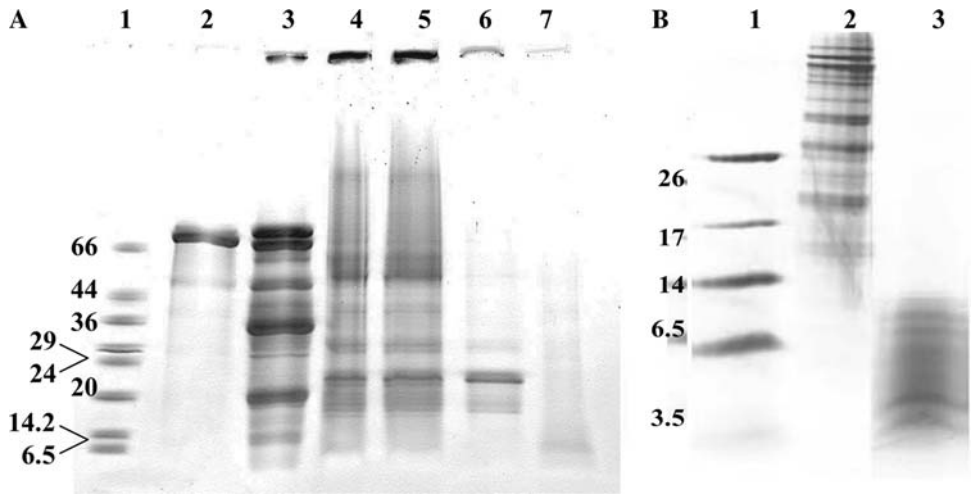

Fig. 4 SDS-PAGE profile of proteins from soybean oil emulsion subjected to various treatments. *Gel A* cream samples were prepared without use of 2-mercaptoethanol. Tris–HCl 4–15% acrylamide linear gradient gel. *1* M.W. marker (66–6.5 kDa range), *2* β-conglycinin marker, *3* commercial soy protein isolate marker, *4* untreated cream, *5* freeze–thaw treated cream, *6* heat-treated cream, *7* enzyme-treated cream. *Gel B* Tris–tricine 16.5% acrylamide resolving gel. *1* M.W. marker (26–3.5 kDa range), *2* untreated cream, *3* enzyme-treated cream

(Table 2). The particle size distribution showed a broader peak than for the enzyme/heat treatment (Fig. 2b). For this combined treatment the peak in the 0.1–1 μm area decreased, as was seen before for all the enzyme-thermal combination treatments. Free oil recovery increased to 46%, the highest value achieved (Fig. 3). Even so, the level of residual cream indicates that this focus on the protein component of stabilization was not sufficient to totally destabilize the cream formed during AEP of full fat flour.

This study has identified the likely stabilizers of the oil emulsions resulting from aqueous extraction of oil from soy flour. Destabilization efforts aimed at the protein component reduced stability but not enough to achieve high conversions to free oil. Additional work needs to be directed at the stabilizing roles of phospholipids and residual polypeptides.

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