Contents lists available at ScienceDirect

Food Chemistry

Low temperature dry extrusion and high-pressure processing prior to enzyme-assisted aqueous extraction of full fat soybean flakes

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ABSTRACT

Oil, protein and solid extraction yields obtained during aqueous extraction processing (AEP) of full fat soybean flakes (FFSF), FFSF extruded at a die temperature of 100 °C and FFSF pressurised at 200 and 500 MPa for 15 min at 25 °C, were compared to those obtained during enzyme-assisted aqueous extraction processing (EAEP) using 0.5% of protease Protex 7L. Without enzyme addition, pretreatment of the FFSF with extrusion and 500 MPa increased and decreased, respectively, the oil extraction yield while protein extraction yield was significantly decreased after both treatments. The best treatment in terms of oil and protein recovery was EAEP of extruded flakes with 90% and 82% of oil and protein extraction yield, respectively, and 17% of free oil. Addition of protease during extraction significantly decreased the yield of isolated soy protein (ISP) due to an increased solubility of the proteins at pH 4.5. ISP from extruded EAEP had higher solubility at pH 7.0 and better functionality. The DSC results, combined with the protein extraction yields, showed that a proportion of the proteins became insoluble after extrusion and 500 MPa treatment, while only those extracted from 500 MPa FFSF had a reduced native state.

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1. Introduction

Aqueous extraction processing (AEP), which uses water as an extraction medium, is an alternative method for solvent oil extraction that has been applied to various oilseeds, including soybean, rapeseed and peanut (Rosenthal, Pyle, & Niranjan, 1996). The first reports on this process dated from the 1950s, with major work done in the 1970s. Since then, investigations on this topic have continued, but interest in this environmentally-friendly process has recently re-emerged, mainly because of increased regulatory concerns and potential opportunities to add value to the co-products (fibre and protein). This technology has other inherent advantages, including lower capital investment requirements and increased safety compared to hexane extraction, and might therefore correspond to the need to construct smaller processing plants in developed and developing countries (Lusas, Lawhon, & Rhee, 1982; Olsen, 1982). Hexane and aqueous extraction from oilseeds have in common that broken cells enhance/accelerate oil recovery (Nelson, Wijeratne, Yeh, Wie, & Wei, 1987; Wiese & Snyder, 1987). Cell wall disruption, which accelerates mass transfer and extraction kinetics, can be obtained either by mechanical or enzymatic treatment, or a combination of both. Interestingly, it is the combinatory pretreatment of soybean flakes by extrusion, followed by protease-assisted aqueous processing, that is the more efficient

process to date for aqueous extraction of soybean oil (Freitas, Hartman, Couri, Jablonka, & Carvalho, 1997; Lamsal, Murphy, & Johnson, 2006). Extrusion is a mechanical process exposing material to high temperature, shear force and pressure over a short period of time (Nelson et al., 1987; Phillips, 1989; Rhee, Kuo, & Lusas, 1981). This combination of extreme processing conditions promotes both cell disruption and protein denaturation that seems to favour aqueous extraction of oil and proteolytic attack (Freitas et al., 1997; Lamsal et al., 2006). However, the exact mechanisms of this efficient combinatory process are still unclear. Better understanding of it would be beneficial to its transfer to other oilseeds and could provide co-products with desired properties.

Pretreatments other than extrusion, such as ultrasonication, pulsed electric fields, microwave heating and γ-irradiation, were applied in an attempt to improve oil extraction yield for various oilseeds prior to AEP, enzyme-assisted aqueous extraction (EAEP), or solvent extraction (Guderjan, Elez-Martinez, & Knorr, 2007; Shah, Sharma, & Gupta, 2005; Valentova, Novotna, Svoboda, Schwartz, & Kas, 2000). High-pressure processing (HPP) of food, which consists of applying pressures up to 600 MPa (∼87,000 psi), is a newly developed, non-traditional technology. This process has mainly been investigated as an efficient non-thermal treatment to increase shelf-life of food products and inactivate food deterioration enzymes, while maintaining product nutritional characteristics and appearance (Norton & Sun, 2008). In few cases HPP has been studied for its potential to promote the release of compounds from their matrix (Kato, Katayama, Matsubara, Omi, & Matsuda,

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2000). There are currently no studies investigating the extractability of oil during aqueous extraction of soybean flakes pretreated with high pressure.

Maximum recovery of the oil fraction has been the driver for AEP optimisation; however, the simultaneous extraction of the soy proteins constitutes another major advantage of the process. The pretreatment applied to the soybean flakes to improve the oil extraction yield during aqueous extraction processing may affect the properties of the extracted proteins. In other words, a pretreatment that may be beneficial to a maximum oil extraction yield may be detrimental to the properties of the extracted proteins. Soy protein isolates obtained from defatted soybean material are often treated with protease to improve their functionality, particularly to increase their solubility over a wider pH range (Henn & Netto, 1996; Puski, 1975). On the other hand, protein denaturation/precipitation is promoted during extrusion. Therefore, the combination of pressure, high shear and high temperature applied to FFSF during extrusion, followed by the addition of protease during AEP, could be potentially detrimental or beneficial, respectively, to the functionality of the protein fractions recovered from the process. Besides, extensive literature is available on the properties of isolated proteins from defatted soy meal, which is the co-product obtained from soybean oil extraction, and the starting material used by the industry to produce edible soy product ingredients. Scarce information is available on the properties of soy proteins obtained from full fat soybean. The objectives of this study were to first compare the extraction yields of oil, protein and solids during aqueous extraction, assisted or not with protease treatment, of FFSF pretreated with dry extrusion and high-pressure processing. AEP and EAEP of FFSF served as baseline controls for evaluating effects of each pretreatment. Second, the effects of processing on the recovery and composition of the isolated soy proteins and their physicochemical and functional properties were determined.

2. Materials and methods

2.1. Full fat soy flakes preparation

FFSF were prepared at the Center for Crops Utilization Research at Iowa State University from variety 92M91 soybeans harvested in 2006 in Iowa, USA. The soybeans were cracked in a roller mill (Model: 10X 12SGL, Ferrell-Ross, Oklahoma, OK) and aspirated by using a cascade aspirator (Kice Metal, Wichita, KS) to separate the hulls. The de-hulled soybeans were conditioned to 60 °C using a triple-deck seed conditioner (French Oil Mill Machinery Co., Piqua, OH), and were flaked using a smooth-surfaced roller mill (Roskamp Mfg Inc., Waterloo, IA) to ~0.30 mm thickness. The flakes were sealed and stored in plastic bags at 4 °C prior to use. Before extrusion and high-pressure processing, the soy flakes were adjusted to the desired moisture level of ~15% by adding water to the flakes in a Gilson mixer (Model: 59016A, St. Joseph, MO). The moisture-adjusted flakes were then placed in double polyethylene bags and kept at 4 °C until used. These flakes contained 19% oil (dry basis) and 32% crude protein (dry basis).

2.2. Extrusion

The extrusion was carried out in a Micro 18 twin-screw extruder (American Leistritz Extruders, Somerville, NJ, USA). The unit was equipped with a die having one 4 mm diameter, 12 mm long opening. The length and diameter of each screw were 540 and 18 mm, respectively. The screw configuration used in the experiments consisted of conveying elements ($L/D = 10$), kneading element ($L/D = 4.4$), conveying element ($L/D = 1.6$), kneading element ($L/D = 4.4$), conveying element ($L/D = 1.6$), kneading ele-

ment ($L/D = 2.2$) and a conveying element ($L/D = 5$). The barrel consisted of six independently controlled heating barrels. The barrel temperatures, measured via Fe–CuNi thermo elements inserted in the bottom of each barrel, during the process were: feed barrel (30 °C), barrel 1 (70 °C), barrel 2 (100 °C), barrel 3 (100 °C), barrel 4 (100 °C) and barrel 5 (100 °C). Soy flakes were fed into the unit with an Accu-rate dry material feeder (Accu-rate Inc., Whitewater, WI, USA) and processed via the intermeshing co-rotating screw at a constant rpm of 100. Processed material was fed through until equilibrium conditions were reached before material was collected for experimental use. Extruded soy pellets (~80 g) were collected directly in half the amount of the water needed for extraction. The water was at room temperature.

2.3. High-pressure processing

One hundred grammes of soy flakes and 300 g of distilled water were transferred into a polyester bag (Sealpaks, KAPAK, Minneapolis, MN) and sealed such that the headspace was kept to a minimum (Multivac Inc., Kansas City, MO). The samples were pressurised at 200 and 500 MPa at an initial temperature of 25 °C for a dwell time of 15 min using a Food-Lab 900 High-Pressure Food Processor (Stansted Fluid Power Ltd., Stansted, UK). The sample holder had a 6.5 cm internal diameter and 23 cm height. The rates of pressurisation and depressurisation were 260 and 500 MPa/min, respectively. The pressurisation fluid was a 1:1 mixture of 1,2 propanediol to water (GWT Global Water Technology Inc., Oakbrook Terrace, IL). The temperature increase of the pressurisation fluid due to adiabatic heating was ~3 °C/100 MPa. Each treatment was conducted in triplicate.

2.4. Aqueous extraction and separation of the different fractions

Extractions were conducted in a temperature-controlled water bath (Acrylic open bath with Isotemp 2150 circulator, Fisher Scientific, USA) at a constant speed of 300 rpm with lab-stirrer LR400C (Fisher Scientific, USA) at a flake to water ratio of 1:10. After 1 h of reaction at 50 °C and pH 7.0, the pH was raised to 8.0 and the extraction was carried out for another 15 min. This procedure constituted the standard aqueous extraction process (AEP). For the enzyme-assisted aqueous extraction processing (EAEP), Protex 7L was added at a dose of 0.5% (w/w, on the dry basis of soy flake). Protex 7L (EC 3.4.21.62 and EC 3.4.24.38) is a bacterial neutral protease preparation, with mainly endopeptidase activities, derived from the controlled fermentation of a non-genetically modified strain of *Bacillus amyloliquefaciens*. Its optimum pH varied from 6.0 to 8.0, and optimum temperature from 40 to 60 °C. This enzyme was kindly provided by Genencor International Inc. (Rochester, NY). During AEP and EAEP, the pH was maintained at a constant value with the addition of 2 N NaOH. Separation of the liquid and insoluble residue was carried out by centrifugation with a JS 4.0 swinging bucket rotor (Beckman Coulter Inc., USA) at $3000 \times g$ for 15 min at room temperature. After centrifugation, the insoluble fraction was oven-dried at 130 °C overnight and the liquid fractions (cream, free oil layer and skim) were transferred into a funnel and stored at 4 °C overnight. The skim was then separated from the cream and free oil layer (Fig. 1). The cream and free oil fractions were transferred into 30 ml tubes and allowed to decant again at 4 °C overnight. When a small amount of residual skim appeared at the bottom of the cream layer after refrigerated overnight storage, it was collected and added to the previously collected skim fraction.

2.5. Proximate analysis

Oil, crude protein and solids analysis were carried out on the skim, insoluble fraction, cream layer and FFSF. Total oil and crude
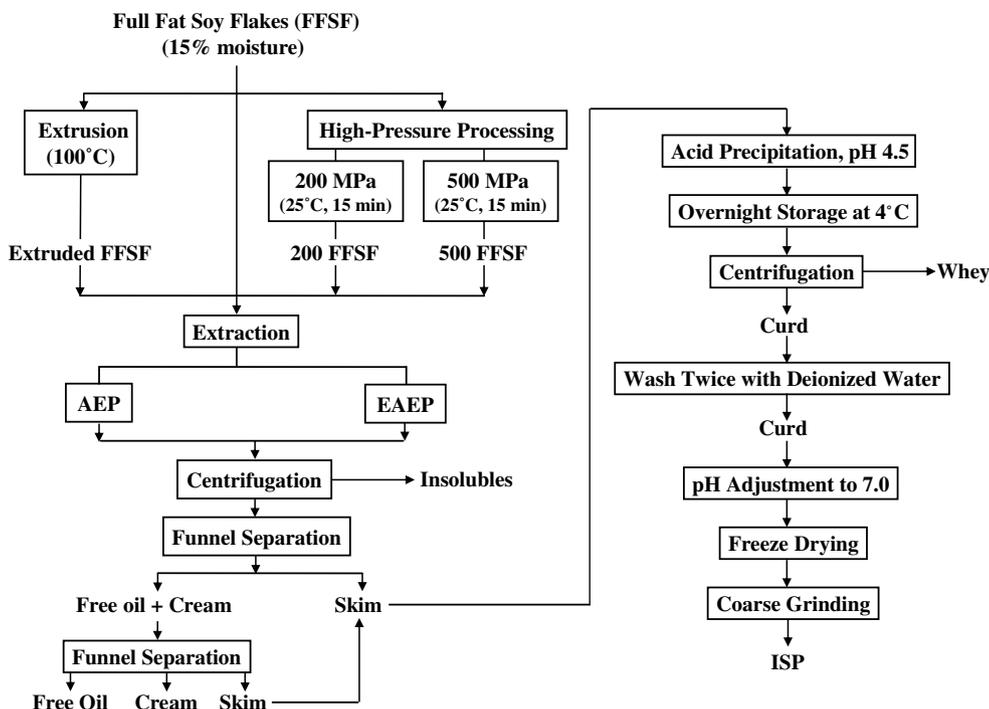
Full Fat Soy Flakes (FFSF)
(15% moisture)

Fig. 1. Process flow diagram for pretreatment of full fat soybean flakes (FFSF), followed by aqueous extraction processing (AEP) and enzyme-assisted aqueous extraction processing (EAEP), and preparation of isolated soy protein (ISP) from skim fraction.

protein contents were determined by using the Mojonnier Method (AOCS method 922.06 for solids and skim and AOCS method 995.19 for the cream layer) and the Kjeldahl method (AACC Standard Method 46-08 with a conversion factor of 6.25), respectively. For total solids, samples were heated in a vacuum oven at 110 °C for 3 h (AACC Method 44-40).

2.6. Extraction yield

The extraction yields of the oil, solids and protein were calculated as

$$\text{Extraction Yield } (\%) = 100 - \left[\frac{(C_f \times W_f)}{(C_{SF} \times W_{SF})} \times 100 \right]$$

where C_{SF} is the concentration of oil, protein or solid (%, g/100 g) in the soy flakes, W_{SF} is the weight of soy flakes (g, as is), C_f is the concentration of oil, protein or solids (%, g/100 g) in the insoluble fraction and W_f is the weight of the insoluble fraction (g, as is).

The partitioning of oil was calculated as the quantity of oil (g) in a selected fraction (i.e., skim, cream and free oil) divided by the amount of oil (g) extracted, multiplied by 100. A similar calculation was done to determine the partitioning of protein and solids in the skim and the cream fractions.

The isolated soy protein (ISP) extraction yield and total protein extraction yield were calculated as

$$\text{Yield}_{ISP}(\%) = \frac{(C_{ISP} \times W_{ISP})}{(C_{skim} \times W_{skim})} \times 100$$

$$\text{Yield}_{Total\ Protein}(\%) = \frac{(C_{ISP} \times W_{ISP})}{(C_{SF} \times W_{SF})} \times 100$$

where C_{skim} and C_{ISP} are the protein concentrations in the skim milk and isolated soy protein fraction (%, g/100 g), respectively, and W_{skim} and W_{ISP} are the weights of the skim milk and isolated soy protein fraction (g, as is), respectively.

2.7. Rheological measurements

Rheological measurements were performed on a 10% protein dispersion of the isolated soy protein fraction. The measurements were carried out in a controlled-stress rheometer, RS-150 (Haake, Karlsruhe, Germany) using a cone-plate sensor system (60 mm diameter, 2° angle) over the range 10/s to 500/s. The experimental flow curves were modelled by using the power law model: $\sigma = K(\dot{\gamma})^n$ where σ was shear stress (Pa), K was the consistency coefficient (mPa s^n), $\dot{\gamma}$ was shear rate (s^{-1}), and n was the flow index. Samples were tested a minimum of three times. The rheological behaviour was measured at 4 °C.

2.8. Isolated soy protein preparation

After adjustment of the pH of the skim fraction to 4.5, the samples were stored at 4 °C overnight and centrifuged at $10,000 \times g$, 4 °C for 15 min (Fig. 1). The precipitate was washed twice with distilled water and centrifuged again under the same conditions. The washed precipitate was dispersed in water and neutralised to pH 7.0, freeze-dried, sealed in a plastic bag and kept at 4 °C prior to further analysis.

2.9. Differential scanning calorimetry

Differential scanning calorimetry (DSC) measurements were performed with an Exstar 6000, Seiko SII calorimeter (Seiko Instruments Inc., Torrance, CA, USA). Twenty five microgrammes of 10% isolated soy protein dispersion (w/w), prepared in 0.01 M phosphate buffer (pH 7.0), were hermetically sealed in aluminium pans. A pan with buffer served as a reference. The samples were heated at the rate of 10 °C/min from 25 to 120 °C. The thermal denaturation enthalpy, ΔH, was expressed in J/g protein. All assays were replicated at least four times.

2.10. Isolated soy protein solubility at pH 7.0

Freeze-dried samples of 0.50 g were suspended in distilled water at 1% concentration and stirred for 1 h at room temperature. When necessary, the pH was readjusted to pH 7.0. Twenty-four millilitres of the dispersions were loaded into 50 ml centrifuge tubes and centrifuged at 10,000×g for 10 min at 20 °C. Crude protein content in the supernatant was determined using the Kjeldahl method. Solubility was the percentage of the original protein that was solubilised. Quadruplicate determinations were made.

2.11. Water- and fat-holding capacities of isolated soy protein

Water-holding capacity (WHC) and fat absorption capacity (FAC) were assessed by the following procedure: 1.25 g of isolated soy protein sample was dispersed in 0.01 M phosphate buffer, pH 7.0 and soybean oil for WHC and OHC, respectively, at a concentration of 5%. The samples were stirred for 20 min. Twenty millilitres of the slurry were transferred into a 50 ml centrifuge tube and then centrifuged at 1074×g for 30 min at 25 °C. The amount of water or oil retained after removal of the supernatant was determined. WHC was expressed as the amount of water retained by 1.0 g of sample (dry basis), and as the amount (g) of water retained by 1.0 g of insoluble proteins (Hutton & Campbell, 1981). The OHC was determined as oil retained by 1.0 g of sample (dry basis).

2.12. Surface hydrophobicity

Supernatants obtained from solubility determination at pH 7.0 were diluted with 0.01 M phosphate buffer, pH 7.0, at 0.1 mg/ml concentration. From this solution, several dilutions were made with the same buffer at concentrations from 3.66 to 100 µg/ml. H_o was determined by using a hydrophobicity fluorescence probe, 1-anilino-8-naphthalene-sulfonic acid magnesium salt monohydrate (ANS; ICN Biomedicals Inc., Aurora, Ohio, USA). Forty microlitres of 8.0 mM ANS in 0.01 M phosphate buffer were added to 3 ml aliquots of diluted samples. The zero point corresponded to 3 ml of phosphate buffer with 40 µl of ANS solution and 15 µl of ANS with 3 ml of methanol corresponded to a fluorescence of 80. The fluorescence intensity was measured by using a Turner Quantech spectrophotometer (Barnstead Thermolyne, Dubuque, Iowa, USA) at excitation and emission wavelengths of 360 and 515 nm, respectively. A plot of initial slope of the FI versus percentage protein concentration (calculated by linear regression analysis) was used as an index of surface hydrophobicity.

2.13. Statistical analysis

Each treatment was applied independently three times, and each parameter calculated from or determined after each treatment was the average of at least three determinations. The General Linear Model, PROC GLM in SAS System (version 9.1, SAS Institute Inc., Cary, NC, USA) was used to compare means and calculate least significant differences (LSD) at $p < 0.05$.

3. Results and discussion

3.1. Extraction yield

Three fractions are generated during aqueous extraction processing (AEP) of full fat soybean flakes (FFSF): a fibre-rich fraction, a hydrophobic phase containing the majority of released oil called cream, and a hydrophilic phase containing mainly solubilised sugars and proteins, called skim. Extraction yield and partitioning of oil, protein and solids between the fractions are summarised in Table 1. Only 60.0% of the total oil available was recovered in the aqueous extract of FFSF. Extrusion, followed by AEP, promoted the release of oil from the cotyledon cells into water, increasing the oil recovery to 67.7%, thus confirming the efficiency of extrusion in releasing oil during AEP (Freitas et al., 1997; Lamsal et al., 2006). Extrusion also had a marked effect on protein extractability, decreasing it to 45.3% from 73.5% for FFSF. Among the pressurised samples, an increase of pressure from 200 to 500 MPa decreased both oil and protein recoveries by ∼17%.

Extrusion is known to decrease protein solubility, which has been related to the formation of insoluble aggregates, involving both covalent disulphide bonds and non-covalent interactions (Jeunink & Cheftel, 1979; Liu & Hsieh, 2008; Zhu, Riaz, & Lusas, 1996). Some discrepancy in the involvement of disulphide bonds over non-specific hydrophobic and electrostatic interactions exists and can be attributed to the methods used to determine these interactions, as well as differences in extrusion temperatures (Liu & Hsieh, 2008; Zhu et al., 1996). Changes in extruded proteins occurred because the treatment involves enough energy to break down covalent high-energy bonds, such as disulphide (bond energy 21.1 kJ/mol), along with relatively weak non-covalent bonds such as hydrophobic bonds (Hayakawa, Linko, & Linko, 1996). High pressure can only break down weak non-covalent bonds and, therefore, high pressure-induced mechanisms are expected to differ from the extruded ones. The susceptibility of a protein to high pressure denaturation is closely related to its structure and consequent compactness of the pressure-perturbed structure. The effect of high-pressure processing on soy protein solubility, for pressures higher than 400 MPa, reported in the literature, is contradictory, with both increased and decreased solubility being reported (Lakshmanan, de Lamballerie-Anton, & Jung, 2006; Puppo et al., 2004). The 38% and 11% decrease in protein extraction yield after extrusion and 500 MPa treatment, respectively, indicated that both treatments promoted protein insolubilisation, but to different extents. Under the experimental conditions used, protein insolubilisation by a pressure of 500 MPa was found to be less severe than that promoted by extrusion.

The extent of protease hydrolysis depends on many factors, including the accessibility of the protease to its hydrolysis site (Jung, Murphy, & Johnson, 2005; Marsman, Gruppen, Mul, & Voragen, 1997). After harvesting, the unit operations applied to the soybeans were cleaning, cracking, dehulling, conditioning and flaking. During these steps, a maximum temperature of 60 °C was used, which is below the denaturation temperature of the two major soy proteins, glycinin and β-conglycinin (German, Damodaran, & Kinsella, 1982). The addition of Protex 7L during AEP was unsuccessful in releasing more oil, protein or solids from the FFSF, which agreed with Rosenthal, Pyle, Niranjan, Gilmour, and Trinca (2001). These results could be attributed to the physical and/or structural inaccessibility of the enzyme to the proteins, due to their location inside the unbroken cotyledon cells, and to the compact structure of the proteins while in their native state (Marsman et al., 1997; Yamauchi, Yamagishi, & Iwabuchi, 1991). The highest oil extraction yield was obtained with enzyme-treated extruded FFSF, increasing from 67.7% with AEP to 89.8%. This combinatory extrusion pretreatment, followed by EAEP, released 17% of the initial oil in the FFSF as free oil, which was the highest amount obtained. This treatment also improved the protein extraction yield, which increased when compared to AEP of extruded FFSF, from 45.3% to 75.4%. Therefore, after protease addition, the protein extraction yield of extruded FFSF became similar to EAEP of FFSF (Table 1). This extraction yield was slightly, but significantly, lower than that obtained with AEP and EAEP of FFSF at 200 MPa. However, the 200 MPa pretreatment did not promote changes that enhanced oil extraction yield. With 500 MPa FFSF, a similar trend to that obtained with extruded FFSF was observed with increases in both oil

Table 1
Oil, protein and solid extraction yield and their partitioning.

Starting material	Extraction	Extraction yield (%)			Partitioning of extracted material (%)						
		Oil	Protein	Solid	Oil			Protein		Solid	
					S	C	FO	S	C	S	C
FFSF[A]	AEP	60.0[b]	73.5[c]	58.5[a,b]	60.0[e]	40.0[d]	0[a]	99.2[a,c]	0.8[a,c]	91.5[d]	8.5[a,b]
	EAEP	59.9[b]	75.6[c]	61.0[b]	35.2[c]	64.8[f]	0[a]	98.4[a,b]	1.6[c,d]	82.5[c]	17.5[c]
Extruded FFSF	AEP	67.7[c]	45.3[a]	52.3[a]	14.2[a]	81.2[h]	5.1[b]	99.9[c]	0.2[a]	70.3[a]	29.7[e]
	EAEP	89.8[d]	75.4[c]	71.1[c]	17.5[b]	69.3[g]	17.0[c]	99.6[c]	0.4[a,b]	76.0[b]	24.0[d]
Pressurised FFSF 200 MPa	AEP	64.2[b,c]	81.5[d]	64.7[b,c]	81.4[h]	18.6[a]	0[a]	99.5[c]	0.5[a,b]	94.4[e]	5.6[a]
	EAEP	63.1[b,c]	81.2[d]	63.7[b]	43.2[d]	56.8[e]	0[a]	98.2[a]	1.9[d]	83.5[c]	16.5[c]
Pressurised FFSF 500 MPa	AEP	52.5[a]	65.6[b]	53.7[a]	78.5[g]	21.5[b]	0[a]	98.6[a,c]	1.4[b,c,d]	91.7[d]	8.4[a,b]
	EAEP	61.2[b,c]	74.3[c]	58.5[a,b]	64.3[f]	35.7[c]	0[a]	99.2[a,c]	0.8[a,c]	88.8[d]	11.2[b]
	LSD	7.2	5.4	7.0	2.5	2.7	3.7	1.1	1.0	3.2	3.2

[A] FFSF, full fat soybean flakes; S, skim; C, cream; FO, free oil. The protein and solid contents of free oil were not determined. Values in a column sharing the same superscript are not significantly different at $p < 0.05$. LSD, least significant difference.

and protein extractability after enzyme addition when compared to 500 MPa AEP.

The rupture of cell walls is a critical step in improving oil extraction yield during AEP, and major cellular disruption explained the observed 13% oil extraction yield increase between AEP of extruded FFSF and untreated FFSF (Freitas et al., 1997; Lamsal et al., 2006). The remaining 32.3% oil in the insoluble fraction of extruded FFSF after AEP could be due to the presence of some undisrupted cells and/or sequestration of oil by other mechanisms into the insoluble fraction. The significant decrease in protein extraction yield between FFSF and extruded FFSF suggests that the oil released during extrusion might be physically entrapped in newly formed protein aggregates or that new interactions were formed between proteins and the released oil. An increase of oil extractability to 89.8% with proteolytic hydrolysis supports these assumptions, and illustrated that the protease was efficient in breaking new extrusion-induced interactions. This enhancement of *in vitro* protein hydrolysis is in agreement with Marsman et al. (1997) who reported a sharp increase of protein released after protease addition to extruded soybean meal. With the 500 HPP treatment, the small increase in the oil extraction yield with the protease treatment to a value similar to that obtained with EAEP of FFSF suggests that the treatment did not promote rupture of the cotyledon cells, while the increased protein solubility showed that the protease was effective in breaking aggregates induced by the application of this pressure.

3.2. Partitioning of oil

The state of the extracted oil (i.e., free or involved in an oil-in-water emulsion) and its repartition into the liquid fractions are important parameters of AEP. Recovery of the oil as free oil is the preferred option since no additional demulsification step will be required. Recovery of free oil was only observed for extruded FFSF. The protease addition during extraction of extruded FFSF increased the free oil yield, determined as the amount of free oil over the initial weight of oil in the FFSF, from 7% to 17% (results not shown). It was worth noting that the repartition of extracted oil between the skim and cream fractions varied, depending on the starting materials and use of enzyme. For AEP of FFSF, 60% of the extracted oil was partitioned into the skim. Interestingly, the enzyme addition did not promote higher oil extraction yield, but the oil extracted went preferentially into the cream fraction. Similar observation applied to 200 MPa FFSF. For 500 MPa FFSF, the majority of the oil was still partitioned into the skim fraction after enzyme addition but there was a significant increase to 35% in the cream compared to 21% with AEP. For the extruded FFSF, the partitioning of the oil in the skim remained after enzyme addition. Scarce information is

available on protein/lipid interaction in colloidal dispersion of soybean material (Guo, Ono, & Mikami, 1997; Ono, Takeda, & Shuntang, 1996). To study the stability of soymilk, Guo et al. (1997) submitted it to thermal treatment from 50 to 95 °C, and analysed (after high speed centrifugation) the particulate, soluble and "floating" fractions, which would be equivalent to our cream. Changes in the lipid distribution in the different fractions started at 70 °C. The increased lipid content of the skim and cream was related to denaturation of β-conglycinin followed by glycinin. Phospholipids were also identified as a key component in the particle stability in soymilk (Ono et al., 1996). The mechanisms behind the oil repartition in the fractions recovered with our experimental conditions will require further investigations. Our attempts to increase oil extractability by increasing temperature during AEP were unsuccessful, illustrating that the oil/protein interactions were not destabilised with low speed centrifugation (results not shown).

The protein extracted went into the skim fraction except for 0.5% to 1.9%, which was partitioned into the cream. Stability of the cream fraction obtained with AEP of FFSF has been previously reported and attributed to the presence of proteins, along with phospholipids (Chabrand, Kim, Zhang, Glatz, & Jung, 2008). It was interesting to observe that during EAEP of FFSF and 200 MPa FFSF, almost twice as much protein was partitioned into the cream fraction. Proteins are known to be excellent emulsifiers and their concentration at the interface, as well as their structural conformation, or molecular weight, i.e., large aggregates or small polypeptides, determine oil-in-water emulsion stability (Agboola, Singh, Munro, Dalgleish, & Singh, 1998; Damodaran, 2004). The oil partitioning results suggest that the cream fraction recovered from EAEP of extruded flakes was less stable, since the higher amount of free oil recovered corresponded to a decrease in the oil content of the cream fraction. The absence of free oil for the other process conditions applied illustrated the stability of the cream emulsion.

3.3. Characterisations of isolated soy protein

Isolated soy proteins (ISP) are traditionally obtained from defatted soybean meal in two steps. The proteins are first solubilised into water during extraction at neutral pH and 50 to 60 °C. The proteins are then recovered from this water extract, called the skim, by isoelectric precipitation. The isolated soy proteins (ISP) are called soy protein isolate (SPI) if their protein content is higher than 90%, dry basis (Lusas & Rhee, 1995). None of the mechanical pretreatments (i.e., extrusion and high-pressure), applied to the FFSF before AEP modified the ISP recovery, which was around 80% (Table 2). Enzyme addition during AEP decreased the ISP recovery yield, regardless of the pretreatment applied. A 10% recovery decrease occurred for EAEP ISP of extruded FFSF, 20% with

Table 2
Protein yields, and protein and oil composition of isolated soy protein.

Starting material	Extraction	Yield		Content	
		ISP[A] (%)	Total (%)	Crude protein (%, db)	Oil (%, db)
FFSF	AEP	79.2[d]	61.6[e]	79.2[b]	20.8[c,d]
	EAEP	62.1[b]	46.7[c,d]	82.8[b,c]	14.5[a,b,c]
Extruded FFSF	AEP	80.0[d]	38.3[b]	92.3[d]	10.7[a]
	EAEP	70.9[c]	51.0[d]	87.1[c,d]	14.9[a,b,c]
Pressurised FFSF 200 MPa	AEP	81.5[d]	64.6[e]	77.3[b]	21.6[d]
	EAEP	63.7[b]	42.5[b,c]	81.0[b,c]	18.7[b,c,d]
Pressurised FFSF 500 MPa	AEP	82.1[d]	51.1[d]	79.1[b]	14.2[a,b]
	EAEP	38.6[a]	24.8[a]	65.5[a]	28.3[e]
	LSD	7.0	4.6	7.0	6.6

[A] ISP, isolated soy protein. Values ($n = 3$) for a given fraction sharing the same superscript are not significantly different at $p < 0.05$. LSD, least significant difference.

Table 3
Denaturation temperatures and enthalpies of glycinin and β-conglycinin of isolated soy protein.

Starting material	Extraction	Peak temperature (°C)		Enthalpy ΔH (mJ/mg)	
		β-Conglycinin	Glycinin	β-Conglycinin	Glycinin
FFSF	AEP	75.1[a,b]	89.0[b]	0.6[d]	5.3[c]
	EAEP	74.5[a,b]	86.8[a]	0.3[a,b]	6.0[c]
Extruded FFSF	AEP	74.9[a,b]	90.2[b,c]	0.5[c,d]	8.1[d]
	EAEP	75.7[b]	89.0[b]	0.2[a,b]	7.1[d]
Pressurised FFSF 200 MPa	AEP	77.1[b]	91.2[c]	0.3[b,c]	3.7[b]
	EAEP	72.2[a]	89.2[b,c]	0.1[a]	7.8[d]
Pressurised FFSF 500 MPa	AEP	75.6[a,b]	90.4[b,c]	0.2[a,b]	0.3[a]
	EAEP	76.1[b]	88.4[a,b]	0.3[a,b]	0.5[a]
	LSD	3.5	2.1	0.2	1.0

Each value is the mean of 10 measurements. For each observation, means followed by different letter (s) are significantly different ($p < 0.05$).

FFSF and 200 MPa FFSF were used, and the largest ISP recovery decrease of 40% was obtained with 500 MPa FFSF. This decrease in ISP yield can be explained by an increase in the protein solubility at pH 4.5 and can be attributed to the formation of small polypeptides and/or changes in protein configuration due to the protease action (Jung et al., 2005; Puski, 1975). The total protein extraction recovery for AEP of FFSF was 62% (Table 2), which agrees with yield obtained during SPI production of full fat meal (L'Hocine, Boye, & Arcang, 2006). Only 38% of the total protein was recovered when extrusion of FFSF was performed prior to AEP. This low protein extraction yield was the consequence of significantly decreased protein recovery from the insoluble fraction during extraction (Table 1). A similar explanation applied to the total protein yield obtained with AEP of 500 MPa FFSF. The addition of enzyme during extraction decreased the total protein recovery of the process in all cases except extrusion. Forty-two to 51% of the total proteins were recovered with EAEP of FFSF, extruded FFSF and 200 MPa FFSF. Both extrusion and 500 MPa treatments significantly decreased the extraction of proteins from FFSF, but the addition of protease during extraction was most beneficial to the extruded FFSF for which the protein extraction yield increased by 65% (from 45 to 75%), while decreasing the ISP yield by only 12.5%. On the other hand, the adding enzyme to 500 MPa FFSF only increased the protein extraction yield by 4%, while reducing the ISP yield by 53%. Overall, recovery of the protein extracted during EAEP by acidic precipitation did not appear to be the most appropriate method. Other processes such as membrane filtration might be more suitable.

3.4. Composition of ISP

The crude protein and oil content of the isolated proteins ranged from 65% to 92% and 10% to 29%, respectively (Table 2). The isolated proteins obtained during extraction of extruded FFSF, with and without enzyme, had the highest crude protein content, while ISP from EAEP of 500 MPa FFSF had the lowest. For each fraction, a decrease of the crude protein content was concomitant with an increase of the oil content.

3.5. Thermal properties

The glycinin and β-conglycinin of all ISPs exhibited thermal transitions at onset and peak temperatures of ~69.0 and 75.1 °C, and 80 and 89.0 °C, respectively (Table 3). Surprisingly, extrusion did not decrease the denaturation enthalpy of extracted glycinin and β-conglycinin. A non-uniform effect of extrusion processing on protein structure might explain why some proteins became insoluble, as reported above, while those extracted into water maintained their denaturation enthalpy. This hypothesis is supported by the result of Marsman et al. (1997) reporting total denaturation of glycinin and β-conglycinin in the insoluble fraction of extruded soybean meal. On the other hand, the denaturation temperature of soy proteins increased with decreased moisture conditions. Denaturation temperatures of 170.7 and 135.6 °C were observed for β-conglycinin and glycinin, respectively, for SPI with a moisture content of 29% (Kitabatake, Tahara, & Doi, 1989). In our study, a barrel temperature of 100 °C was applied to FFSF adjusted to a moisture content of 15%. The proteins should have maintained, at least partially, their enthalpies of denaturation. Further investigations will determine which mechanism, or to what extent both mechanisms, occurred during extrusion. Interestingly, compared to what was observed for AEP of extruded FFSF, ISP proteins recovered during AEP of pressurised FFSF had a decreased enthalpy of denaturation. The treatment of FFSF at 200 MPa decreased the enthalpy denaturation of extracted glycinin and β-conglycinin by 30% and 47%, respectively. ISP from 500 MPa FFSF had the lowest denaturation enthalpy of glycinin and β-conglycinin. While major changes in protein usually occur for pressures higher than 400 MPa, some protein structural changes can occur even at relatively low pressure, i.e., 100–200 MPa range (Hayakawa et al., 1996). Accordingly, our results suggested the occurrence of some structural modifications, including protein denaturation, of soy proteins treated at 200 MPa, which slightly enhanced the protein extractability (Table 1). It was established that reactivity to HPP differed between glycinin and β-conglycinin, and with level of pressure applied. At 200 MPa, partial unfolding of the globulins, with exposure of hydrophobic and sulfhydryl groups, probably occurred while, at 500 MPa, formation of aggregates between basic subunits of glycinin and β-conglycinin promoted protein precipitation (Molina, Papadopoulo, & Ledward, 2001).

The solid content of the sample submitted to extrusion and HPP was probably an important factor in the observed protein changes induced by the treatment: the higher solid/protein concentration promoting interactions between constituents having newly exposed sites (Fischer et al., 2001; Wang, Johnson, & Wang, 2004; Zhu et al., 1996). In our study the low flakes to water ratio (1:3) and low moisture content of the flakes (15%) used for HPP and extrusion, respectively, probably favoured protein/protein and protein/other constituent interactions. Another factor that might have greatly impacted the interactions, including protein interactions, during treatment of full fat soy flakes, was the location of the proteins in the soy structure, i.e., inside the cotyledon cells or solubilised into the water phase.

The reduced denaturation enthalpy of 7S and maintained enthalpy of 11S of the EAEP ISPs can be attributed to the structure and, also, spatial conformation of native β-conglycinin (7S) which favours protease hydrolysis compared to native glycinin (11S).

Similar reduced denaturation enthalpies were observed for proteins extracted from EAEP extruded FFSF. For the 200 and 500 MPa EAEP ISPs, denaturation enthalpy of both glycinin and β-conglycinin, were decreased and maintained, respectively, when compared to corresponding pressurised AEP ISPs. It was worth noting that, in a few cases, an increase in the denaturation enthalpy value for glycinin was observed, suggesting some protein changes that increased the stability of the 11S towards temperature. Changes in the heat capacity of proteins that are recorded via DSC reflect protein unfolding. These changes are believed to originate from the disruption of the forces stabilizing the native protein structure. Denaturation enthalpy, however, represents the net product of positive and negative contributions due to denaturation and aggregation, respectively. The observed increased stability suggests that newly formed soluble aggregate had higher thermostability.

3.6. Physicochemical and functional properties

Changes in conformations/interactions of extracted proteins due to processing were confirmed by ISP solubility and surface hydrophobicity results (Table 4). Rearrangements of the proteins with burying of the hydrophobic sites occurred after extrusion and HPP treatments, as illustrated by the decreased surface hydrophobicity of soluble proteins. When aqueous extraction was carried out with enzyme, proteins unfolded as they denatured due to enzyme attack, exposing the hydrophobic regions of the protein interior. The extent of the increased exposure of the hydrophobic sites depended on the process applied; a small increase in the surface hydrophobicity of the EAEP ISP was observed when compared to the corresponding AEP ISP. The extruded and 200 MPa ISPs had increased surface hydrophobicity of ~35%, and surface hydrophobicity of 500 MPa ISPs increased by more than 50%. These changes should be considered carefully as they are not indicative of the extent to which the protein structure changes, since rearrangement of the structure due to processing might occur and involve shelter of some hydrophobic regions.

The solubility of the AEP ISP increased from 71.3% to 93.9% when extrusion pretreatment was applied. This result illustrated that the decreased surface hydrophobicity of the extruded ISP promoted protein solubility and is in agreement with good protein dispersibility reported for SPI recovered from extruded/expelled residue (Wang et al., 2004). HPP at 200 and 500 MPa decreased the solubility of the AEP ISP, with the 500 MPa AEP showing the lowest solubility of 19.5%. Except with extrusion pretreatment,

adding enzyme during extraction increased the solubility of the ISP when compared to AEP ISP, with 38% and 121% increase for 200 and 500 MPa ISP, respectively.

High-pressure treatments did not modify the ability of the ISP AEP to retain water compared to that of FFSF, when the WHC was expressed in g of water retained per g of sample (dry basis) (Table 4). Adding enzyme during the extraction of FFSF and pressurised FFSF significantly reduced the ability of the proteins to bind water. Interestingly, lowest WHC was obtained with EAEP and AEP ISP of extruded FFSF. Water retention of proteins relates to both the number and type of polar groups and protein conformation (Hutton & Campbell, 1981). The protein content of the samples also impacts the WHC results. Based on the surface hydrophobicity and DSC results, it was unexpected that HPP at 500 MPa did not modify the ability of the proteins to bind water. Therefore the loss of soluble proteins was taken into account in the WHC calculation (i.e., WHC was expressed as g/g insoluble protein). With this change in the calculation, the 500 MPa pretreatment did significantly modify the ability of protein to bind proteins, while the trend for the other ISPs remained the same.

The effect of treatment on OHC of ISP did not follow the same trend as did WHC, confirming the different nature of interactions involved in protein/lipid interactions compared to protein/water interactions (Hutton & Campbell, 1981). Overall, adding enzyme decreased the ability of the ISP to bind oil only when the extrusion and 500 MPa treatment were applied to FFSF prior to the aqueous extraction.

Except with extrusion, the 10% AEP ISP dispersions had a shear-thinning behaviour with n values less than 1.0, and a flow behaviour closer to a Newtonian fluid when the proteins were recovered after EAEP (Table 5). Interestingly, extrusion pretreatment changed the behaviour of the extracted proteins from shear-thinning to almost Newtonian fluid, regardless of enzyme use during extraction. Extrusion pretreatment significantly reduced the apparent viscosity of the isolated proteins from 46.2 mPa s for the AEP ISP to 8.6 mPa s. Significantly increased viscosity was observed for the 500 MPa AEP ISP, which increased its viscosity twofold compared to AEP ISP, while the treatment at 200 MPa did not modify the viscosity of the samples. The consistency index variation followed the same trend as did the apparent viscosity results.

The relationship between WHC, solubility, viscosity and surface hydrophobicity of proteins has not been consistent in the literature (Wagner & Anon, 1990). In our study, it can be observed that decreased WHC (g/g sample) of EAEP samples was consistent with a decrease in their apparent viscosities and that no changes in WHC coincided with no changes in apparent viscosities for AEP and EAEP samples. The significant increase of apparent viscosity

Table 4
Protein solubility in water at pH 7.0, water- and oil-holding capacity, and surface hydrophobicity of isolated soy protein.

Starting material	Extraction	Protein characteristics			
		H_0[A]	Solubility (%)	WHC (g/g S.[B]) (g/g IP.[C])	OHC (g/g S.[B])
FFSF	AEP	23.9[e]	71.3[d]	28.6[d] 15.90[d]	28.0[d]
	EAEP	26.9[f]	84.7[f]	10.0[b] 10.14[bc]	24.9[cd]
Extruded FFSF	AEP	18.1[c]	93.9[g]	3.5[a] 7.58[abc]	27.7[d]
	EAEP	24.8[e]	87.7[f]	4.4[a] 6.34[ab]	22.8[bc]
Pressurised FFSF 200 MPa	AEP	16.4[b]	58.5[c]	26.2[d] 11.22[bc]	22.1[abc]
	EAEP	21.9[d]	81.0[e]	12.6[bc] 9.12[abc]	18.6[ab]
Pressurised FFSF 500 MPa	AEP	15.0[a]	19.5[a]	28.9[d] 6.58[ab]	22.7[bc]
	EAEP	23.8[e]	43.1[b]	16.5[c] 5.79[a]	18.2[a]
	LSD	1.3	3.6	4.9	4.2

Values ($n = 3$) for a given fraction sharing the same superscript are not significantly different at $p < 0.05$. WHC, water-holding capacity; OHC, oil-holding capacity.
[A] Surface hydrophobicity.
[B] S., sample.
[C] IP., insoluble proteins.

Table 5
Flow behaviour parameters of 10% water dispersion of isolated protein.

Starting material	Extraction	Flow behaviour parameters		
		Apparent viscosity (mPa s)	n value	k value (mPa s^n)
FFSF	AEP	46.2[b]	0.6[a,b]	58.0[c]
	EAEP	7.8[a]	0.9[d]	1.5[a]
Extruded FFSF	AEP	8.6[a]	1.0[d]	1.1[a]
	EAEP	5.0[a]	1.0[d]	0.7[a]
Pressurised FFSF 200 MPa	AEP	43.0[b]	0.7[b]	33.3[b]
	EAEP	7.2[a]	0.9[d]	1.1[a]
Pressurised FFSF 500 MPa	AEP	113.4[c]	0.6[a]	152.1[d]
	EAEP	38.2[b]	0.7[c]	65.2[c]
	LSD	22.3	0.1	22.8

Values for a given fraction sharing the same superscript are not significantly different at $p < 0.05$.

of ISP recovered after 500 MPa AEP can be explained by swelling of proteins.

Overall, adding enzyme during aqueous extraction of FFSF promoted a significant increase in oil and protein solubility only if severe pretreatments (i.e., extrusion, 500 MPa) were applied to the flakes. In addition, using protease improved only the oil extractability yield of extruded FFSF, the protein extraction yield being similar to that obtained with AEP. An economic analysis will determine if this 50% increase in extracted oil and the modified properties of proteins recovered from the process justify the enzyme cost for the treatment.

Acknowledgements

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