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Pilot Plant Recovery of Soybean Oleosome Fractions by an Enzyme-Assisted Aqueous Process

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Abstract An aqueous enzymatic procedure for oleosome fractionation from 25 g of soy flour was developed in our laboratory. This fractionation procedure was evaluated with 75 kg using pilot plant equipment to evaluate the effect of the scale-up on the recovery, proximate composition, soybean storage protein profiles, and subcellular microstructure of oleosome fractions. The process included enzymatic hydrolysis, grinding, and centrifugation, respectively. Pilot-scale grinding and centrifugation of the slurry were accomplished with a Stephan® Microcut mill grinder and a three phase decanter. A blender and swinging bucket rotor were used for the laboratory-scale fractionation. The oleosome fractions recovered in the pilot plant were similar in oil and protein content to those obtained in the laboratory. The pilot-scale process resulted in a significantly higher oil yield of 93.40% as total oleosomes compared to that of 76.83% achieved in the laboratory. Urea–SDS gel electrophoresis of proteins extracted from the oleosomes and supernatant from the pilot-scale fractionation had similar profiles to those obtained in the laboratory. Electron microscopy verified that the structure of isolated oleosomes was virtually identical with that of in situ oleosomes. This work confirms that large-scale fractionation of oleosomes from full fat soybean flour can be accomplished.

Keywords Soy oleosomes · Oil yield · Pilot plant process

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Introduction

Most oilseeds contain stored lipid reserves in spherical organelles known as oil bodies, oleosomes, lipid bodies or spherosomes [1–5]. In the soybean, the diameter of oil bodies in situ ranges from 0.2 to 0.5 μm [1, 6]. They consist of a triacylglyceride core encapsulated by a monolayer of phospholipids in which unique proteins, oleosins, are embedded to stabilize the structure of the oil body and to prevent coalescence of the oil in the cell cytoplasm [7–9]. Oleosins, with a molecular weight between 16 and 24 kDa, represent approximately 4% of the oil body weight and 6–15% of the total seed protein [1, 6, 7, 9, 10]. Iwanaga et al. [11, 12] demonstrated that oleosomes could be used in food products as ingredients in place of emulsified soybean oil in dressings, sauces, dips, beverages and desserts. The advantage of using oleosomes in foods is that they required neither emulsifiers nor homogenization of the oil.

Several groups have developed procedures to isolate oleosomes from oilseeds for purification and characterization. Those procedures involved hydrating whole seeds, with subsequent grinding, filtering, and centrifugation [8, 13–18]. However, when we evaluated these procedures without repeated grinding and re-extraction, less than 45% of oil was recovered as oleosome fraction from full-fat soybean flour [19]. Kapchie et al. [19] demonstrated that a combination of cell wall hydrolyzing enzymes, mechanical disruption of cell walls and flotation centrifugation enhanced the oleosome recovery to 84.65% on a laboratory-scale. While promising, this study was a laboratory-scale with oleosomes production in grams. To generate enough oleosomes for further food and non-food utilization studies and demonstrate that oil can be extracted from soybeans efficiently without hexane or other petroleum

solvents, a pilot-scale study is needed. The objective of this study was to evaluate the effect of the scale-up of our oleosome fractionation process [19] on the proximate composition, oil and proteins recoveries, soybean storage protein profiles, and subcellular microstructure of the fractions. We focused on the recovery rather than the purification of oleosome fractions. We have already demonstrated that oil from isolated oleosomes may be quantitatively recovered [20].

Experimental Procedures

Full-fat soybean flour was obtained in 2009 from Natural Products Inc. (Grinnell, IA, USA) and stored at 4 °C for one week prior to use. The flour was not thermally treated. This flour had a particle size distribution of 0.63–52.48 μm (57.29%), 60.25–549.54 μm (41.12%) and 630.95–954.99 μm (1.58%), as measured by a laser light scattering instrument (Mastersizer 2000s, Malvern Instruments, Ltd., Chicago, IL, USA).

The enzymes used were: Multifect® Pectinase FE (E.C. 4.2.2.10, pectinase, optimal pH 3.85, optimal temperature 45 °C); Multifect® CX B (E.C. 3.2.1.4, cellulase complex standardized on β-glucanase, optimal pH 5.0, optimal temperature 40 °C); and Multifect® CX G (E.C. 3.2.1.4, cellulase, optimal pH 4.0, optimal temperature 55 °C) were acquired from Genencor Inc. (Genencor, a Danisco company Rochester, NY, USA).

Analytical grade reagents used were bought from Fisher Scientific (Pittsburgh, PA, USA) or Sigma (St. Louis, MO, USA). Food grade reagents (C&H® pure cane sugar, Carey® Salt) were purchased locally.

Laboratory-scale oleosome fractionation from 25 g of soybean flour was carried out according to the procedure by Kapchie et al. [19]. The difference between that procedure and current one was the replacement of cellulase A and Multifect CX3L with Multifect CX B and Multifect CX G, respectively, in the cell wall hydrolyzing enzyme cocktail, due to the discontinuation of former enzymes by Genencor. Moreover, the filtration step was eliminated, and only 0.1 M potassium acetate containing 0.5 M NaCl and 0.4 M sucrose was used during the process. The first extraction of oleosomes (Extraction 1) involved incubating the soybean flour and enzymes in 0.1 M potassium acetate pH 4.6, 0.5 M NaCl, 0.4 M sucrose, (1/6, dw/v) at 57 °C for 15 h, grinding with the Warning grinder for 3 min at maximum speed, and centrifuging with the swinging bucket rotor JS-4.0 (Beckman Avanti® J-20 Series Centrifuge, Beckman Instruments Inc., Fullerton, CA, USA) for 45 min at 2,278×g. The high osmotic and high ionic strength buffer was used to prevent disruption of organelle membranes [13–15, 19]. After the first extraction, the insoluble fraction and oleosome-free supernatant were mixed, ground and centrifuged again to recover residual oleosomes from the insoluble fraction. Three additional successive extractions were carried out (Extractions 2–4). The oleosomes from four extractions were combined to form a final oleosome fraction.

Pilot-scale oleosome fractionation from 75 kg of soybean flour was done in the Center for Crops Utilization Research pilot plant, Iowa State University. A 200 gallon (740 L) jacketed tank with a bottom sweep-arm agitator (Walker Stainless Equipment Company Inc., New Lisbon, WI, USA) rotating at 35 rpm was used for enzyme treatment (Fig. 1). Seventy-five kilogram of soy flour and 2,250 mL of 1% (v/w) of Multifect pectinase FE, Multifect CX B and Multifect CX G each were added to 445 L of 0.1 M potassium acetate pH 4.6, 0.5 M sodium chloride, 0.4 M sucrose and 0.05% benzoic acid. The pH of the buffer was adjusted with 2 N NaOH by using the Thermo Scientific Orion 3-Star Plus pH portable meter (Fischer Scientific, Pittsburgh, PA, USA). Then, the slurry was incubated at 57 °C for 15 h. The heating and temperature control was by a Microtherm water heater, Model 250-24, Chromolox (Pittsburgh, PA, USA). Benzoic acid at 0.05% was used to inhibit the potential microbial growth over prolonged isolation process [21]. The slurry was further ground and blended by feeding once through a Stephan Microcut® mill, which was equipped with a slanted-tooth cutting wing, with a tooth gap of 0.5 mm. The slurry was then pumped to a Centrysis® three-phase decanter, Model 10/4 (Centrysis, Kenosha, WI, USA). The operation parameters were as follows: decanter bowl centrifuge speed at 5,130 rpm (3,500×g) with scroll differential speed of 1.0 rpm; 160 mm liquid end Weir plate setting; 14 mm neutral of the third phase drainage tube setting; soy slurry feeding speed at 18.6 kg/min by a Moyno-progressive cavity pump (Electric Pump Model 1FFCA SSE SAA, Des Moines, IA, USA). In order to overcome the low yield of oleosome due to short residence time in the decanter, the insoluble and supernatant fractions were recombined manually in the mixing tank in a batch of 18.3 kg (collected every 15 min) for the insoluble fraction, and 13.5 kg (collected every 1.25 min) for the supernatant fraction. They were mixed along with the slurry in the tank. In this way, the slurry was re-circulated through the decanter with the oleosome fraction as the only continuous product. Two centrifugation periods were carried out. In the first centrifugation (Extraction 1), one batch of oleosomes was recovered in the first 6 h of re-circulation. After 6 h of the first centrifugation, the insoluble (Residue 1) and supernatant (Supernatant 1) fractions and the residual slurry inside the decanter bowls were carefully collected and mixed together to do a second centrifugation. This second step of oleosome extraction (Extraction 2) was carried out using the same procedure of re-circulation extraction as

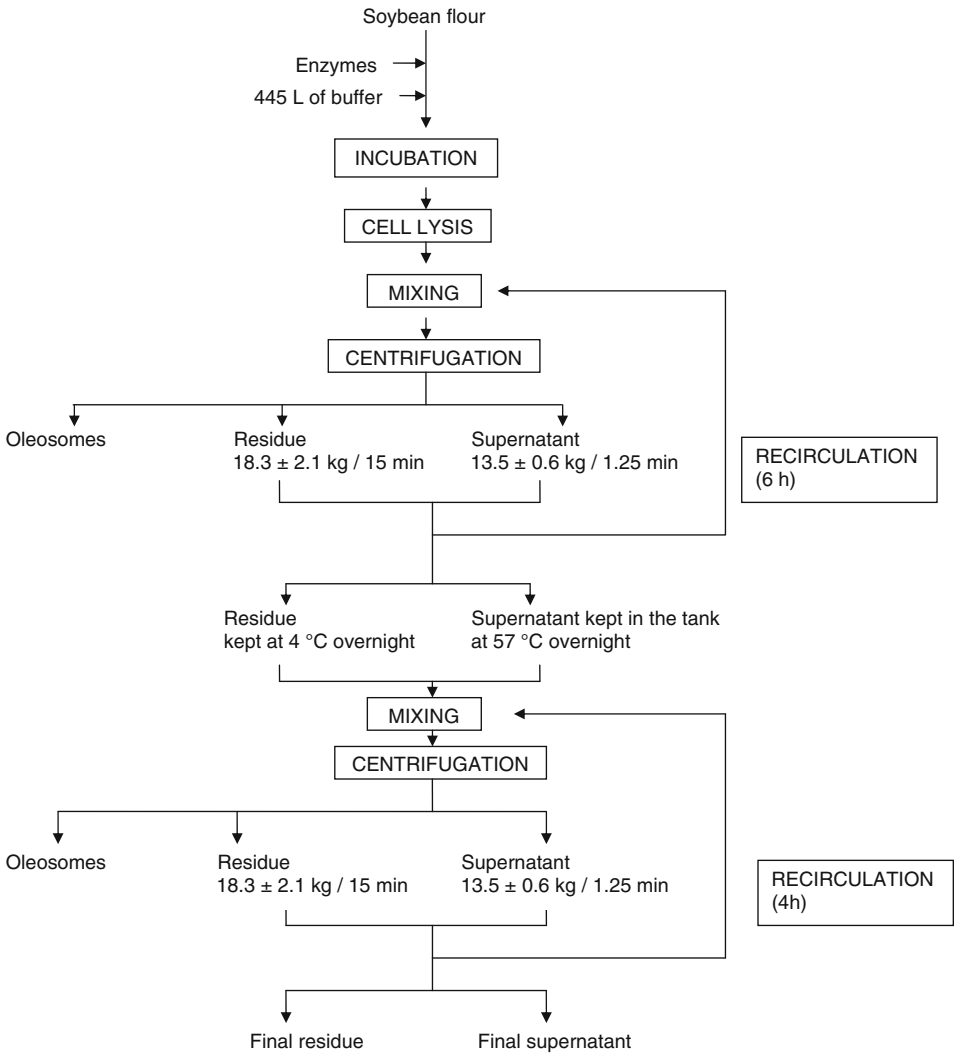

Fig. 1 Pilot-scale oleosome fractionation procedure from soy flour

described above. The centrifugation in the second extraction took about 4 h before sampling showed the supernatant was oleosome-free. One batch of oleosomes was collected in the second extraction. At the end of the second extraction, the supernatant (Supernatant 2) and insoluble residue (Residue 2) were collected as the final by-products. The oleosomes from the two extractions were combined to form the final oleosome fraction.

Proximate Analysis

Proximate analysis was conducted on the oleosome, supernatant and residue fractions collected during the laboratory- and pilot-scale processes. The dry matter content was determined by drying a 5–10 g sample in a force-convection oven at 105 °C for 48 h [22]. Oil contents were determined from the aforementioned dried samples using a Goldfische apparatus [22] and hexane according to the AOAC method [23]. Protein contents were evaluated with the Dumas method by using a rapid N III Nitrogen Analyzer (Elementar Americas, Inc. Mt. Laurel, NJ, USA), and were calculated as total nitrogen × 6.25 [24]. The ash content of different fractions was determined using an AACC method [22]. The carbohydrate contents were determined by difference [25]. The mass balances (or recoveries) were expressed as the percentages of each component in each fraction relative to the initial amounts in soybean flour on a dry weight basis.

Urea–SDS-PAGE and Gel Density Image Analysis

Samples of starting soy flour, oleosomes, and supernatants from the laboratory- and pilot-scale processes were prepared for the urea–SDS-PAGE according to Beisson et al. [10]. Twenty micrograms of a low molecular weight marker protein (6.5–66 kDa) was loaded as a standard, while 70 μg protein from soybean flour, oleosome or supernatant fractions was loaded per well. Electrophoresis was run as suggested by Lamsal et al. [26] with an SDS–Tris-glycine buffer system, 4% stacking gel and 13%

resolving gel (Biorad Mini Protean II Gel). Gels were scanned on an Amersham Pharmacia Biotech ImageScanner flatbed scanner with transparency module Software version 4.

Preparation of Sample for Transmission Electron Microscopy

One gram of fresh soy flour and isolated oleosomes from the laboratory- and pilot-scale extractions were fixed overnight at 4 °C in 3% glutaraldehyde, 2% paraformaldehyde in 0.1 M cacodylate buffer at pH 7.4. The samples were rinsed five times in cacodylate buffer and post-fixed for 2 h in 1% osmium tetroxide, 0.1 M cacodylate at pH 7.4 at room temperature. Fixation was followed by five buffer rinses, one de-ionized water rinse, and en bloc staining with aqueous 2% uranyl acetate for 30 min at room temperature. The samples were dehydrated in a graded ethanol series, transferred to pure acetone, and then infiltrated and embedded into Spurr's resin. Sections having silver to gold interference colors were cut with a diamond knife using a Leica UC6 ultramicrotome (Mager Scientific, PA, USA) then were collected on copper grids and examined with a JEOL 2100 scanning transmission electron microscope at an accelerating voltage of 200 kV (Japan Electron Optics Laboratory, Peabody, MA).

Statistical Analysis

The laboratory-scale oleosome extraction was repeated in triplicate, whereas the pilot-scale was a single determination to establish proof of concept. All analyses on the resulting fractions were performed in triplicate. The General Linear Model ANOVA, Least Significant Difference and Duncan tests in SAS System (version 9.2, SAS Institute, Inc., Cary, NC, USA) were used to compare data means at $p < 0.05$.

Results and Discussion

The laboratory-scale oleosome fractionation was conducted on 25 g of full-fat soy flour, whereas 75 kg was used for the pilot-scale. The results of the laboratory-scale reported here represent the control for the pilot-scale. We needed to repeat the experiments since Genencor changed the composition of the cellulases since our first report [19].

Ninety-three percent of the oil from the initial amount in the soy flour was recovered in the total oleosome fraction after 10 h of centrifugation in the pilot plant (Table 1). Oleosome isolation in the pilot plant was conducted in two centrifugation steps. Extraction 1 represents the first 6 h of re-circulation of the slurry in the decanter centrifuge. This gave one batch each of oleosomes, supernatant (Supernatant 1), and insoluble residue (Residue 1), respectively. Extraction 2 refers to the last 4 h of centrifuge re-circulation of the mixture constituted by combining supernatant 1 and residue 1. This gave a second batch each of oleosomes, final supernatant (Supernatant 2), and final residue (Residue 2), respectively. Oleosomes from extraction 1 and 2 were combined and analyzed as total oleosomes, whereas supernatant 2 and residue 2 were analyzed as the final by-products. The oil recovery from oleosomes collected in the first 6 h (76.69%) was 4.75 times higher than that collected the last 4 h (16.70%). While these results suggest that most of oleosomes were extracted during the first 6 h, the last 4 h were necessary to optimize the yield of oleosomes

Table 1 Mass balance of dry mass, oil, protein, ash and carbohydrate of pilot-scale oleosome fractionation

	Recoveries* (%)				
	Mass	Oil	Protein	Ash	Carbohydrate
Oleosomes					
Extraction 1**	21.43 ± 0.01^{e}	76.69 ± 0.92^{b}	17.86 ± 0.29^{e}	19.03 ± 0.82^{e}	17.40 ± 0.21^{e}
Extraction 2***	24.71 ± 0.27^{d}	16.70 ± 1.17^{c}	27.64 ± 0.30^{d}	22.98 ± 0.50^{d}	25.30 ± 1.46^{d}
Supernatant 1**	67.85 ± 0.86^{a}	16.59 ± 3.03^{c}	71.07 ± 0.8^{a}	70.99 ± 0.80^{a}	73.31 ± 1.64^{a}
Supernatant 2***	45.52 ± 0.13^{c}	4.94 ± 2.21^{e}	51.04 ± 0.77^{b}	54.08 ± 2.32^{b}	51.08 ± 0.76^{b}
Residue 1**	11.87 ± 0.10^{f}	10.11 ± 0.25^{d}	9.23 ± 0.27^{f}	10.80 ± 0.19^{f}	13.91 ± 1.12^{f}
Residue 2***	7.07 ± 0.06^{g}	1.25 ± 0.59^{f}	4.99 ± 0.12^{g}	6.97 ± 1.06^{g}	7.65 ± 0.16^{g}
Total oleosomes	46.32 ± 0.29^{b}	93.40 ± 0.88^{a}	44.71 ± 0.41^{c}	41.01 ± 0.39^{c}	42.71 ± 0.37^{c}

Oleosomes from extraction 1 and 2 are total oleosomes, Supernatant 2 and Residues 2 are the final by-products. Mean ± SD for a given component and fraction sharing the same lettered superscript in a column are not significantly different at $p < 0.05$, $n = 3$

db dry basis

* Percentage of each component relative to the initial amount in soybean flour

** Fraction collected the first 6 h

*** Fraction collected the last 4 h

isolated. After 10 h of oleosome extraction, most of the protein and ash from the initial amount in the starting material were recovered in the final supernatant at 51.04 and 54.08%, respectively. Only 4.94 and 1.25% of oil from the initial amount in the soy flour remained in the final supernatant and residue, respectively (Table 1). The laboratory and pilot plant fractionation procedures were similar, except that four extraction steps and the swinging bucket rotor centrifuge rotating at $2{,}278\times g$ (45 min × 4) were used for the laboratory-scale. Results showed that 76.83% of oil from the total in soy flour was recovered in the total oleosome fraction of the laboratory-scale compared to 93.40% achieved in the pilot plant (Table 2). A significant amount of oil was trapped in the supernatant and residue of the laboratory-scale (14.23 and 9.02%, respectively) (Table 2). This demonstrates that the oleosome fractionation process as designed in the pilot plant (Fig. 1) is an improvement over the predicted yield of oleosomes isolated in laboratory. The increase in yield was probably due to two reasons. One is the unique mechanism of horizontal decanter centrifuge where the slurry was constantly blended by the conveyor screw inside the rotating bowl. Combined with the re-circulation of the partially depleted oleosome slurry, such a mixing action inside the decanter centrifuge, may release more oleosomes from the residue than the closed batch laboratory centrifuge. The second probable reason for the increase in oleosome yield was that the centrifugation speed ($3{,}500\times g$) in the pilot plant decanter was higher than the one of the closed batch laboratory centrifuge ($2{,}278\times g$).

Dry matter, oil, protein and ash contents of first and second extractions of the pilot-scale process were significantly different for oleosome, supernatant and residue fractions (Table 3). Dry matter and oil contents of the

Table 2 Mass balance of dry mass, oil, protein, ash and carbohydrate of laboratory-scale oleosome fractionation

	Recoveries* (%)				
	Mass	Oil	Protein	Ash	Carbohydrate
Oleosomes**					
Extraction 1	26.54 ± 0.07^{b}	61.83 ± 1.67^{b}	24.50 ± 0.24^{c}	15.03 ± 0.77^{d}	21.01 ± 0.52^{d}
Extraction 2	7.34 ± 1.28^{d}	10.26 ± 1.08^{d}	8.61 ± 0.89^{e}	5.87 ± 0.83^{e}	6.28 ± 2.03^{e}
Extraction 3	3.81 ± 0.28^{e}	4.28 ± 0.07^{e}	3.70 ± 0.21^{f}	3.26 ± 0.24^{f}	3.77 ± 0.39^{f}
Extraction 4	2.75 ± 0.27^{f}	1.69 ± 0.19^{f}	2.39 ± 0.52^{g}	2.26 ± 0.22^{g}	3.08 ± 0.35^{f}
Total oleosomes	40.45 ± 1.38^{a}	76.83 ± 2.65^{a}	39.58 ± 1.46^{b}	26.45 ± 1.10^{b}	34.16 ± 2.55^{b}
Supernatant***	40.50 ± 2.06^{a}	14.23 ± 1.78^{c}	48.75 ± 1.68^{a}	51.65 ± 3.30^{a}	40.42 ± 0.79^{a}
Residue****	19.87 ± 1.30^{c}	9.02 ± 0.24^{d}	14.43 ± 1.50^{d}	21.45 ± 1.34^{c}	23.85 ± 1.21^{c}

Mean ± SD for a given component sharing the same lettered superscript in a column are not significantly different at $p < 0.05$, $n = 3$

db dry basis

* Percentage of each component relative to the initial amount in soybean flour

** Oleosome fraction collected from the first to fourth extraction

*** Supernatant collected after the fourth extraction

**** Insoluble residue obtained after the fourth extraction

Table 3 Proximate composition of pilot-scale oleosome fractionation

	Dry matter (%)	Oil (%, db)	Protein (%, db)	Ash (%, db)	Carbohydrate (%, db)
Oleosomes					
Extraction 1*	42.67 ± 0.02^{a}	35.33 ± 0.37^{a}	23.33 ± 0.49^{b}	8.05 ± 0.98^{b}	39.87 ± 1.38^{b}
Extraction 2**	30.90 ± 0.49^{b}	7.86 ± 2.45^{b}	24.39 ± 0.24^{a}	13.70 ± 0.07^{a}	56.03 ± 0.53^{a}
Supernatant 1*	27.65 ± 0.34^{a}	2.45 ± 0.47^{a}	22.58 ± 0.03^{a}	16.36 ± 0.06^{a}	58.59 ± 0.56^{b}
Supernatant 2**	26.86 ± 0.07^{b}	1.36 ± 0.04^{b}	21.12 ± 0.17^{b}	17.06 ± 0.65^{a}	60.72 ± 0.76^{a}
Residue 1*	33.56 ± 0.30^{a}	8.51 ± 0.15^{a}	15.72 ± 0.21^{b}	12.39 ± 0.13^{b}	63.39 ± 0.08^{b}
Residue 2**	29.03 ± 0.27^{b}	1.78 ± 0.86^{b}	16.17 ± 0.27^{a}	13.48 ± 0.02^{a}	68.55 ± 0.74^{a}

Oleosomes from extraction 1 and 2 are total oleosomes, Supernatant 2 and Residues 2 are the final by-products. Mean ± SD for a given component and fraction sharing the same lettered superscript in a column are not significantly different at $p < 0.05$, $n = 3$

db dry basis

* Fraction collected the first 6 h

** Fraction collected the last 4 h

oleosome fraction were lower in the second extraction than those in the first extraction. As more oleosomes were harvested over centrifugation time, less were available to be picked up by the collector tube in the three phase decanter resulting in dilution of second oleosome fraction with supernatant. The dilution of the oleosome fraction after the first extraction was also noticed in the laboratory-scale (Table 4). There was a decrease in dry matter and oil contents of the oleosome fractions from the first to the fourth extraction. As expected, at the end of oleosome centrifugation in the pilot- or the laboratory-scale, the insoluble residue had the highest carbohydrate content (63–68%), while the supernatant had the highest protein content (21–26%). The oil and protein contents of the total oleosome fractions from pilot-scale were not significantly different than that predicted by the laboratory results. Although this section demonstrated that our oleosome fractionation process can be efficiently transferred from laboratory to pilot plant, the protein composition and structural analysis of resulted fractions are needed to prove that the scale-up of our fractionation process did not affect the integrity of isolated oleosomes.

Protein Profiles

Several studies [10, 15, 18] reported that seed storage proteins are always evident in the oleosome preparations prior to purification. Figure 2 presents the soybean storage protein profiles determined by urea–SDS gel electrophoresis from soybean flour, oleosomes and supernatant in the pilot and laboratory-scales. All of the major soybean storage proteins subunits, α', α, and β subunits of the β-conglycinin and the acidic and basic subunits of glycinin as well as lipoxygenase, were present in the soy flour (Lane 2). These results were consistent with previous reports for glycinin and β-conglycinin in whole soybeans [27]. Proteins profiles from the oleosome and supernatant fractions from the pilot plant- (Lanes 3–8) or the laboratory-scale processes (Lanes 9–10) were similar. Lipoxygenase disappears in the oleosome and supernatant fractions. Most of the α'subunit of β-conglycinin disappears while new peptide bands were observed at 23 kDa and minor bands at 6.5 kDa. These results indicated that the protease activity was present in the Multifect pectinase FE as we have reported previously [28]. In fact, when a protease inhibitor cocktail for yeast and fungi proteases was included in the oleosome extraction protocol [28], the α', α subunits of the β-conglycinin and lipoxygenase were still present. However, the protease in the commercial pectinase was necessary for oleosome fractionation since we obtained very low yields of intact oleosomes in the presence of the protease inhibitor or if we substituted a highly purified pectinase for Multifect pectinase FE (data not shown). Similar degradation patterns of the storage proteins were observed by Jung et al. [29] when using 5 and 10% of Multifect pectinase FE to improve protein extraction yield from defatted soy flakes.

The presence of soy storage proteins in the pilot- and laboratory-scales oleosome preparations (Lanes 3–4, 6–7, 9) agrees with reports by Beisson et al. [10], Millichip et al. [15], and Chen and Ono [18]. Their studies showed that extensive washing of oleosome preparation in salt solutions followed by density gradient centrifugation resulted in oleosome purification. We can obtain highly purified intact oleosomes by washing the oleosome fractions with the sucrose–NaCl–acetate used in this report to remove the debris and soy storage protein contaminants (manuscript in preparation). Soybean oleosins should be found between 18 and 24 kDa according to Chabrand et al. [30]. On our SDS-PAGE, the oleosins would be in the same MW region as

Table 4 Proximate composition of laboratory-scale oleosome fractionation

	Dry matter (%)	Oil (%, db)	Protein (%, db)	Ash (%, db)	Carbohydrate (%, db)
Oleosomes*					
Extraction 1	45.02 ± 0.01^{a}	32.74 ± 0.31^{a}	20.48 ± 0.22^{d}	7.63 ± 0.04^{f}	35.78 ± 0.08^{f}
Extraction 2	35.19 ± 0.17^{b}	18.40 ± 2.43^{b}	24.24 ± 0.55^{b}	10.82 ± 0.04^{e}	44.53 ± 0.41^{e}
Extraction 3	31.64 ± 0.64^{c}	14.50 ± 0.90^{c}	21.54 ± 1.19^{c}	11.54 ± 0.01^{c}	52.18 ± 1.85^{d}
Extraction 4	29.92 ± 1.54^{d}	8.10 ± 1.41^{d}	21.54 ± 1.06^{c}	11.08 ± 0.01^{d}	59.38 ± 0.07^{b}
Supernatant**	23.65 ± 0.06^{f}	4.50 ± 0.36^{f}	26.63 ± 0.13^{a}	17.17 ± 0.18^{a}	58.11 ± 0.00^{c}
Residue***	26.94 ± 2.04^{e}	5.81 ± 0.44^{e}	16.04 ± 0.75^{e}	14.54 ± 0.07^{b}	63.59 ± 0.26^{a}

Mean ± SD for a given component sharing the same lettered superscript in a column were not significantly different at $p < 0.05$, $n = 3$

db dry basis

* Oleosome fraction collected from the first to fourth extraction

** Supernatant collected after the fourth extraction

*** Insoluble residue obtained after the fourth extraction

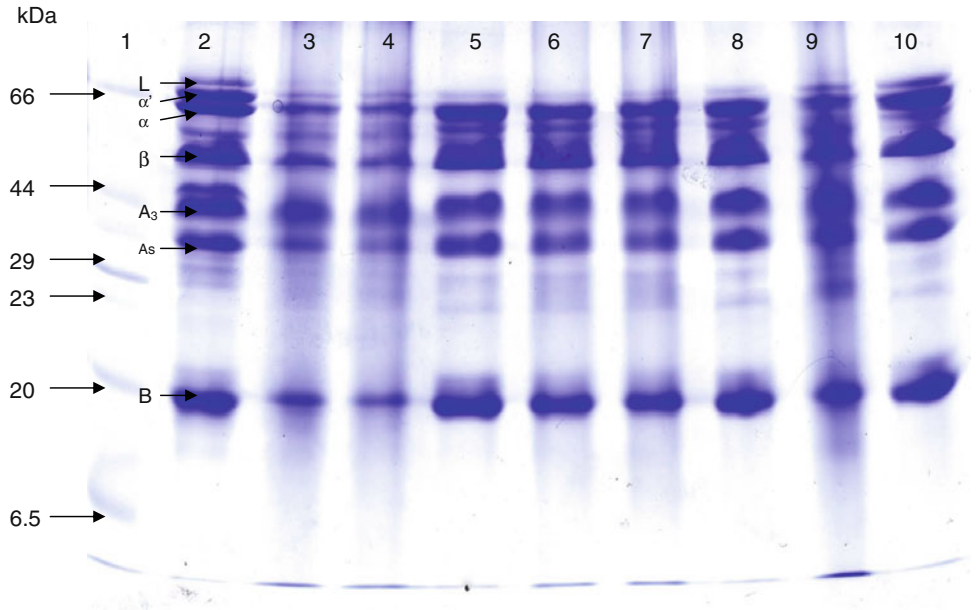

Fig. 2 Urea–SDS gel electrophoresis of proteins from soybean flour, oleosome and supernatant fractions. *Lane 1* low molecular markers (6.5–66 kDa) and calculated location of molecular weight 23 kDa, *lane 2* soy flour, *lanes 3–4* pilot-scale oleosomes collected the first 6 h, *lane 5* pilot-scale supernatant collected the first 6 h, *lanes 6–7* pilot-scale oleosome fractions collected the last 4 h, *lane 8* pilot-scale supernatant collected the last 4 h, *lane 9* laboratory-scale oleosome fraction, *lane 10* laboratory-scale supernatant, *L* lipoxygenase, *α'*, *α* and *β* subunits of *β*-conglycinin, *A* acidic peptides of glycinin, *B* basic peptides of glycinin. 20 μg protein loaded in *lane 1*, 70 μg protein loaded in each *lane* from *2* to *10*

the glycinin basic subunits, 20 kDa, and may be obscured. These results demonstrate that the scale-up of our oleosome fractionation process does not affect the protein profiles of oleosome and supernatant fractions [28]. Additionally, we have shown that the soybean storage proteins can be recovered in good yield from the supernatant of our scale-up process [28].

Although the protein profile from oleosome and supernatant fractions from the pilot or laboratory-scale processes were similar, the distribution of the protein in these fractions is significantly different ($\alpha = 0.05$) (Table 5). Densitometric analysis of the gels proteins in Fig. 2 was used to quantify the distribution of glycinin and *β*-conglycinin in soybean flour, oleosomes and supernatants from the laboratory- and pilot-scales. The amount of glycinin and *β*-glycinin as a percent of the total starting soy flour protein was 31.15 and 29.38%, respectively (Table 5). Most of glycinin and *β*-conglycinin from whole seed were recovered in the supernatant as presented in Tables 1 and 2. These data are in accordance with our previous report on recycling of aqueous supernatants in soybean oleosome fractionation [28]. The glycinin and *β*-conglycinin represented 7–10 and 6–9% of the total oleosome mass. The proteins recovery from our oleosome preparation is higher than that reported by Tzen et al. [14]. The purity of our oleosome preparation can be improved by washing with the sucrose–NaCl–acetate isolation media used to fractionate the soy flour (manuscript in preparation).

Microstructures

Soybean oleosomes are 0.2–0.5 μm diameter spherules consisting of a lipid core bounded by a single fine-line membrane [1–6, 31]. Electron microscopy analysis was performed to evaluate the effect of our fractionation procedure scale-up on the integrity of isolated oleosomes (Fig. 3). Oleosomes in the dry soybean flour represent the control. The electron micrographs reveal that the laboratory- or pilot-scale preparations show isolated oleosomes slightly contaminated with membrane debris. This is in accordance with reports by Jacks et al. [32] showing that the peanut oleosomes preparation is rich in the storage proteins and membrane debris prior to the purification and supports results from Fig. 2. Isolated oleosomes in the laboratory or pilot plant products were spherical subcellular organelles 0.2–0.5 μm in diameter filled with lipid and surrounded by an intact membrane. They were identical in size to the oleosomes in situ [1–6, 31]. The isolated oleosomes were subjected to freeze/thaw or heating, and did not release free oil as would be expected for an oil-in-water emulsion stabilized by soy

Table 5 Distribution of glycinin and β-conglycinin fractions of starting soybean flour, oleosomes and supernatants of the laboratory- and pilot-scales

	% of total mass (wb)		
	Acidic	Basic	Glycinin
Soy flour	14.30 ± 0.00[a]	16.85 ± 0.00[a]	31.15 ± 0.00[a]
Oleosomes			
Laboratory-scale	3.82 ± 0.00[e]	4.05 ± 0.00[e]	7.87 ± 0.00[e]
Pilot-scale	4.45 ± 0.16[d]	6.12 ± 0.31[d]	10.57 ± 0.49[d]
Supernatant			
Laboratory-scale	6.89 ± 0.00[c]	9.52 ± 0.00[c]	16.41 ± 0.00[c]
Pilot-scale	8.63 ± 0.80[b]	11.17 ± 0.57[b]	19.81 ± 1.38[b]

	α'	α	β	β-Conglycinin
Soy flour	8.28 ± 0.00[a]	8.15 ± 0.00[a]	12.93 ± 0.00[a]	29.38 ± 0.00[a]
Oleosomes				
Laboratory-scale	1.11 ± 0.00[d]	2.26 ± 0.00[e]	3.22 ± 0.00[e]	6.60 ± 0.00[d]
Pilot-scale	1.49 ± 0.21[c]	3.77 ± 0.5[d]	4.94 ± 1.25[d]	9.08 ± 0.68[c]
Supernatant				
Laboratory-scale	2.94 ± 0.00[b]	7.167 ± 0.00[b]	8.38 ± 0.00[c]	18.50 ± 0.00[b]
Pilot-scale	1.63 ± 0.21[c]	6.28 ± 0.33[c]	10.54 ± 0.59[b]	18.46 ± 0.46[b]

Mean ± SD for a given component and fraction sharing the same lettered superscript in a column are not significantly different at $p < 0.05$, $n = 2$

wb wet basis

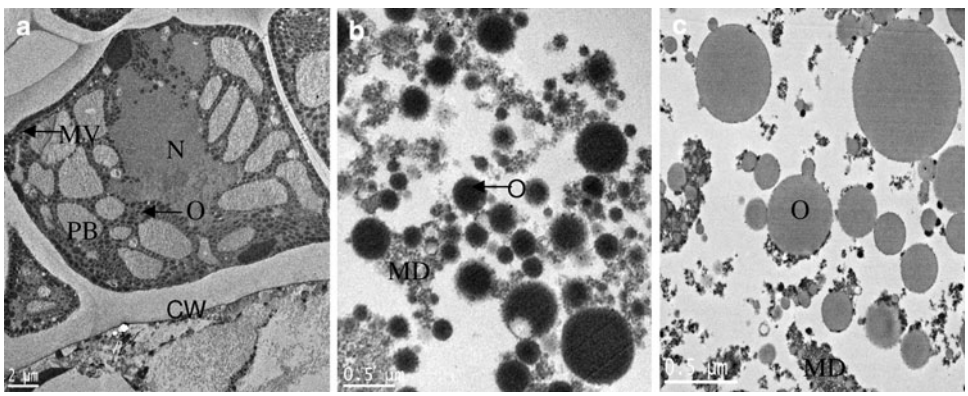

Fig. 3 Electron micrographs of oleosomes in **a** dry soybean flour, **b** laboratory-scale process preparation, **c** pilot-scale process preparation. *CW* cell wall, *O* oleosomes, *MV* cell ultrastructure (mitochondria or endoplasmic reticulum), *MD* membrane or proteins debris, *N* nucleus, *PB* proteins body

protein and lecithin [20, 30, 33, 34]. Our results demonstrated that the scale-up of our fractionation process does not affect the size or and shape of isolated oleosomes. The resulting isolated oleosomes were found to be intact.

Conclusion

The pilot plant fractionation procedure significantly increased the yield of oleosomes isolated from soybean flour to 93.40% compared to laboratory-scale process. The proximate composition of oleosome recovered in the pilot plant-scale was comparable to that of the laboratory-scale. The protein profiles of oleosome and supernatant fractions from the pilot- and laboratory-scale are similar. Electron microscopy shows that oleosomes isolated by the laboratory- or the pilot plant process were essentially identical to those of in situ oleosomes.

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