Flaking and Extrusion as Mechanical Treatments for Enzyme-Assisted Aqueous Extraction of Oil from Soybeans

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ABSTRACT: Flaking and extruding dehulled soybeans were evaluated as a means of enhancing oil extraction efficiency during enzyme-assisted aqueous processing of soybeans. Cellulase, protease, and their combination were evaluated for effectiveness in achieving high oil extraction recovery from extruded flakes. Aqueous extraction of extruded full-fat soy flakes gave 68% recovery of the total available oil without using enzymes. A 0.5% wt/wt protease treatment after flaking and extruding dehulled soybeans increased oil extraction recovery to 88% of the total available oil. Flaking and extruding enhanced protease hydrolysis of proteins freeing more oil. Treating extruded flakes with cellulase, however, did not enhance oil extraction either alone or in combination with protease. Discrepancies in oil extraction recoveries were encountered when merely considering crude free fat because some oil became bound to denatured protein during extrusion and/or sample drying. Bound fat was unavailable for determination by using the hexane extraction method, but was accounted for by using the acid hydrolysis method for total oil determination. Oil extraction recovery from extruded soybean flakes was affected by oil determination methods, which was not the case for unextruded full-fat soy flour.

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KEY WORDS: Aqueous extraction, extrusion, enzyme treatment, oil extraction, protease, soybeans, soy oil, soy protein.

The vast majority of soybean oil is extracted by using organic solvents, hexanes (a mixture of hexane isomers usually containing >60% *n*-hexane) being the most efficient solvent, achieving oil recoveries in excess of 95% and defatted meal residual oil contents of <1% (1). Currently, direct hexane extraction is the most cost-effective oil recovery method for soybeans. Hexane, however, has characteristics that make it challenging to use, including high flammability, making it costly to use safely. The loss of hexane during oilseed processing is a major source of hexane emissions to the atmosphere (2). In recent years, the U.S. Environmental Protection Agency has enacted emission standards for the vegetable oil extraction industry, imposing stringent restrictions and financial penalties on hexane losses. Viable alternatives to hexane extraction are to use extruding-expelling or drying and screw pressing, and in case of soybeans (3), these processes leave 7–10% residual oil contents in the meal. Both of these mechanical procedures also denature protein, rendering it deficient in functional properties for food use.

Aqueous extraction processing (AEP) uses water as an extraction aid or medium and has been extensively studied as an alternative to hexane extraction (4). AEP is based on insolubility of oil in the medium rather than its dissolution and uses the same principle as hot water flotation as used in palm oil extraction (5). Once the seed is ground in water, the oil is released and floats as an emulsified cream and/or free oil (6). There has been a resurgence of interest in AEP because it is regarded as "green" processing with little environmental impact, and there may be opportunities to add value to co-products and use the method as the front-end to a soybean biorefinery. AEP has been evaluated with and without incorporating enzyme hydrolysis of proteins and degradation of cell wall components for various oil-bearing materials such as sunflower seeds (7,8), rapeseed (9), coconut (10), corn germ (11), rice bran (12), palm kernels (13), soybeans (14–16), and peanuts (17). Oil extraction recoveries typically range from 65 to 75% of the total available oil. This comparatively low oil extraction recovery and high oil content in the residue have discouraged commercial adoption of AEP.

Critical steps in improving oil extraction during AEP of oil-bearing seeds are those operations used to rupture cell walls and release the oil so that it can be recovered as an emulsified cream, or even more preferably, as free oil. Enzymes have been shown to be helpful in such separations, and interest in enzyme-assisted AEP is increasing as enzyme costs decline. Most of the prior work on AEP with or without enzymes has used ground material (full-fat flour) prior to extraction. Grinding alone does not completely rupture cell walls, which is a key barrier to recovering oil by AEP. Rosenthal *et al*. (16) reported that oil recovery improved by reducing full-fat soybean flour to smaller particles; oil extraction increased from 33 to 64% of total when flour particles were reduced from 400 to 100 μm. Yoon *et al*. (18) obtained 62% oil extraction when flour particle size was reduced to <150 mesh. Very fine grinding, however, may produce smaller oil globules, smear oil over protein and fiber particles, and make a more stable emulsion cream phase that must be broken to recover free oil. We believe that

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other forms of mechanically treating oil-bearing materials that do not require extensive grinding while achieving greater cell distortion, such as flaking and extruding (or expanding), are worthy of investigation.

During flaking, conditioned (moistened and heated) soybean cotyledons are passed through a small gap between two counter-rotating smooth rolls turning at different speeds, thus imparting compression and shear (19). These distort and disrupt cell walls and reduce the thickness of plasticized cotyledons. This is acceptable for conventional oil extraction with hexane, but for AEP, further mechanical treatment of flaked material may be necessary. Extruding flakes provides vigorous thermal/mechanical treatment that may be helpful in rupturing cell walls (cell distortion) and expelling some free oil (3). At the same time, heat denatures the protein, which can sequester some of the free oil. We hypothesized that combining flaking and extruding would be effective in increasing oil recovery in subsequent enzymatic extraction in an aqueous medium (AEP). The only prior report of using extrusion as a pretreatment for enzyme-assisted AEP has been Frietas *et al*. (20), who reported improved oil recovery when extruding unflaked and unground, dehulled soybean cotyledons. Extruding dehulled soybeans without flaking, however, does not achieve as extensive cell distortion as does extruding flaked soybeans. We believe achieving extensive cell distortion (21) is critical in AEP, perhaps even more so than in traditional hexane extraction. Our approach was similar to that used to expand soybean for hexane extraction (22,23), except we extruded directly into water. Unlike previous work, we also used total oil measurements to quantify oil extraction recoveries, which we believe are much more accurate.

Extrusion enhances the action of enzymes on cell components by increasing the surface area and denaturing protein, which increases the susceptibility of protein to enzyme attack and reduces the stability of the resulting difficult-to-break oil-rich cream emulsion (cream phase). Proteases degrade oleosin, a lipophilic protein, which surrounds lipid globules and comprises lipid body membranes, to facilitate oil release (6). Mixtures of cellulases, hemicellulases, and pectinases have been used, recovering as much as 75% of the total available oil but not with flaked and/or extruded materials, which may enhance cell wall and membrane degradation (barriers to oil release). The objectives of the present study were: (i) to evaluate different mechanical treatments, including flaking and extrusion, of full-fat soybean flakes in AEP; and (ii) to determine the effectiveness of proteases and cellulases to enhance oil recovery when using flaked and extruded full-fat soybean flakes.

EXPERIMENTAL PROCEDURES

Full-fat soy flakes and flour. Full-fat soy flakes were prepared from variety IA1008 soybeans harvested in 2002. The soybeans were cracked by using a corrugated roller mill (model 10X12SGL; Ferrell-Ross, Oklahoma City, OK) and aspirated by using a cascade aspirator (Kice Metal, Wichita, KS) to separate into meats and hulls fractions. The meats were conditioned to 60ºC by using a triple-deck seed conditioner (French Oil Mill Machinery Co., Piqua, OH). The conditioned meats were flaked to approximately 0.30 mm thickness by using a smooth-surfaced roller mill (Roskamp Champion, Waterloo, IA). The flakes contained 23.8% oil (dry basis), 42.7% protein (dry basis), and 9% moisture (as-is basis). The flakes were sealed in plastic bags and stored at 4°C until used. During initial extrusion experiments, once-pin-milled full-fat soy flour was used for comparison by using a pin mill (model UT-03; Bauermeister, Inc., Memphis, TN).

Extrusion. A twin-screw extruder (18-mm screw diameter, Micro 18; American Leistritz Extruders, Somerville, NJ) was used for all extrusion trials. For initial experiments, full-fat soy flour and flakes were extruded at 15% feed moisture, 100 and 120°C barrel temperature, and 100 rpm rotational speed with a high-shear geometry screw. The extruded pellets were dried to 6% moisture under a hood. The dried pellets were either ground in a coffee grinder or used unground. Full-fat soy flour and flakes were also extruded directly into a water bath.

Screening extrusion conditions. Based on encouraging results from the initial experiments, operating conditions for extruding full-fat soy flakes were screened for maximal oil recovery. Three parameters observed to affect oil recovery were process temperature, shear as manifested by screw geometry and rotational speed, and feed moisture content. By keeping the screw geometry constant, a 2^3 factorial experiment for determining the effects of temperature (T), moisture content (M), and screw rotational speed (R) was designed.

Eight variable combinations (runs) for one replicate were needed. Only four runs were possible each day due to the time required for subsequent aqueous extraction and separation. To account for day-to-day variability, two experimental blocks of four runs each were formed such that the third-level variable interaction was confounded with this variability (24). The extrusion parameters and their levels were temperature (100 and 120°C), flake moisture (12 and 14%), and screw rotational speed (100 and 150 rpm). The high-shear geometry screw was kept the same throughout the experiment.

About 20 kg full-fat soy flakes (9% moisture) were adjusted to the desired moisture level by spraying water onto the flaked meats in a Gilson mixer (model # 59016A; St. Joseph, MO). The moisture-adjusted flakes were then placed into double polyethylene bags and kept at 4°C until used. About 200 g of pellets were directly extruded into a bath of water at 100 rpm screw speed (63 g/min feed rate) and at 150 rpm screw rotational speed (93 g/min feed rate), and AEP oil recoveries were compared. Three replicates of each treatment (total 24 runs) were carried out, and means were reported. Flake moisture contents were measured during every extrusion run, and the means ± 1.0 SD were 14.1 ± 0.4% for high- and 11.8 ± 0.36% for low-moisture flakes.

AEP simulation and oil recovery determination. AEP simulation using either full-fat soy flakes or extruded pellets was carried out in a 3-L glass reaction vessel (model CG-1929-16; ChemGlass Inc., Vineland, NJ) following procedures described by Rosenthal *et al*. (15). A 10:1 wt/wt water-to-pellet ratio,

50°C extraction temperature, 8.0 slurry pH, and 200-rpm impeller speed (model BDC 3030 stirrer; Caframo, Wiarton, Ontario, Canada) were used. The impeller had two 5-cm diameter propellers—one placed 2.5 cm from the bottom of the reactor and another 10 cm above it—which provided gentle stirring action. The extraction time was 15 min after the pH was adjusted; longer extraction times have not been shown to improve oil recovery (16). The slurry was separated into an aqueous fraction comprised of extracted oil (free oil and cream emulsion) and skim milk and an insoluble precipitate fraction by centrifuging at 3000 × *g* and 20°C for 20 min. The insoluble fraction was either oven-dried (130°C for 7 h) or freeze-dried, and oil and protein contents were determined. Oil or protein extraction recoveries were calculated as the difference between total oil or protein in the insoluble fraction compared with the starting flakes: extraction recovery, % = 100 × [(oil or protein in starting sample) – (oil or protein in insoluble fraction)]/oil or protein in the starting material.

Extrusion- and enzyme-assisted AEP. The best combination of parameters from the 2^3-factorial experiments in the extrusion screening experiments was selected for extrusion and enzyme-assisted AEP. One cellulase (Experimental Cellulase A, 308004, from *Aspergillus* sp.) and one commercial protease (Multifect Neutral from a proprietary bacterial source, 1738 AZO/g) from Genencor International, Inc. (Rochester, NY) were used either alone or in combination. Enzyme selection was based on literature that suggested prior cellulase treatment enhances the effectiveness of protease in freeing oil (8). The enzymes [1% wt/wt Cellulase A, 0.5% wt/wt Multifect Neutral, and a combination of 0.5% wt/wt Cellulase A and 0.5% wt/wt Multifect Neutral] were evaluated with extruded full-fat soy flakes for oil extraction. Enzyme concentrations were expressed as weight percentages per weight of dry flakes. The combination of enzymes was added either at the start of the extraction period or Cellulase A at time 0 min followed by Multifect Neutral after 30 min. The enzyme concentrations selected were based on preliminary experiments and did not represent optimal levels.

The enzyme-treated slurry was stirred in 3-L glass beakers for 1 h at 50°C and pH 7.0, which were optimal for the enzymes used. The slurry pH was then increased to 8.0 and stirred for 15 min to conform to previously used AEP conditions. The treated slurry was then centrifuged at 3000 × *g*, and the insoluble fraction was oven-dried at 130°C for 7 h. The oil and protein contents of insolubles were determined and recoveries calculated.

Oil, protein, and moisture contents. Total oil contents were determined on 2-g samples of full-fat soy flour, full-fat soy flakes, extruded full-fat flakes, and insoluble fractions by using the acid hydrolysis Mojonnier method (AOAC Method 922.06) unless stated otherwise (25). Protein contents were determined by using a combustion-type nitrogen analyzer (Elementar Americas, Mt. Laurel, NJ) and the Dumas method (AOAC Method 993.13) (25). The nitrogen values were multiplied by 6.25 to estimate protein content. Moisture contents (or % total solids) were determined by heating samples in a forced-draft oven at 130°C for 3 h.

Statistical analysis. The General Linear Model, PROC GLM, in SAS system (version 8.2, SAS Institute, Inc., Cary, NC) was used to compare means at $P < 0.05$.

RESULTS AND DISCUSSION

Extruding flakes and flour into water without enzymes. Previous work on aqueous extraction of oil from soybeans has mostly used full-fat flour, achieving oil recoveries <65% (16,18) for nonenzyme-assisted aqueous extraction. Extruding full-fat flour for aqueous extraction of oil was beneficial under certain conditions (Fig. 1). Only 50% of the total available oil was recovered in the aqueous extraction of unground full-fat soy flour extrudates. Grinding disrupted the porous pellets, which were about 2–3 mm in diameter and 5–7 mm in length,

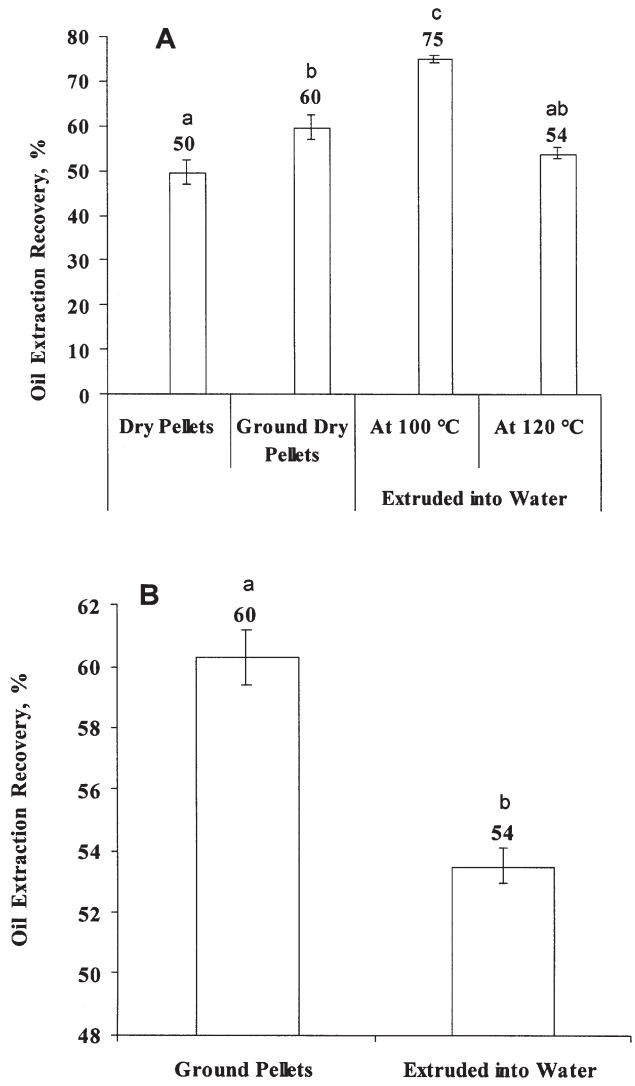

FIG. 1. Oil extraction recoveries during aqueous extraction processing (AEP) of extruded full-fat flour (A) and extruded full-fat flakes (B) under different conditions. Flour was extruded at 100 or 120°C and flakes were extruded at 120°C. AEP was carried out at 50°C, pH 8, 15 min for both extruded flour and flakes. Oil extraction recoveries sharing the same letter were not statistically different at $P < 0.05$.

TABLE 1
Aqueous Extraction Processing Recoveries for Oil and Protein and Corresponding Variable Effects in a 2^3 Factorial Experiment[a]

	Variables			Oil extraction recovery (%)		Protein extraction recovery (%)	
Run	*T*	*M*	*R*	Mean ± SE	Variable effect	Mean ± SE	Variable effect
(1)	100	12	100	53.2 ± 3.7		50.6 ± 1.6	
T	120	12	100	46.4 ± 2.3	–9.8[a]	32.9 ± 1.3	–21.6[a]
M	100	14	100	55.2 ± 5.9	1.7[b]	58.7 ± 0.6	0.9[b]
TM	120	14	100	45.3 ± 4.6	–1.8[b]	28.4 ± 1.6	–2.7[b]
R	100	12	150	49.9 ± 4.2	–3.2[b]	54.4 ± 0.4	1.8[b]
TR	120	12	150	40.6 ± 3.9	–1.5[b]	34.4 ± 0.5	2.4[b]
MR	100	14	150	55.0 ± 5.9	1.3[b]	53.7 ± 1.9	–0.9[b]
TMR	120	14	150	41.68 ± 10.7	–0.3[b]	35.2 ± 2.5	3.5[c]

[a]Variables are *T*, barrel temperature, °C; *M*, flakes moisture content, % wet basis; and *R*, screw rotational speed, rpm. Run codes represent one-, two- or three-level variable interactions. SE, standard error of mean ($n = 3$). Variable effects sharing the same superscript were not statistically different at $P < 0.05$.

and increased oil recovery to 60%. Oil extraction from full-fat soy flour, when extruded at 100°C directly into water, significantly improved (75% of total oil recovered).

The free oil that was present immediately post-extrusion/expansion was exposed to water without extensive interaction with denatured protein and other emulsifying agents such as lecithin. Increasing the extrusion temperature to 120°C, however, was not helpful and oil recovery was only 54%. Frothing of free oil was observed at 120°C, but not at 100°C, and a slick of free oil formed on top of the aqueous phase. This observation, however, did not translate into higher oil recoveries at higher extrusion temperature, likely owing to more protein denaturation and sequestering of oil before water could compete for the oil. Extruding full-fat soy flakes at 120°C (Fig. 1, bottom) also had a similar trend. The recoveries when extruded at 120°C were similar for both extruded flour and flakes. Since we were able to extrude full-fat soy flakes with the lab-scale extruder at low moisture, we used flakes instead of flour in further extrusion experiments. It is an industry practice to expand (extrude) full-fat soy flakes, not flour, for hexane extraction.

Screening extrusion conditions. Mean oil and protein extraction recoveries, their SE, and statistical main and interaction effects of variables are presented in Table 1. Oil extraction recoveries ranged from 41 to 55% for different processing conditions. Protein extraction recovery ranged from 28 to 58%. The 95% Bonferroni confidence intervals for statistical effects on oil and protein recoveries were 6.9 and 3.15, respectively. Extrusion temperature significantly and negatively affected oil recovery. Other variable effects and their interaction effects were not significant.

For protein extraction, the effect of temperature and the three-level interaction between variables were significant, with temperature having a negative effect. Extruding at higher temperature resulted in lower protein extraction. The large statistical effect for protein (–21.6) highlighted the importance of temperature during extraction, with higher temperature causing proteins to denature and become insoluble. The three-level variable interaction, although positive, was confounded with the block effect by design, rendering it unusable. The results suggested that for the higher oil and protein extraction recoveries, extrusion temperature needed to be low. The best extrusion conditions for full-fat soy flakes with our lab extruder were 100°C barrel temperature, 14% flake moisture content, and the high-shear screw rotational speed of 100 rpm. These extrusion conditions were kept constant for subsequent enzyme-assisted AEP.

Extrusion- and enzyme-assisted AEP. Mean oil and protein recoveries for enzyme-assisted AEP of extruded full-fat soy flakes are shown in Figure 2. Cellulase A at a 1% level was not effective with extruded flakes in increasing oil and protein extraction over the no-enzyme control. Protease treatment alone (0.5% Multifect Neutral) or in combination with cellulase (0.5% each enzyme), added either together or sequentially, significantly improved oil and protein extraction recoveries. Cellulases are effective in breaking down cell walls and facilitating oil release (6,7), but the compression and high shear generated during flaking and extrusion in our study must have disintegrated cell wall materials to the extent that further cellulase treatment was not effective.

Proteolytic enzymes hydrolyze the proteins in cell membranes as well as inside the cytoplasm, such as oleosins (6). They affect the cytoplasmic network, largely composed of proteins in case of soybeans, thereby making the network less compact. A less compact cytoplasmic network facilitates easier removal of oil and protein by aqueous media. The data in Figure 2 show increased extraction of protein and oil achieved due to protease treatment. Oil and protein extraction recoveries for 0.5% Multifect Neutral over the control increased by 29 and 48%, respectively. Our 88% oil extraction recovery with protease was similar to values reported by Freitas *et al.* (20) from unflaked, dehulled soybeans following extrusion and enzyme-assisted AEP by using a cellulase and a protease, although we used acid hydrolysis determination of total fat and they used a free fat determination, in which we have less confidence (see later discussion about bound and free fat). Comparison of the amounts of enzyme required or incubation period is not meaningful given the variation between the studies in extrusion conditions, starting material, and enzyme activity and concentration used. Extrusion of flaked soybeans is clearly effective in extracting more oil during enzyme-assisted AEP (Fig. 3). Extrusion alone increased the oil recovery from 46 to 71%. Protease action on unextruded flakes

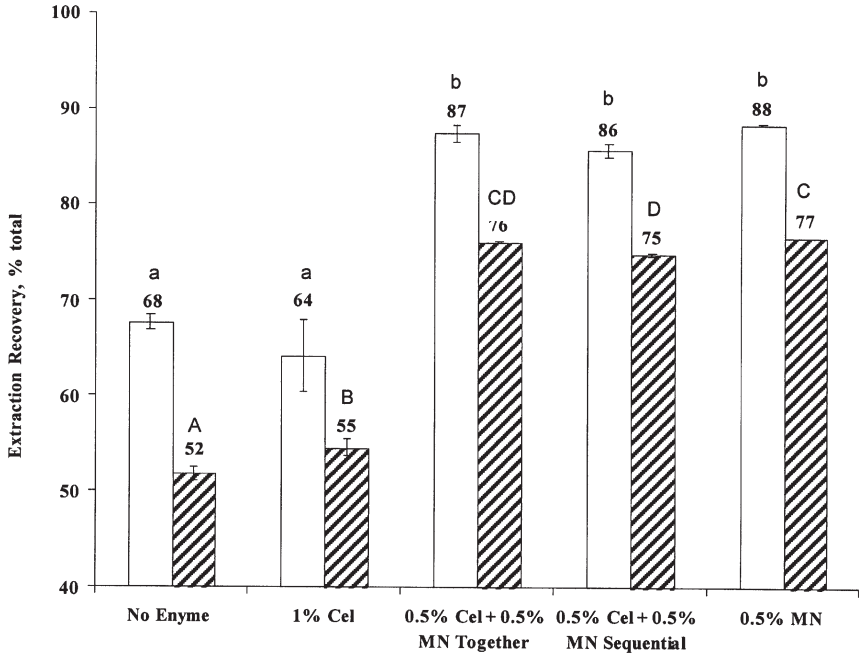

FIG. 2. Oil (open bars) and protein (hatched bars) extraction recoveries for enzyme-assisted AEP of extruded full-fat soy flakes. Cel, Cellulase; MN, Multifect Neutral. Extrusion conditions: 100 rpm screw rotational speed, 100°C barrel temperature, and 14% flake moisture content. Extraction recoveries sharing the same letter (lowercase for oil and uppercase for protein) were not statistically different at $P < 0.05$. For other abbreviation see Figure 1.

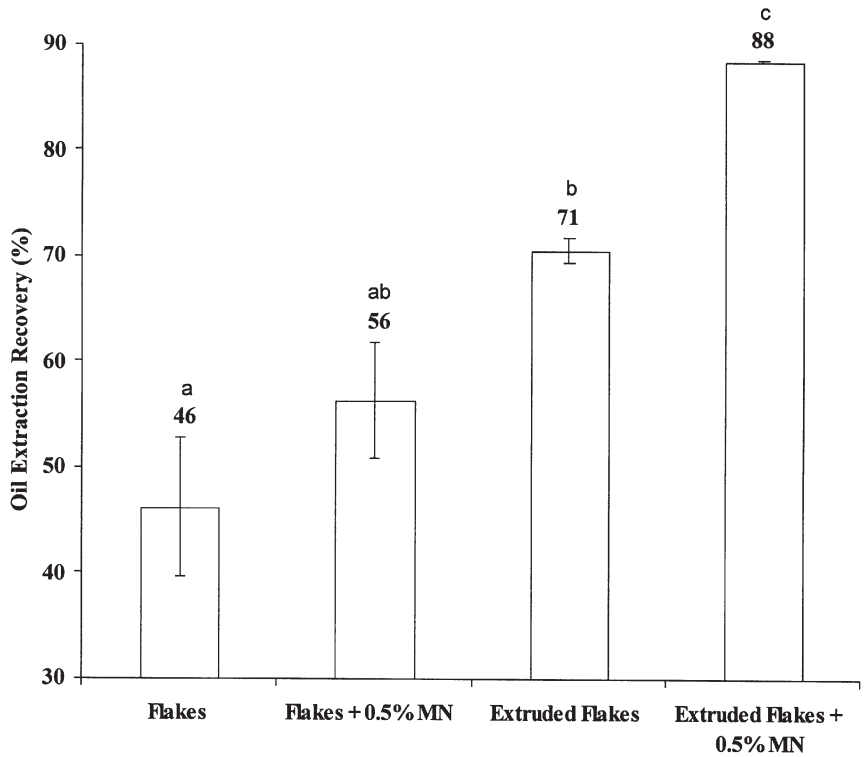

FIG. 3. Oil extraction recoveries for full-fat flakes, full-fat flakes with 0.5% protease (MN, Multifect Neutral), extruded full-fat flakes (no enzymes), and extruded full-fat flakes with 0.5% MN w/w. Extrusion conditions: 100 rpm screw rotational speed, 100°C barrel temperature, and 14% flake moisture content. Oil extraction recoveries sharing the same letter were not statistically different at $P < 0.05$.

was not as effective as it was with extruded flakes. Extrusion facilitated protease action resulting in oil recovery increasing from 71 to 88%. Our results compare favorably, or are even slightly higher, with oil extraction recovery of 78 (15) and 86% (16) from full-fat soy flour during AEP.

Whereas the results in Table 1 suggested conditions effective in increasing oil recovery from extruded full-fat soy flakes in a lab-scale extruder, the results in Figures 2 and 3 emphasized the importance of extrusion in facilitating enzymatic action. It was surprising, however, to observe the difference between the oil extraction recoveries of 55.2% (Table 1, Run M conditions) and 68% (Fig. 2) for no-enzyme extruded flakes. Even though the extrusion conditions were the same for these sets of experiments, some extraction details and drying conditions for the insoluble fraction differed. AEP conditions for the 2^3 factorial experiments were pH 8, 50°C for 15 min in a 3-L glass reactor and those for enzyme-assisted AEP were pH 7, 50°C for 1 h followed by pH 8, 50°C for 15 min in 3-L glass beakers. The insoluble fractions were freeze-dried in 2^3 factorial experiments and oven-dried at 130°C for 7 h in enzyme-assisted AEP experiments. So, as described below, we looked into these differences to ascertain whether they affected oil extraction recoveries.

Comparisons of pH and extraction time. Full-fat soy flakes were extruded (100°C barrel temperature, 100 rpm screw rotational speed, and 14% flake moisture content) directly into water. The pH of the 1:10 w/w extrudate/water slurry was adjusted to 7, 8, or 9 with 2 N NaOH and stirred for 15 min, 1, 2, or 3 h. The insoluble fraction after centrifugation was oven-dried at 130°C for 7 h. The oil content was determined by Mojonnier method, and oil extraction recovery was calculated. Each experiment was duplicated and means were compared. There were no significant differences ($P < 0.05$) in oil extraction recoveries at pH 7, 8, or 9 extracted for 15 min or 1, 2, or 3 h. At pH 7, the mean and SE of oil recoveries when extracted for 15 min and 1, 2, and 3 h were 67 ± 3, 72 ± 2, 70 ± 1, and 70 ± 1%, respectively. At pH 8, oil extraction recoveries for 15 min and 1, 2, and 3 h extraction times were 69 ± 0.3, 73 ± 0.6, 73 ± 0.1, and 72 ± 0.5%, respectively. Raising the pH to 9.0 did not result in oil extraction recovery greater than 75% at any extraction time. Rosenthal *et al.* (16) reported no increase in oil extraction after 15 min at pH 8 during AEP of full-fat soy flour. Thus, there were no effects due to pH and extraction time on variation in oil extraction recoveries seen during earlier experiment sets.

Comparison of extraction vessels and insoluble fraction drying methods. AEP of full-fat soy flour and extruded full-fat soy flakes was carried out in either a 3-L glass reactor vessel (model CG-1929-16; ChemGlass, Inc.) or in a simple 3-L glass beaker for 15 min at pH 8 and 50°C. The reactor had a U-shaped bottom with a water jacket, whereas the beaker had a flat bottom with an external water bath to maintain the temperature. After centrifuging, the insoluble fraction from each vessel was divided into two replicates. One replicate was oven-dried (7 h at 130°C) and the other was freeze-dried. The oil contents of both freeze-dried and oven-dried insoluble fractions were determined by using the acid hydrolysis Mojonnier method as well as by using the 5-h hexane-extraction Goldfisch method (26) and the recoveries compared. The Goldfisch method extracts only hexane-soluble free fat, in contrast to total (free and bound) fat determined by the Mojonnier method. Extraction vessel shape did not significantly affect oil extraction recoveries from full-fat soy flour and flakes. The mean ± SE of oil extraction recoveries from full-fat soy flour using beaker and reactors were 66 ± 3 and 63 ± 6%, respectively. Extracting extruded full-fat soy flakes in a beaker or a reactor had mean ± SE of oil extraction recoveries of 68 ± 2 and 67 ± 2%, respectively.

The method used to dry the insoluble fraction affected the oil extraction recoveries from extruded full-fat soy flakes, but not from full-fat soy flour (Fig. 4). Any binding of fat by the insoluble fraction affected oil extraction recoveries. The oil extraction recoveries determined by using the Mojonnier and Goldfisch methods were not significantly different for both drying procedures used on the insoluble fraction from full-fat soy

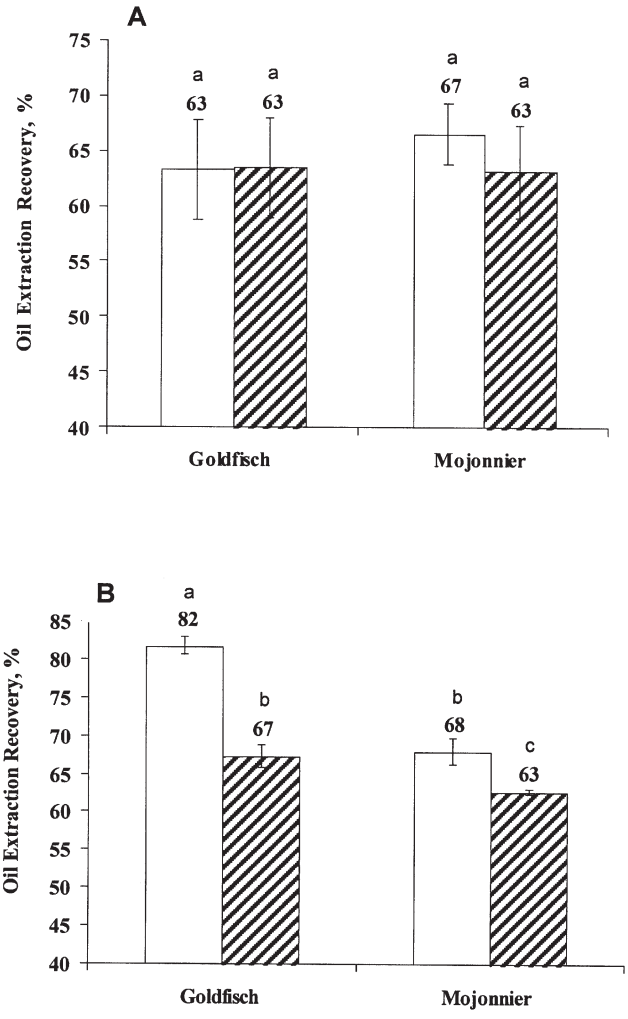

FIG. 4. Effects of drying methods used on the insoluble fractions on oil extraction recovery from full-fat soy flour (A) and extruded full-fat soy flakes (B) by Goldfisch and Mojonnier oil determination methods. Plain bars represent oven-dried insolubles and hatched bars represent freeze-dried insolubles. Oil extraction recoveries sharing the same letter were not statistically different at $P < 0.05$.

flour. On the other hand, the difference in oil extraction recoveries for full-fat soy flake insolubles was significant (14% for oven-dried samples, but just 4% for freeze-dried samples). The insoluble fraction from extruded full-fat soy flakes contained mostly heat-denatured protein, which may have bound some oil freed during extrusion. Oven-drying the insoluble fraction from extruded full-fat soy flakes may have caused the denatured protein to bind the fat and rendered it unextractable by hexane when using the Goldfisch method. The bound fat was freed by acid hydrolysis at elevated temperature when using the Mojonnier method resulting in higher oil contents. This resulted in lower calculated oil extraction recoveries when using the Mojonnier method for oil determination.

Freeze-dried extruded full-fat soy flake insolubles, by contrast, had a more porous structure from which hexane was able to extract more oil during Goldfisch determination. The oil extraction recovery by using the Goldfisch methods on freeze-dried insolubles was not significantly different from the oil extraction recovery when using the Mojonnier method on both oven- and freeze-dried insoluble fractions. The Goldfisch method risks overestimating oil extraction recoveries when calculated by difference, especially in the case of oven-dried extruded full-fat soy flakes. In previous AEP reports, Goldfisch- or Soxhlet-type hexane-extraction methods have been used without recognizing the potential for binding free oil. Our results show that such methods may have overestimated oil extraction recoveries in prior research.

When considering the difference in oil extraction recoveries from full-fat soy flakes extruded at 100°C barrel temperature, 100 rpm screw rotational speed, and 14% flake moisture content between 2^3 factorial experiments and enzyme-assisted AEP control experiments, our data show the difference in insoluble drying method (freeze- or oven-drying) was responsible for the discrepancy. The 68% oil extraction recovery in Figure 4B obtained for extruded full-fat soy flakes (Mojonnier) was consistent with oil recoveries for the enzyme-assisted AEP control experiments.

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