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ORIGINAL PAPER

Scale-up of Enzyme-Assisted Aqueous Extraction Processing of Soybeans

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Abstract The effects of scaling-up enzyme-assisted aqueous extraction process (EAEP) using 2 kg of flaked and extruded soybeans as well as the effects of different extrusion and extraction conditions were evaluated. Standard single-stage EAEP at 1:10 solids-to-liquid ratio (SLR) was used to evaluate the effects of different extruder screw speeds and whether or not collets were extruded directly into water. Increasing extruder screw speed from 40 to 90 rpm improved oil extraction yield from 85 to 95%. Oil, protein, and solids extraction yields of 97, 86, and 78% were obtained when extruding directly into water and 95, 84, and 77% when not extruding into water. When not extruding into water, standard single-stage EAEP (1:10 SLR) yielded 95, 84, and 77% of total oil, protein, and solids extraction, respectively, and two-stage countercurrent EAEP (1:6 SLR) yielded 99, 94, and 83% total oil, protein, and solids extraction, respectively. These yields were similar to those previously obtained in the laboratory (0.08 kg soybeans), but higher oil contents were observed in the skim fractions produced at pilot-plant scale for both processes. Modifying processing parameters improved the oil distribution among the fractions, increasing oil yield in the cream fraction (from 76 to 86%) and reducing oil yield in the skim fraction (from 23 to 12%). Steady-state oil extraction was achieved after two 2-stage extractions. Two-stage countercurrent EAEP is particularly attractive due to reduced water usage compared to conventional single-stage extraction.

Keywords Aqueous processing · Scale-up · Enzyme · Oil extraction · Protein extraction · Soybeans

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Introduction

The use of water as an extraction aid or medium for physical separation of oil and protein from different oil-bearing seeds has been widely reported in the literature [1–6]. In this process, known as the aqueous extraction process (AEP), extraction of oil from other seed components is based on the insolubility of the oil rather than dissolution as when using organic solvents [7]. Due to increasingly restrictive environmental regulations and health concerns regarding oil extraction with hexane [8], a resurgence of interest in AEP has recently occurred [7]. AEP is regarded to be an environmentally friendly process [7] where oil and protein can be simultaneously recovered [2].

AEP typically yields low oil recovery (~60%) [7] compared to hexane extraction (>95%) [9]. Low extraction efficiency is related to difficulties in rupturing cell walls (the barrier to extraction) and releasing free oil to be washed out of cells with water [10]. Aqueous extraction efficiency has been improved by employing enzymes [11, 12] and mechanical treatments such as flaking and

extruding [6, 13]. Combining flaking, extruding, and hydrolyzing with protease has achieved up to 97% oil extraction [6] compared with 65% [14] obtained for AEP using full-fat soybean flour.

When enzymes are used to assist AEP (enzyme-assisted aqueous processing, EAEP) of soybeans, oil and protein are distributed among three fraction: free oil, skim (a protein- and sugar-rich aqueous phase), and an oil-rich cream emulsion. De-emulsifying the cream to recover free oil and recovering the oil present in the skim fraction are challenges to maximizing total oil recovery. Total cream de-emulsification in standard single-stage EAEP using flaked and extruded soybeans (low solids-to-liquid ratio, 1:10) was achieved by using enzyme (2.5% of Protex 6L) or chemical (adjusting to pH 4.5) treatments [6]. Although the oil present in the skim fraction is considered extracted from the starting material, no viable methods are yet available to recover this oil. Therefore, minimizing the amount of oil in the skim and maximizing the oil in the cream and free-oil fractions are desired.

Most of the prior work on EAEP of soybeans has achieved high oil and protein extraction yields when using relatively low solids-to-liquid ratios (SLR), generally 1:10, and single-stage extraction [6, 13, 15]. Using such large amounts of water produces large volumes of skim that must be concentrated [16] and/or evaporated to recover protein and carbohydrates. Since greater oil and protein extraction are achieved at low solids-to-liquid ratios, reducing the amount of water used in the process without losing extraction efficiency is a challenge to making EAEP commercially viable. Employing two-stage countercurrent EAEP of flaked and extruded soybeans as a means of reducing water usage was recently proposed by Moura and Johnson [17]. Two-stage countercurrent EAEP achieved much greater oil, protein and solids extraction with approximately one-half of the normal water use than using standard single-stage EAEP. Up to 98 and 96%, 92 and 87%, and 80 and 77% oil, protein and solids extraction were obtained using two-stage countercurrent EAEP and standard single-stage EAEP, respectively. The 40% water reduction facilitated by using two-stage countercurrent EAEP represents an important energy savings in the recovery of protein and carbohydrates present in the dilute skim fraction. Two extraction stages were adequate to achieve oil extraction similar to hexane extraction.

Generally, the oils produced by EAEP have low phosphatide levels (making possible physical refining), low peroxide values, and similar free fatty-acid contents compared to those obtained by conventional processes [18–20]. Less severe exposure of the oil to heat during EAEP seems to improve oxidative stability of corn germ and soybean oils compared to conventional hexane extraction or screw-press processes [19, 20].

Since prior research on EAEP of soybeans has been performed at quite small scale, generally using 0.08–0.1 kg of extruded soybeans flakes, which produces ~1 L of slurry when using 1:10 solids-to-liquid ratio (SLR) [6, 13, 15], we decided to scale-up two-stage countercurrent EAEP [17] to identify any potential problems that might occur at industrial scale. Two-stage countercurrent EAEP was scaled-up from 0.08 to 2 kg of extruded soybeans flakes using 1:6 SLR, producing ~11 L of slurry. The objectives of the present study were (1) to determine the effects of different extruder screw speeds in a large-scale extruder on extraction efficiency and the impact of extruding directly into water or not extruding into water when using standard single-stage EAEP; and (2) to identify scale-up issues for two-stage countercurrent EAEP that must be solved before commercial adoption.

Materials and Methods

Full-fat Soybean Flakes

Full-fat soybean flakes were prepared from variety 92M91-N201 soybeans (Pioneer, Johnston, IA, USA) harvested in 2007. The soybeans were cracked into four to six pieces by using a corrugated roller mill (model 10X12SGL, Ferrel-Ross, Oklahoma City, OK, USA), and the hulls were removed from the meats (cotyledons) by aspirating with a multi-aspirator (Kice, Wichita, KS, USA). The meats were conditioned at 60 °C using a triple-deck seed conditioner (French Oil Mill Machinery, Piqua, OH, USA) and flaked to approximately 0.25 mm thickness by using a smooth-surface roller mill (Roskamp, Waterloo, IA, USA). The moisture content of the flakes (~8%) was increased to 15% by spraying water onto the flakes while mixing in a Gilson mixer (model 59016A, St. Joseph, MO, USA).

Extruding Soybean Flakes

A twin-screw extruder (ZSE 27-mm diameter twin-screw extruder, American Leistritz Extruders, Somerville, NJ, USA) was used to extrude the flakes. High-shear geometry screws were used in co-rotational orientation. The extruder barrel (1,080 mm length) was composed of 10 heating blocks set to achieve the temperature profile of 30–70–100–100–100–100–100–100–100–100 °C. The extruder was manually fed to achieve an output rate of 10.5 kg/h of extruded flakes. Screw speeds of 40, 60, 80 and 90 rpm were evaluated in relation to extraction efficiency as well as extruding directly into water or not extruding into water. When extruding directly into water, about 1 kg of extruded flakes was collected. Depending on the process used, additional water was added to achieve different SLRs.

When not extruding directly into water, the collets were cooled to room temperature, placed in polyethylene bags and stored in a cold room at 4 °C until extracted. The extruded flakes contained 22.8% oil (as is), 35.0% protein (as is), and 10.0% moisture.

Enzyme Treatment

Protex 6L, obtained from Genencor Division of Danisco (Rochester, NY, USA), was used. Protex 6L is a bacterial alkaline endoprotease derived from a strain of *Bacillus licheniformis* and has highest activity at pH 7.0–10.0 and 30–70 °C temperature. The 0.5% enzyme dosage in the extraction was based on the weight of extruded flakes and was selected based on our previous work [6].

Standard EAEP

Standard EAEP employed single-stage extraction using 1:10 SLR (Fig. 1). Standard EAEP was chosen to evaluate extrusion conditions because it was less labor intensive and time consuming than two-stage countercurrent EAEP, and the effects of these treatments on extraction yields should be similar for both processes. About 1 kg of extruded flakes was dispersed into water to obtain 1:10 SLR. The slurry was adjusted to pH 9.0 before adding 0.5% Protex 6L (w/w extruded flakes), and the slurry was stirred for 1 h at 120 rpm and 50 °C. The reaction was carried out in a 20-L jacketed glass reactor. Following extraction, the slurry was centrifuged at 3,000×*g*. After removing the insoluble fraction, the liquid phase (skim, cream, and free oil) was placed into a separator funnel (5-L jacketed reactor) and allowed to settle overnight at 4 °C. During settling, the liquid phase separated into two fractions (skim fraction and cream + free oil fraction). Standard EAEP was replicated two times for each condition.

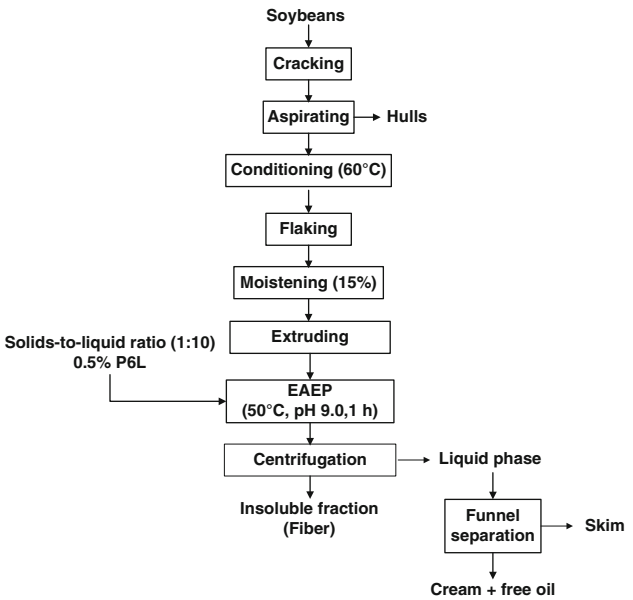

Fig. 1 Process flow diagram for the standard single-stage EAEP of dehulled, flaked, and extruded soybeans

Two-stage Countercurrent EAEP

Two-stage countercurrent EAEP was performed over 4 days. The extruded flakes were subjected to two-stage extraction and the liquid fraction (skim + cream + free oil) obtained in the second extraction stage of one trial was recycled to the first extraction stage of the next trial (incoming fresh flakes) on the following day (Fig. 2). On the first day of extraction, AEP was performed with 2 kg of extruded flakes using 1:6 SLR. The slurry pH was maintained at pH 8.0 and stirred for 15 min at 120 rpm and 50 °C. The reaction was carried out in a 20-L jacketed glass reactor. The slurry obtained in the first extraction stage was centrifuged at 3,000×*g* to remove the insoluble fraction. The liquid phase was separated by using a separatory funnel (5-L jacketed reactor) into skim and cream. The insoluble fraction obtained in the first extraction stage (first insolubles) was then subjected to EAEP. Prior to

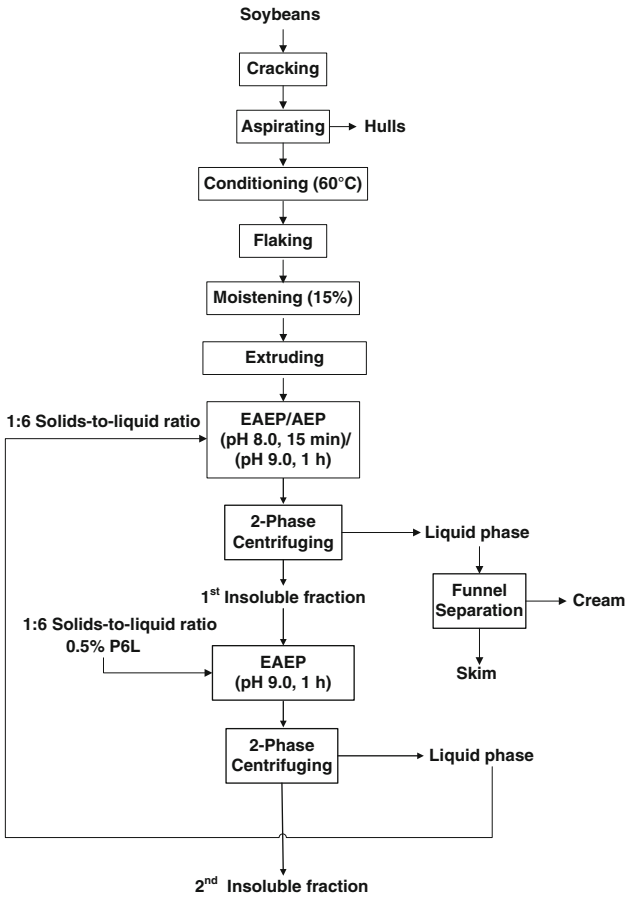

Fig. 2 Process flow diagram for two-stage countercurrent EAEP of flaked and extruded soybeans

EAEP, the first insoluble fraction was dispersed in water to obtain 1:6 SLR. The slurry pH was adjusted to 9.0 before 0.5% Protex 6L (wt/extruded flakes) was added and stirred for 1 h at 120 rpm and 50 °C. The slurry obtained in the second extraction stage was centrifuged to separate the insoluble and liquid fractions. The liquid phase was recycled to the first extraction stage on the next day. The extractions on the second, third, and fourth trials were performed in the same manner as the first trial. Two-stage countercurrent EAEP was performed in two sets of experiments. Each experimental set was composed of 4 days with one two-stage extraction performed each day. Except for evaluating the insoluble fraction daily, only the samples collected on the last day (fourth extraction trial) of each set of experiments (duplicate samples) were analyzed for chemical composition and mass balances of oil, protein, and solids (dry matter).

Another set of experiments was performed with some modification to the procedure described above. The use of enzyme in the first extraction stage on the first trial was evaluated in order to determine if steady-state extraction (equilibrium in oil extraction within each stage) could be achieved sooner. Extraction conditions in the first extraction stage were changed from 15 min at pH 8.0 to 1 h at pH 9.0 in an attempt to improve the oil distribution among the fractions (less oil in skim). Similar to the first set of experiments, two-stage countercurrent EAEP was performed over 4 days with one two-stage extraction each day. The insoluble fraction was collected each day, and all samples (cream + free oil and skim fractions) collected in the second, third, and fourth extraction trials (triplicate samples) were analyzed to determine mass balances of oil, protein, and solids.

Oil, Protein, and Solids Recoveries

Analyses of oil, protein, and solids (dry-matter) contents were carried out on the skim, insoluble, and cream fractions as well as the extruded flakes. Total oil contents were determined by using the acid hydrolysis Mojonnier method (AOCS method 922.06), protein contents by using the Dumas method and a conversion factor of 6.25 (vario MAXCN Elementar Analysensysteme, Hanau, Germany), and total solids by weight after drying samples in a vacuum-oven at 110 °C for 3 h (AACC method 44–40). The extraction yields were expressed as percentages of each component in each fraction relative to the initial amounts in the extruded flakes. All chemical analyses were performed in duplicate.

Statistical Analyses

The experiment was a completely randomized design. The data were analyzed by analysis of variance (ANOVA) using mixed models from the SAS system (version 8.2, SAS Institute, Cary, NC). Means were compared using *F*-protected contrasts, and the level of significance was set at $P < 0.05$.

Results and Discussion

Effects of Extrusion Conditions on Oil, Protein, and Solids Extraction when Scaling-up Standard EAEP

Extrusion parameters are likely to affect extraction efficiency because they affect the extent of cell rupture and facilitate enzyme action in the aqueous medium [15]. Our previous results [6, 17] were obtained by using a small laboratory twin-screw extruder (18-mm screw diameter). We decided to scale-up to a pilot-plant twin-screw extruder (ZSE 27-mm screw diameter), and it was necessary to confirm optimum operating conditions that had been previously optimized using the small laboratory extruder [13].

Figure 3 shows the effects of different screw speeds on extraction efficiencies. Protein and solids extractions were not significantly affected when increasing the rotational speed of the extruder screws from 40 to 90 rpm; however, oil extraction increased from 85 to 95% (statistically different at $P < 0.05$). Increasing screw speed up to 90 rpm will increase cell-wall disruption facilitating enzyme accessibility and thus oil release. For that reason, 90 rpm screw rotational speed was used in the following experiments.

The effects of extruding soybean flour directly into water without enzyme in AEP were previously determined by Lamsal et al. [13]. Oil extraction increased from 60 to 75% when extruding soybean flour directly into water, however, the effect of extruding into water with enzyme assistance (EAEP) was not evaluated. The possibility of omitting extruding directly into water would simplify the EAEP process in industrial application. Therefore, we decided to evaluate the effects of extruding flakes into water or not extruding into water when using standard single-stage EAEP. Figure 4 shows that extruding directly

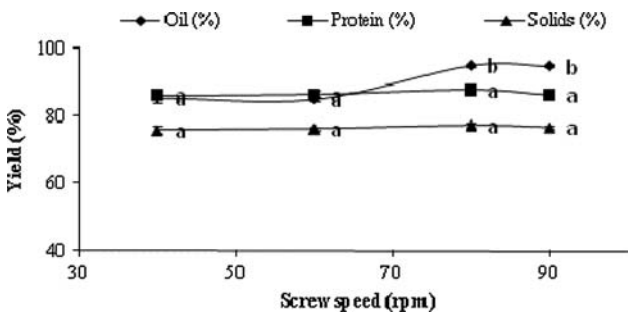

Fig. 3 Effects of extruder screw speed on oil, protein, and solids extraction yields using standard EAEP

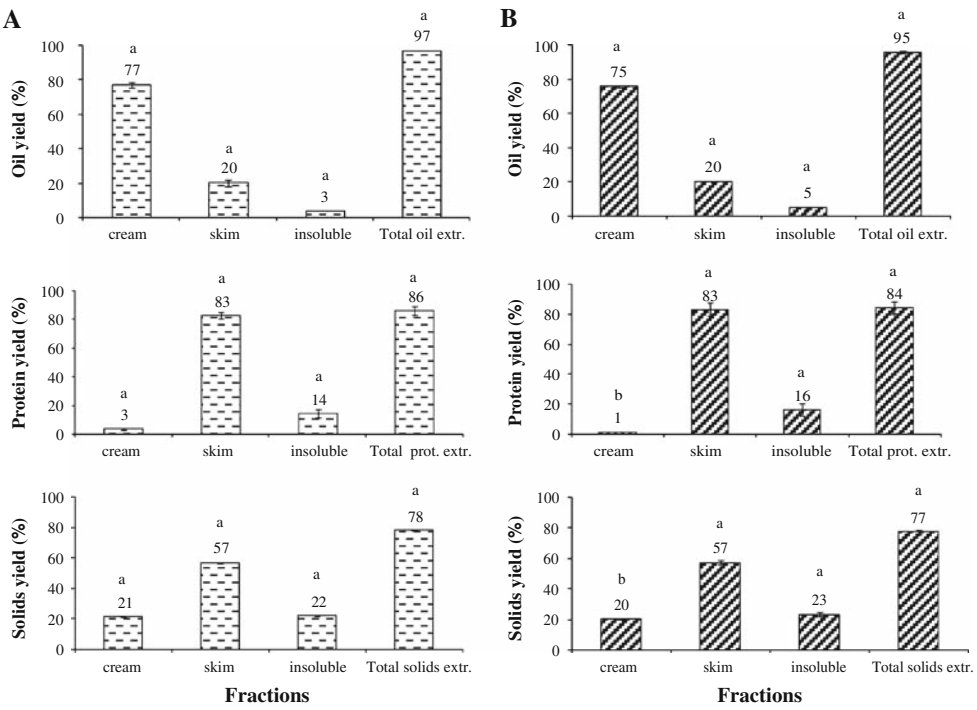

Fig. 4a, b Effects of extrusion conditions on oil, protein, and solids extraction using standard single-stage EAEP. **a** Extruding into water, **b** dry extrusion (not extruding into water). Means within the same fraction followed by *different letters* are statistically different ($P < 0.05$)

into water (Fig. 4a) or not doing so (Fig. 4b) yielded nearly the same extraction of oil, protein, and solids. Oil, protein, and solids extraction yields of 97 and 95%, 86 and 84%, 78 and 77% were obtained when extruding directly into water or not extruding into water, respectively. No statistical difference in total extraction yields was observed at $P < 0.05$. The benefit of extruding directly into water on oil extraction yield observed by Lamsal et al. [13] was greater than observed in our experiment; they extruded soy flour prior to AEP, we extruded soy flakes prior to EAEP. Therefore, we decided not to extrude directly into water when scaling-up two-stage countercurrent EAEP.

The pilot-plant scale-up of standard single-stage EAEP (using 1 kg of extruded flakes) gave similar oil, protein, and solids extraction yields to those obtained at smaller laboratory scale using 0.08 kg of extruded flakes [6]. The oil distribution in the fractions produced when scaling-up the process, however, was different. The skim fraction obtained in standard EAEP at the laboratory scale contained approximately 14% of the oil [6], while the skim fraction generated in the pilot-plant process simulation contained 20% of the oil (Fig. 4).

Scaling-up Two-stage Countercurrent EAEP

Compared with our previous findings for two-stage countercurrent EAEP [17], similar results were obtained when scaling-up from the laboratory to pilot plant. Except for using a different extruder (higher capacity and different screw configuration) and not extruding directly into water, reaction parameters were maintained (Fig. 2). The second insoluble fractions were analyzed for oil contents to determine when extraction attained steady-state. Figure 5 shows that oil extraction reached steady-state after the third extraction trial. A small increase in oil extraction (~0.5%) was observed from the second to the third extraction trial, reaching a constant value thereafter. Since in the first extraction trial only fresh water was used in the first extraction stage (without enzyme) and the skim fraction containing enzyme was recycled for the first time in the second extraction trial, we expected to reach steady-state extraction after the third extraction trial. For this reason, samples obtained in the fourth extraction trial were analyzed for oil, protein, and solids contents when scaling-up two-stage countercurrent EAEP.

In the scale-up trials, two-stage countercurrent EAEP extracted 99% of the oil, 94% of the protein, and 83% of the solids in the extruded flakes (Fig. 6a). These pilot-plant results were consistent with those obtained in the

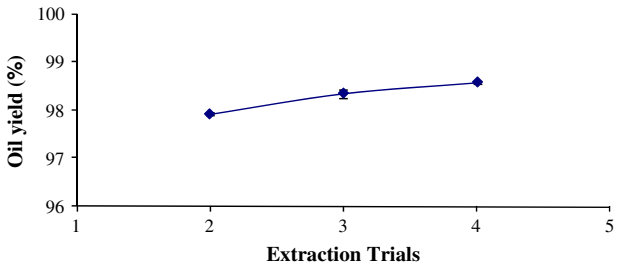

Fig. 5 Oil extraction efficiency of two-stage countercurrent EAEP for different consecutive extraction trials

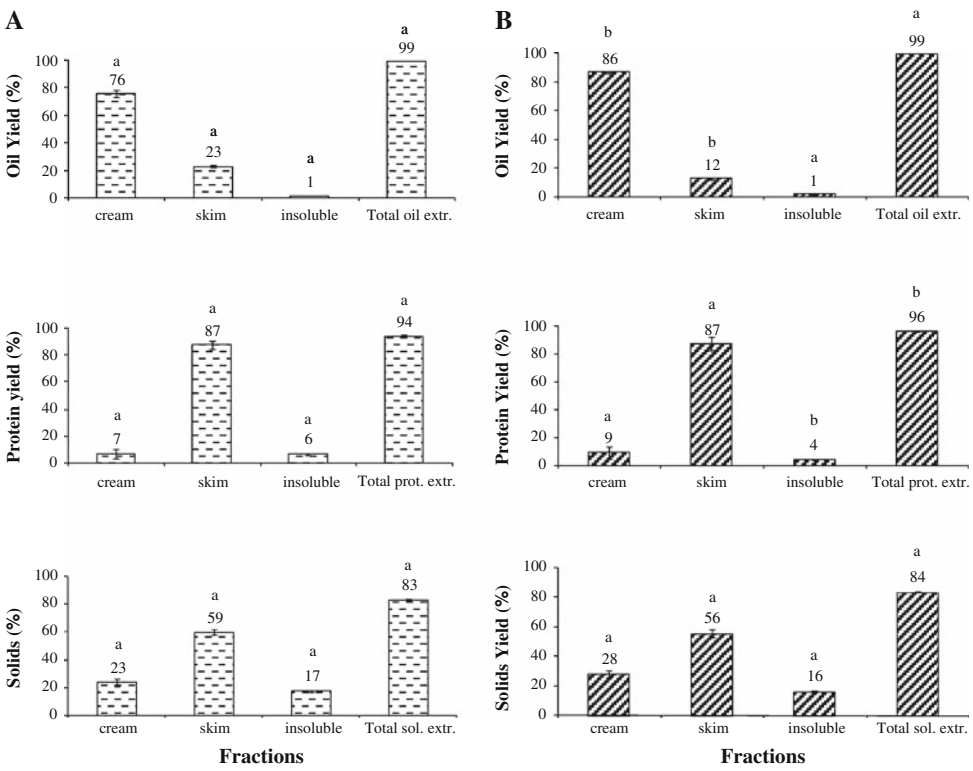

Fig. 6a, b Effects of extraction conditions in pilot-plant two-stage countercurrent EAEP. **a** pH 8.0, 15 min; **b** pH 9.0, 1 h. Means within the same fraction followed by *different letters* are statistically different ($P < 0.05$)

laboratory (0.08 kg of extruded flakes) where oil, protein, and solids extractions were 98, 92, and 80%, respectively [17]. In addition to achieving similar extraction results at pilot-plant scale as those obtained in the laboratory, pilot-plant scale-up produced skim fractions with higher oil yield (23%) compared to skim fractions obtained in the laboratory (13%) [17]. Similar trends were also observed when scaling-up standard EAEP.

In order to reduce the amount of oil present in the skim fraction of two-stage countercurrent EAEP by shifting more oil to the cream, new extraction conditions were evaluated. We also evaluated the time necessary to reach steady-state extraction when using enzyme in the first extraction stage. The normal conditions for the first extraction stage (pH 8.0, 15 min) were modified (pH 9.0, 1 h). As can be seen in Fig. 6b, these changes achieved similar oil and solids extraction (not statistically different at $P < 0.05$) and slightly higher protein extraction yield (statistically different at $P < 0.05$) (Fig. 6a). Despite achieving similar total oil extraction, an important improvement was observed in the oil distribution among the fractions produced when using the latter extraction conditions. Increasing time and pH in the first extraction stage increased oil yield in the cream + free oil fraction from 76 to 86%, consequently reducing the oil yield in the skim fraction from 23 to 12%. Differences in oil yield within the same fractions (cream and skim) from both treatments were statistically different at $P < 0.05$. This is a very important finding because no method is yet available to recover the oil present in the skim fraction as free oil, even though this oil is considered to be extracted from the insoluble fraction. Low oil content in the skim is critical.

Figure 7 shows the effects of adding enzyme in the first extraction stage on the first extraction trial. Similar extraction yields were obtained after the first extraction trial, and steady-state was achieved by the second extraction trial. Steady-state sample collection could begin with the second extraction trial, instead of the fourth extraction trial (Fig. 5). Despite achieving steady-state extraction on the second extraction trial, we recommend sample collection beginning with the third experimental trial due to the recycling of the skim fraction that occurs with the second trial.

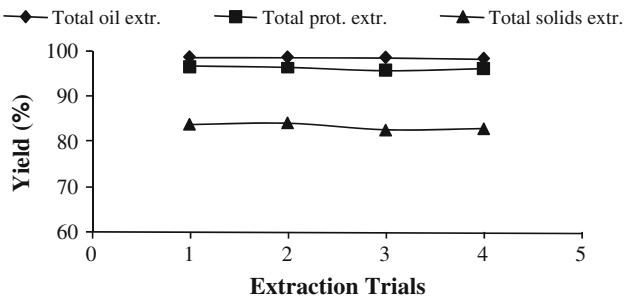

Fig. 7 Effects of adding enzyme in the first extraction stage on extraction efficiency of oil, protein, and solids in two-stage countercurrent EAEP

Oil extractability, i.e., oil present in all three liquid phases (skim, cream, free oil), achieved when using standard EAEP or two-stage countercurrent EAEP (96–99%) was similar to that achieved by using hexane extraction (95.0–97.5%) [7]; however, oil recovery was about 82% when using standard EAEP because the oil is not recovered from the skim (14%) or from the insoluble fraction (4%) [6]. For standard EAEP processing (1:10 SLR) [6], we demonstrated total cream de-emulsification to obtain free oil is achievable by means of enzymatic or pH adjustment treatments. We expect the cream recovered when using two-stage countercurrent EAEP to respond to de-emulsification in the same manner as the cream recovered from standard EAEP; however, experiments regarding oil distribution among fractions generated by two-stage countercurrent EAEP as well as demulsifying this cream are being conducted.

Conclusions

Extruder rotational screw speed was an important parameter to achieving high oil-extraction efficiency from extruded soybean flakes. Increasing screw speed from 40 to 90 rpm improved oil extraction from 85 to 95%. Within the range of screw speeds evaluated, increased shear seemed to favor cell-wall rupture and greater oil extraction. Extruding directly into water did not achieve greater extraction of oil, protein, or solids when using standard single-stage EAEP. Scaling-up both standard single-stage EAEP (1:10 SLR) and two-stage countercurrent EAEP (1:6 SLR) to pilot-plant level (2 kg) achieved similar oil, protein, and solids extraction yields to those obtained in the laboratory of our previous work (0.08 kg). Scaling-up both processes produced skim fractions with greater oil extraction than were obtained in the laboratory. Standard single-stage and two-stage countercurrent EAEP generated skim fractions containing 14 and 13% of the total oil in the laboratory and 20 and 23% in the pilot plant. Increasing reaction time (15 min to 1 h) and slurry pH (8.0–9.0) in the first extraction of two-stage countercurrent EAEP yielded similar total oil extraction, but the oil distribution among the fractions was altered. The oil yield in the cream + free oil fraction increased from 76 to 86% and the oil of the skim decreased from 23 to 12%. Using enzyme in the first extraction stage enabled steady-state oil extraction to be reached on the second extraction trial.

References

1. Kim SH (1989) Aqueous extraction of oil from palm kernel. J Food Sci 54:491–492
2. Cater CM, Rhee KC, Hagenmaier RD, Mattil KF (1973) Aqueous extraction—an alternative oilseed milling. J Am Oil Chem Soc 51:137–141
3. Rhee KC, Cater CM, Mattil KF (1972) Simultaneous recovery of protein and oil from raw peanuts in an aqueous system. J Food Sci 37:90–93
4. Moreau RA, Johnston DB, Powell MJ, Hicks KB (2004) A comparison of commercial enzymes for the aqueous enzymatic extraction of corn oil from corn germ. J Am Oil Chem Soc 81:1071–1075
5. Zhang SB, Wang Z, Xu SY (2007) Optimization of the aqueous enzymatic extraction of rapeseed oil and protein hydrolysates. J Am Oil Chem Soc 84:97–105
6. de Moura JMLN, Campbell K, Mahfuz A, Jung S, Glatz CE, Johnson LA (2008) Enzyme-assisted aqueous extraction of oil and protein from soybeans and cream de-emulsification. J Am Oil Chem Soc 85:985–995
7. Johnson LA (2008) Oil recovery from soybeans. In: Johnson LA, White PJ, Galloway R (eds) Soybeans: chemistry, production processing, and utilization. AOCS Press, Urbana, pp 331–375
8. Galvin JB (1997) Toxicity data for commercial hexane and hexane isomers. In: Wan PJ, Wakelyn PJ (eds) Technology and solvents for extracting oilseeds and nonpetroleum oils. AOCS Press, Champaign, pp 75–85
9. Johnson LA (2000) Recovery of fats and oils from plant and animal sources. In: O'Brien RD, Farr WE, Wan PJ (eds) Introduction to fats and oils technology. AOCS Press, Champaign, pp 108–135
10. Abdulkarim SM, Lai OM, Muhammad SKS, Long K, Ghazali HM (2005) Use of enzymes to enhance oil recovery during aqueous extraction of *Moringa Oleifera* seed oil. J Food Lipids 13:113–130
11. Rosenthal A, Pyle DL, Niranjan K, Gilmour S, Trinca L (2001) Combined effect of operational variables and enzyme activity on aqueous enzymatic extraction of oil and protein from soybean. Enzyme Microb Technol 28:499–509
12. Dominguez H, Nunez MJ, Lema JM (1993) Oil extractability from enzymatically treated soybean and sunflower: range of operational variables. Food Chem 46:277–284
13. Lamsal BP, Murphy PA, Johnson LA (2006) Flaking and extrusion as mechanical treatments for enzyme-assisted aqueous extraction of oil from soybeans. J Am Oil Chem Soc 83:973–979
14. Rosenthal A, Pyle DL, Niranjan K (1998) Simultaneous aqueous extraction of oil and protein from soybean: mechanisms for process design. Trans Inst Chem Eng Part C 76:23–224
15. Freitas SP, Hartman L, Couri S, Jablonka FH, Carvalho (1997) The combined application of extrusion and enzymatic technology for extraction of soybean oil. Fett/Lipid 99:333–337
16. Cabral LMC, Arruda NP, Trugo LC, Freitas SP, Couri S (2005) Distribuição de Peso Molecular e Perfil de Aminoácidos do Coproduto da Extração Aquosa-enzimática de Oleo de Soja. In: Proceedings of the 6th Latin American symposium of food science, Campinas, Sao Paulo, Brazil
17. de Moura JMLN, Johnson LA (2009) Two-stage countercurrent enzyme-assisted aqueous extraction processing of oil and protein from soybeans. J Am Oil Chem Soc 86:283–289
18. Embong MB, Jelen P (1977) Technical feasibility of aqueous extraction of rapeseed oil—a laboratory study. J Inst Can Sci Technol Aliment 10:239–243
19. Bocevska M, Karlovic D, Turkulov J, Pericin D (1993) Quality of corn germ oil obtained by aqueous enzymatic extraction. J Am Oil Chem Soc 70:1273–1277
20. Maurer D, Mahfuz A, Johnson LA, Jung S (2008) Enzymatic destabilization of natural occurring soy emulsion and oil quality. In: 99th AOCS annual meeting and expo, 18–21 May 2008, Seattle, WA