J Am Oil Chem Soc (2011) 88:1649–1658
DOI 10.1007/s11746-011-1831-y

ORIGINAL PAPER

Pilot-Plant Proof-of-Concept for Integrated, Countercurrent, Two-Stage, Enzyme-Assisted Aqueous Extraction of Soybeans

J. M. L. N. de Moura · D. Maurer · S. Jung · L. A. Johnson

Received: 22 December 2010 / Revised: 6 March 2011 / Accepted: 4 April 2011 / Published online: 13 May 2011
© AOCS 2011

Abstract Proof-of-concept for integrated, countercurrent, two-stage, enzyme-assisted aqueous extraction processing of soybeans was demonstrated on a pilot-plant scale (75 kg extruded flaked soybeans) where the protease used to demulsify the cream was recycled into upstream extraction stages. Oil, protein, and solids extraction yields of 98.0 ± 0.5%, 96.5 ± 0.4%, and 86.8 ± 0.5% were achieved by using the integrated countercurrent process. A three-phase horizontal decanter centrifuge efficiently separated the solids from the two liquid fractions (skim and cream). Fine separation between the two liquid fractions was important to reducing the volume of skim contaminating the cream fraction, thereby reducing the amount of enzyme used for cream demulsification and subsequent extraction. We were able to reduce enzyme use when moving from the laboratory to the pilot-plant scale, which reduced the degree of protein hydrolysis and improved cream demulsification. Enzyme-catalyzed cream demulsification was 91.6% efficient and 93.0% free oil recovery from cream was achieved by using the integrated approach.

Keywords Aqueous extraction · Enzyme recycling · Soybeans · Protein hydrolysis · Pilot plant · Scale-up

J. M. L. N. de Moura · S. Jung · L. A. Johnson (✉)
Center for Crops Utilization Research, Iowa State University, 1041 Food Sciences Building, Ames, IA 50011-1061, USA
e-mail: ljohnson@iastate.edu

D. Maurer · S. Jung · L. A. Johnson
Department of Food Science and Human Nutrition, Iowa State University, Ames, IA 50011-1061, USA

Introduction

Extensive research has been performed on enzyme-assisted aqueous extraction processing (EAEP) from soybeans [1–10] as an alternative to using hexane to extract edible oil from soybeans and a number of recent advances have been achieved [11]. In addition to eliminating the use of hazardous and polluting hexane, this water- and enzyme-based technology enables simultaneous fractionation of soybeans into oil-, protein-, and fiber-rich fractions suitable for converting into food, feed, and fuel. Oil and protein extraction in EAEP of soybeans is limited by the extent of soybean cell wall rupture achieved by mechanical pretreatments and by extraction parameters such as solids-to-liquid ratio (SLR), temperature, slurry pH, residence time, and enzyme efficiency.

High extraction yields with much less water were achieved by employing a countercurrent, two-stage strategy than normal, single-stage, EAEP [4, 5]. Two extraction stages enabled 99% oil extraction (oil removed from solids), which is similar to hexane extraction, and 96% protein extraction from extruded, full-fat soybean flakes (FFSF). Although high extraction was achieved by using countercurrent, two-stage, EAEP, unrecovered oil present in the skim fraction (~14%) and unextracted oil in the insoluble fraction (~3%) reduce overall free oil recovery to ~83% [6] when recycling any unbroken cream back into the demulsification step. Improving the distribution of extracted oil among the resulting fractions (skim, cream, and free oil) by shifting more oil from the skim to the cream or preferably recovering that oil as free oil is essential to increasing free oil recovery and thus economic feasibility. However, shifting more oil from the cream fraction to free oil may produce cream fractions with greater emulsion stability and resistance to demulsification [6].

The amount of cream separated determines the amount of enzyme required to demulsify the cream and greater amounts of contaminating skim in the cream fraction increase the amount of enzyme needed (amount of enzyme is based on mass of cream). Using more enzyme for demulsification not only increases processing cost but our past work showed that more enzyme increases the degree of protein hydrolysis, which we attribute to greater emulsion stability of the cream [10].

Countercurrent, two-stage, EAEP and enzyme-catalyzed cream demulsification were integrated and the complete process was demonstrated on a laboratory scale (2 kg soybeans), wherein the enzyme used to demulsify the cream was recycled upstream into the two extraction stages [10]. Enzyme enters the process in the demulsification step and the skim produced after breaking the cream emulsion (third skim), containing the enzyme, is recycled upstream into the second extraction stage and then into the first extraction stage. Oil, protein, and solids extraction yields of 96.1 ± 1.4%, 89.3 ± 1.0%, and 81.2 ± 2.0% were achieved by using the integrated process. Higher degrees of protein hydrolysis (DH of 7.16 ± 1.19% and 16.37 ± 2.04% for first and second extraction stages, respectively) were achieved by using the integrated process than when not recycling enzyme. Greater extent of hydrolysis likely increased surface hydrophobicity and emulsification capacity of proteins, thereby enabling the skim to emulsify more oil and affecting oil distribution among the fractions. About 81% of the oil in the cream was recovered (cream demulsification efficiency) and overall free oil recovery was reduced to 64% due to more oil being shifted into the skim fraction (32%) and to unextracted oil in the insolubles (4%) [10].

We hypothesize that achieving more complete skim-cream separation, thus producing less cream and reducing the amount of enzyme needed to demulsify the cream, the reduced amount of enzyme recycled into extraction should reduce emulsion formation and difficulty in separating the fractions.

The objectives of the present study were to: (1) test our hypothesis that reducing enzyme use by achieving improved skim-cream separation improves downstream cream demulsification efficiency and overall free oil recovery; (2) demonstrate proof-of-concept for integrated, countercurrent, two-stage, EAEP of soybeans at pilot-plant scale in which enzyme is first used to demulsify the cream and then to enhance oil extraction; and (3) identify possible scale-up issues that could affect extraction yields, distribution of extracted oil among the fractions, cream stability toward demulsification, and overall free oil recovery when moving from the laboratory to pilot-plant scale and using similar equipment that industry would employ.

Materials and Methods

Soybeans

Soybeans (variety 92M91-N201) harvested in 2009 were obtained from Pioneer, a DuPont Business (Johnston, IA, USA). The beans were stored in a cold room at 4 °C in sealed polyethylene bags to prevent moisture absorption.

Processing

Soybean Preparation

The soybeans were cracked into 4–6 pieces by using a corrugated roller mill (model 10X12SGL, Ferrell-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 by using a triple-deck seed conditioner (French Oil Mill Machinery Co., Piqua, OH, USA) and flaked to approximately 0.25 mm thickness by using a smooth-surface roller mill (Roskamp Mfg, Inc., Waterloo, IA, USA) as is done in traditional direct solvent extraction with hexane [12].

Extrusion of Soybean Flakes

The initial moisture content of the flakes (7–9%) was increased to 15% by spraying water onto the flakes while mixing in a Gilson mixer (model 59016A, St. Joseph, MO, USA). Higher moisture content of flakes (~14%) enhances oil and protein extractability from soybeans [2] and also enhances the extrusion process itself, avoiding blockage of the material in the barrel. The moistened FFSF were then extruded by using a twin-screw extruder (ZSE 27-mm diameter twin-screw extruder; American Leistritz Extruders, Somerville, NJ, USA). High-shear geometry screws were used in co-rotational orientation at 90 rpm screw speed. The extruder barrel (1,080 mm length) was composed of ten heating blocks set to achieve the temperature profile 30–70–100–100–100–100–100–100–100–100 °C. The extruder was manually fed to achieve 10.5 kg/h output rate of extruded flakes. The collets were cooled to room temperature, placed in sealed polyethylene bags, and stored in a cold room at 4 °C until extracted. The extruded flakes contained 20.3 ± 1.4% oil (as is), 35.6 ± 1.0% protein (as is), and 88.3 ± 0.3% dry matter solids (as is).

Pilot-Plant: Integrated, Countercurrent, Two-Stage, EAEP of Soybeans

Protex 6L, obtained from Genencor Division of Danisco (Rochester, NY, USA), was used in both extraction stages

and for cream demulsification. Protex 6L, having 580,000 DU/g minimum activity, 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. The 0.5% enzyme dosage for extraction based on the weight of extruded flakes and the 2.5% enzyme dosage for the cream demulsification based on the weight of the cream were selected based on our previous work [3, 6, 13].

Integrated, countercurrent, two-stage, EAEP was performed over 11 trials in the pilot-plant facilities of the Center for Crops Utilization Research, with each trial being composed of two countercurrent extraction stages (Fig. 1). In the first trial, the first EAEP extraction stage was performed with 75 kg of extruded FFSF using 1:6 solids-to-liquid ratio (480 kg of slurry). The solids-to-liquid ratio of 1:6 was selected based in our previous work [4], representing the maximum water reduction while having modest loss in oil and protein extractability compared with 1:10 solids-to-liquid ratio. The initial slurry pH (6.8 ± 0.3) was adjusted to 9.0 by adding 10 N NaOH before adding 0.5% Protex 6L (wt/wt extruded flakes) and stirred for 1 h at 35 rpm and 50 °C. The extraction was carried out in a 760-L jacketed stainless-steel tank reactor (Walker Stainless Equipment Company Inc., New Lisbon, WS, USA). The slurry obtained in the first extraction stage was fed at 11.3 L/min by using a screw pump (Moyno Progressing Cavity Pumps, Springfield, OH, USA) to a continuous, horizontal, three-phase, decanter centrifuge (Centrisys Corporation, Model CS10-4, Beloit, WS, USA) to separate the first insoluble fraction from the first skim and cream fraction (no free oil was observed in the cream fraction). Three-phase centrifugation was carried out at 160 mm weir plate setting, 13.8 mm third phase tube-setting, 800 rpm (9.92 kg/min) feed speed, 5,030 rpm bowl speed (3,500×*g*), and 1 rpm differential scroll speed. After removing the insoluble fraction, the first skim was pumped to a jacketed tank (760-L) and then to a plate-and-frame heat exchanger, both cooled with chilled water. After reaching 14 °C, the skim (outgoing fraction) was placed in 19-L (5-gal) containers and frozen.

The cream fraction was allowed to settle overnight at 4 °C before demulsifying. The cream fraction was kept overnight to achieve a reasonable work schedule for the research staff, since the entire demulsification process took approximately 1 day before producing the skim necessary (containing active enzyme) to be used in the second stage of extraction. A schematic diagram for cream demulsification is shown in Fig. 2. The cream fraction was transferred to a 265-L jacketed reactor and heated to 65 °C, and the pH of the cream was adjusted to 9.0 before adding 2.5% (w/w) Protex 6L. Cream demulsification was carried out with constant stirring at 180 rpm for 1.5 h. After demulsifying the cream, the slurry was centrifuged by using a continuous, disc-stack centrifuge (Alfa Laval, Model BTPX-205SGD, Fort Lee, NJ, USA) to separate the free oil and the aqueous phase, referred to as third skim and contained active enzyme. The centrifugation was carried out with a #66 restrictor plate at 20 psi back pressure on both phases, 500 feed speed setting, and 9,787 rpm bowl speed (8,000–10,000×*g*). The third skim was recycled upstream into the second extraction stage of the next extraction trial. The free oil fraction recovered from cream demulsification was transferred to a 20-L jacketed reactor, cooled to 4 °C, and allowed to settle overnight to remove any residual water and cream, hereafter referred to as fourth skim. The fourth skim was then recycled into the next cream demulsification, enabling more free oil to be recovered from the residual cream present in the fourth skim fraction.

The first insoluble fraction obtained in the first extraction stage was then subjected to a second stage of extraction. The first insoluble fraction was dispersed in water to obtain a 1:6 solids-to-liquid ratio and the same extraction conditions, including same enzyme addition, were used as in the first extraction stage. The slurry obtained in the second extraction stage was centrifuged to separate the final insoluble fraction and the second liquid phase, mainly composed of second skim and some residual cream (second cream). The second liquid phase was recycled to the first extraction stage of the next trial (incoming fresh extruded flakes).

Extractions were carried out in the manner described above for 11 consecutive trials except for adding enzyme to the second extraction stage. From the second trial forward, the third skim obtained from cream demulsification was added into the extraction slurry. No more fresh enzyme was added in either extraction stage. The enzyme used to demulsify the cream was first recycled to the second extraction stage (second extraction trial) and then into the first extraction stage (third extraction trial), being used three times in the entire process (two extraction stages and one cream demulsification step). Samples of skim, cream, and insolubles from each of the 11 different trials were collected and analyzed to determine chemical compositions.

Lipids, Protein, and Solids Recoveries

Oil (lipids), protein, and solids (dry matter) contents were determined on the skim, insoluble, and cream fractions as well as the extruded, full-fat, soybean flakes. Total fat contents were determined by using the Mojonnier acid hydrolysis method (AOCS method 922.06) [14], protein contents by using the Dumas combustion method and the N conversion factor of 6.25 (vario MAXCN Elementar Analysensysteme GmbH, Hanau, Germany) [15], and total solids gravimetrically after drying samples in a vacuum

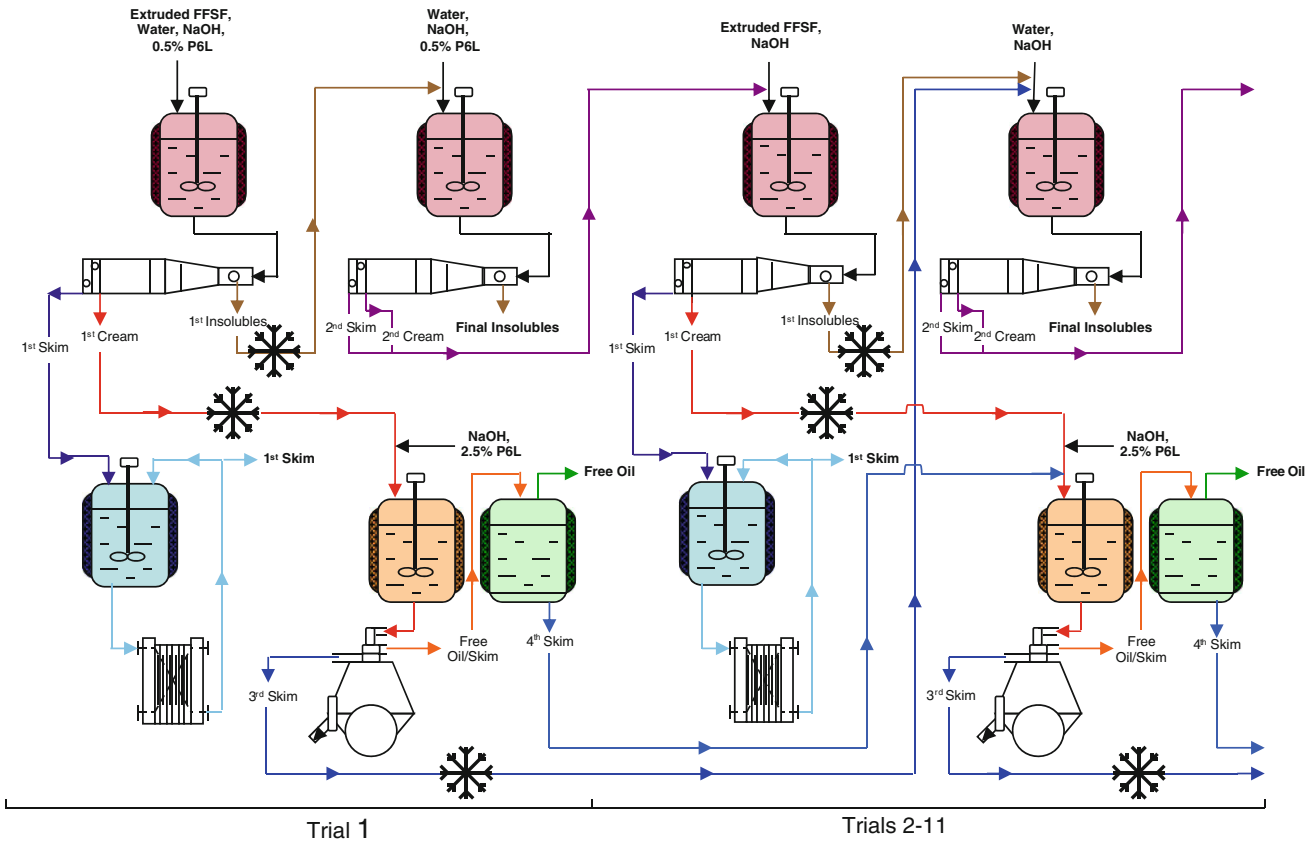

Fig. 1 Schematic representation of the integration of extraction and demulsification stages in countercurrent, two-stage, enzyme-assisted aqueous extraction processing of soybeans

oven at 110 °C for 3 h (AACC Method 44-40) [16]. The extraction yields were expressed as percentages of each component in each fraction relative to the amounts in the initial, extruded, full-fat, soybean flakes. Chemical analyses were performed in duplicate for samples obtained from each of 11 different extraction trials (skim, cream, and insolubles). Mass balances of oil, protein, and solids were calculated based on incoming extruded, full-fat, soybean flakes.

Cream Demulsification Efficiency

Pilot-plant, enzyme-catalyzed, cream demulsification was comprised of two major separation steps: centrifuging the demulsified cream to separate free oil from the remaining skim and cream; and decanting the free oil to separate remaining water and skim (Fig. 2). Only the third skim fraction left the demulsification process (being recycled into the second extraction stage), while the fourth skim fraction was recycled into the next cream demulsification. Recycling these two fractions maximizes oil recovery.

Demulsification efficiency, the amount of free oil (%) obtained after enzyme-catalyzed demulsification of the cream, was determined according to Eq. 1. As shown in the flow diagram for the integrated process (Fig. 3), there were two input streams into the cream demulsification step (the cream from the first extraction stage and the fourth skim containing residual cream from the previous cream

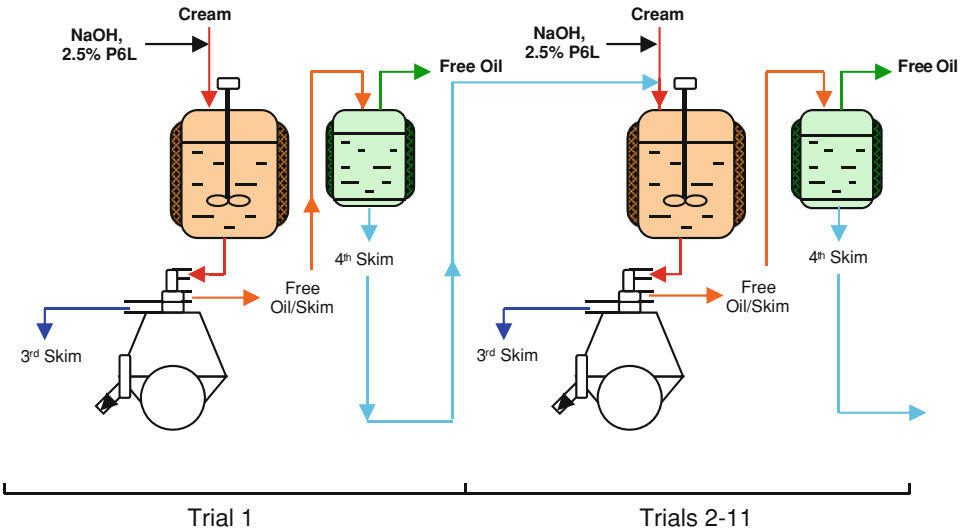

Fig. 2 Schematic representation for cream demulsification in integrated, enzyme-assisted aqueous extraction processing of soybeans

Orange tanks: demulsification tanks, 65 °C, 1.5 h, pH 9

Green tanks: decantation tanks, 4 °C, 16 h

demulsification). Therefore, the amount of oil present in the fourth skim was considered in Eq. 1 to determine cream demulsification efficiency.

$$\text{Demulsification efficiency } (\%) = \frac{\text{free oil (g)} \times 100}{[\text{cream (g)} \times \text{oil content } (\%) + \text{4th skim (g)} \times \text{oil content } (\%)]/100\%} \quad (1)$$

In order to quantify the free oil yield (%) from the demulsification step, including adding the fourth skim fraction, the amount of oil present in the fourth skim was omitted in the denominator of Eq. 2.

$$\text{Demulsified free oil yield}(\%) = \frac{\text{free oil (g)} \times 100}{[\text{cream (g)} \times \text{oil content } (\%)]/100\%} \quad (2)$$

Overall free oil recovery, considering both extraction stages and the cream demulsification step, was calculated relative to the initial amount of oil present in the extruded FFSF as shown in Eq. 3.

$$\text{Overall process free oil recovery } (\%) = \frac{\text{free oil (g)} \times 100}{[\text{extrude soybean flakes (g)} \times \text{oil content } (\%)]/100\%} \quad (3)$$

Degree of Hydrolysis (DH)

The pH during the two stages of extraction was maintained at 9.0 by adding 10 N NaOH and the degree of hydrolysis (DH) was determined based on the amount of NaOH used. DH was determined according to Eq. 4,

$$\text{DH} = [(V_{\text{NaOH}} \times N_{\text{NaOH}})/(\alpha \times \text{MP} \times h_{\text{hot}})] \times 100\% \quad (4)$$

where α is the degree of dissociation of α-amino groups, MP is the mass of protein (g), and h_{hot} is the total number of peptide bonds in the protein (mequv/g protein). The corresponding α for 50 °C and pH 9.0 was 0.98, while h_{hot} was 7.8 [17].

Statistical Analyses

The data were analyzed by using Analysis of Variance (ANOVA) with mixed models from the SAS system (version 8.2, SAS Institute, Inc., Cary, NC, USA). Means were compared by using F-protected contrasts and the level of significance was set at $P < 0.05$.

Results and Discussion

Oil, Protein, and Solids Extraction Yields

To initiate integrated, countercurrent, two-stage, EAEP of soybeans (first trial), fresh enzyme was added to each

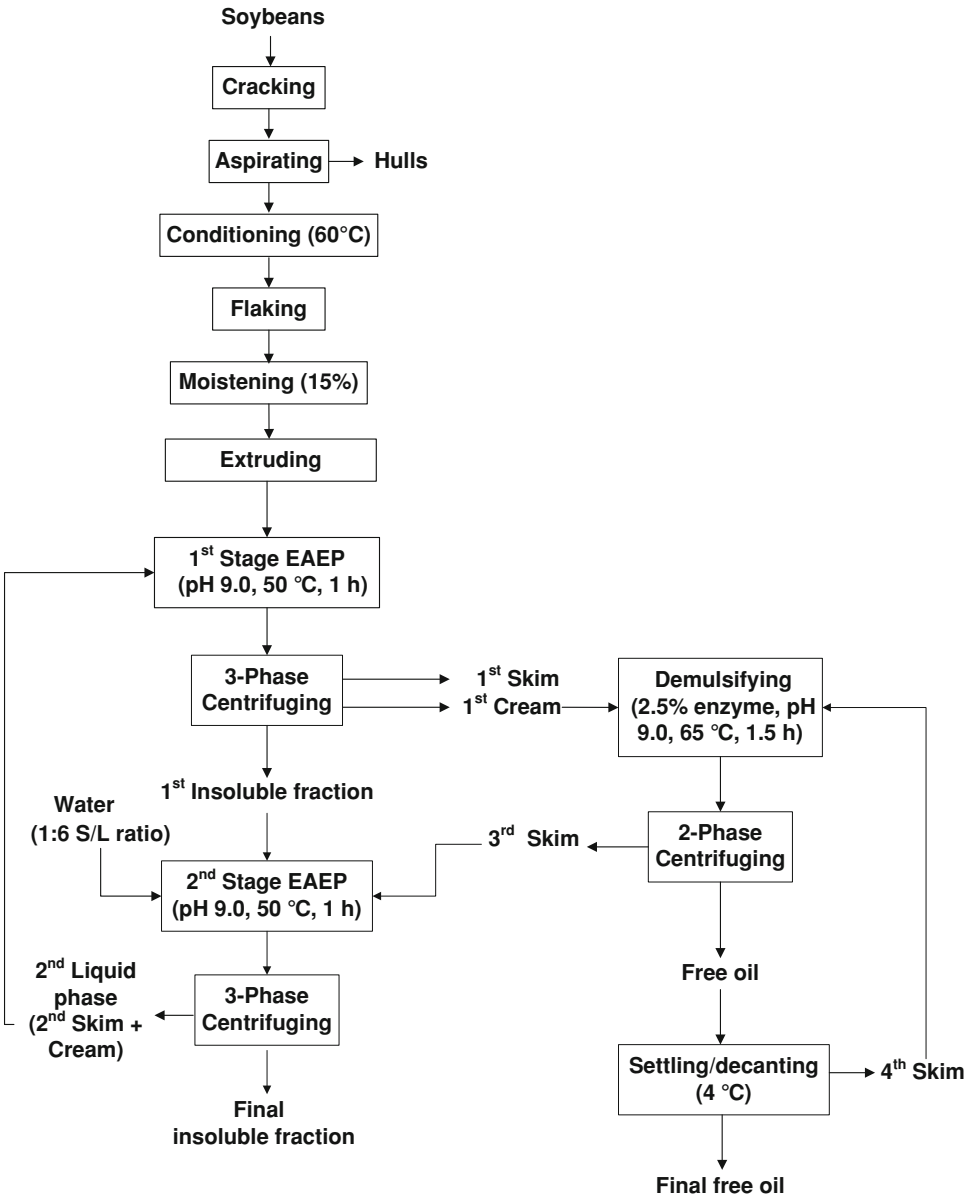

Fig. 3 Process flow diagram for integrated, countercurrent, two-stage, enzyme-assisted aqueous extraction processing of soybeans

extraction stage and to the demulsification step (Fig. 3). During the second trial, the second liquid phase was recycled into the first extraction stage and the skim fraction from the cream demulsification (third skim, containing active enzyme) was recycled into the second extraction stage. During the third trial, the second liquid phase, containing enzyme coming from the cream demulsification step, was used in the first extraction stage. Therefore, the first three trials were non-steady-state extractions. In addition to the three extraction trials necessary to complete enzyme recycling, one additional extraction trial was needed to recycle the enzyme from the first cream obtained after complete enzyme recycling into extraction. For those reasons, extraction Trials 1–4 were considered non-steady-state and extraction Trials 5–11 were considered steady-state, which data in Table 1 support. Therefore, we have seven replications at steady state. Steady-state extraction behavior was in agreement with our previous findings when evaluating the same enzyme recycling strategy at laboratory scale ($\sim$14 kg of slurry vs. 480 kg) [10].

Mean oil, protein, and solids extractions yields of 98.0 $\pm$ 0.5%, 96.5 $\pm$ 0.4%, and 86.8 $\pm$ 0.5%, respectively, were achieved when scaling-up integrated, countercurrent, two-stage, EAEP to the pilot plant (Table 1). Mean extraction yields were higher (statistically different at $P < 0.05$) than those obtained in the laboratory, where 96.1 $\pm$ 1.4% oil, 89.3 $\pm$ 1.0% protein, and 81.2 $\pm$ 2.0% solids were extracted following the same enzyme recycling strategy [10]. We hypothesized from our previous laboratory simulation that the addition of excess enzyme in

Table 1 Effects of enzyme recycling on oil, protein, and solids extraction yields in integrated, countercurrent, two-stage, enzyme-assisted aqueous extraction of soybeans

Extraction trials	Oil yield (%)	Protein yield (%)	Solids yield (%)
1 (pre-steady-state)	98.34	96.55	85.67
2 (pre-steady-state)	98.00	96.49	86.11
3 (pre-steady-state)	97.29	96.50	86.39
4 (pre-steady-state)	98.65	97.56	89.07
5 (steady-state)	97.82	97.07	87.06
6 (steady-state)	97.73	96.59	86.68
7 (steady-state)	98.04	96.65	86.47
8 (steady-state)	98.18	96.26	86.51
9 (steady-state)	97.14	96.12	86.95
10 (steady-state)	98.63	95.86	86.08
11 (steady-state)	98.23	96.81	87.61
Mean of trials 5–11	97.97 ± 0.47	96.48 ± 0.42	86.77 ± 0.49

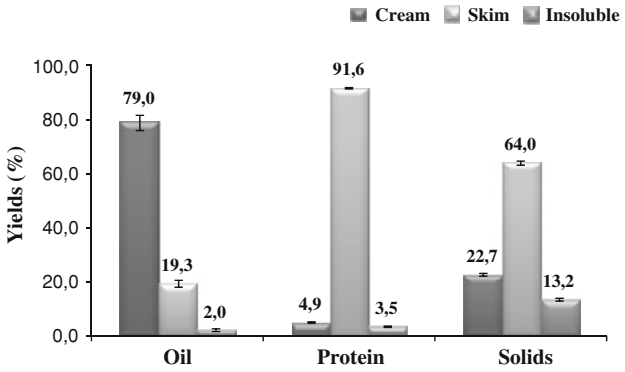

Fig. 4 Effects of enzyme recycling on oil, protein, and solids distribution among the fractions generated in integrated, countercurrent, two-stage, enzyme-assisted aqueous extraction processing of soybeans. Mean and standard deviations are for seven replications at steady-state extraction

extraction because the entire amount of enzyme used to demulsify the cream was recycled into the extraction steps, increased protein emulsification capacity, thereby, making it difficult to separate cream from skim [10]. Using three times the amount of enzyme necessary to perform both extractions adversely affected extraction yields and oil distribution among the fractions when conducted in the lab [10]. When scaling up integrated, countercurrent, two-stage, EAEP in the pilot plant, we used a three-phase centrifuge to separate insolubles, first skim and cream fractions, which produced less skim contamination in the cream fraction than when using the laboratory decanting procedure. The reduced cream production (with less contaminating skim) in the pilot plant reduced the amount of enzyme needed to demulsify the cream, thereby, reducing the amount of enzyme recycled into extraction from three times the optimum amount to twice the optimum amount for extraction. Using less enzyme likely reduced emulsion formation and/or viscosity of the liquid phase, thereby, achieving oil, protein, and solids extraction yields similar to those obtained without enzyme recycling from the cream demulsification step into the extraction steps (99% oil, 96% protein, and 84% solids extraction yields) [5].

When conducting pilot-plant trials, material losses are often quite large. The two extraction stages followed by two centrifugation steps to separate the extraction slurry into insoluble, skim, and cream fractions, generated a mean loss of 11.7 ± 2.4% of the initial slurry (480 kg). This loss was mainly due to material retained in the centrifuge bowls, pumps, and piping. This material loss would not occur in commercial continuous extraction, in which individual batch losses would be avoided due to continuous use.

Oil Distribution Among Fractions

Oil, protein, and solids were distributed as 79, 5, and 23% in the cream and 19, 92, and 64% in the skim, respectively, relative to the initial amounts present in the extruded flakes (Fig. 4). Previous results obtained at laboratory scale for the integrated, countercurrent, EAEP (with enzyme recycling from the cream demulsification) and for the traditional, countercurrent, EAEP (without enzyme recycling from the cream demulsification) are summarized in Table 2. Lower oil yield in the cream (64%) and higher oil yield in the skim (32%) were observed at laboratory scale when using the same enzyme recycling strategy [10]. As previously mentioned, the use of an excessive amount of enzyme in the laboratory-scale experiment may have favored emulsification properties of the protein and peptides, affecting the oil distribution among the fractions. The pilot-plant equipment set-up (three-phase, horizontal, decanter centrifuge) reduced the amount of cream produced by achieving better separation, thereby, reducing the amount of enzyme needed for cream demulsification, which is recycled into the extraction steps.

Improved fraction separation was achieved at pilot-plant scale. More oil was distributed to the cream fraction (79 vs. 64%); producing skim with less oil yield (19 vs. 34%), which is desirable since there is no practical method to recover that oil. As a consequence of reducing the amount of enzyme recycled into the extraction at pilot-plant scale, lower DHs were achieved compared with laboratory scale. DHs of 7.2 ± 1.2% and 16.4 ± 2.0% achieved in laboratory trials [10] were reduced in the current pilot-plant trials to 8.8 ± 2.2% and 10.7 ± 3.0% for first and second extraction stages, respectively. Although no statistical difference at $P < 0.05$ was observed for the DHs corresponding to the first extraction for both experiments (laboratory and pilot plant), the DHs for second extraction

Table 2 Oil and protein distribution among the fractions when recycling enzyme from cream demulsification into the extraction (integrated countercurrent) and not doing so (countercurrent) on a laboratory scale

Integrated, countercurrent, EAEP (laboratory scale)[a]				Countercurrent, EAEP (laboratory scale)[b]			
Yield (%)	Cream	Skim	Insoluble	Yield (%)	Cream	Skim	Insoluble
Oil	64	32	4	Oil	86	12	1
Protein	8	81	11	Protein	9	87	4

[a] Integrated, countercurrent, two-stage, enzyme-assisted aqueous extraction from soybeans on a laboratory scale (with enzyme recycling from cream demulsification [10]

[b] Countercurrent, two-stage, enzyme-assisted aqueous extraction from soybeans on a laboratory scale (without recycling enzyme from the cream demulsification) [5]

were statistically different at $P < 0.05$. Although greater extent of protein hydrolysis is generally associated with higher extraction yields [7], hydrophobicity and emulsification capacity of proteins may have increased with increased DH [17, 18].

Our pilot-plant results were similar to those obtained in the laboratory when not recycling enzyme from the cream demulsification step into extraction [5]. The pilot-plant and laboratory trials achieved similar oil (98 vs. 99%) and protein extraction (96% in both cases), similar DHs ?tul?> (8.8 ± 2.2% and 10.7 ± 3.0% at pilot-plant and 6.4 ± 0.1% and 10.1 ± 1.3% at laboratory-scale for the first and second extraction stages, respectively), and only slightly different oil distributions. Reducing the amount of cream produced by integrated, continuous, two-stage, EAEP in the pilot plant was beneficial in reducing the amount of enzyme recycled into extraction, thereby, avoiding emulsion formation and difficulty in separating the fractions; however, reducing the amount of cream generated left more oil in the skim fraction. Oil yield in the skim fraction increased from 12 to 19% when moving from the laboratory (no enzyme recycling) [5] to the pilot plant (recycling the enzyme), respectively.

Cream Demulsification Efficiency

Compositions and oil yields from enzyme-catalyzed demulsification of each cream obtained during the 11 trials are presented in Table 3. Cream composition was relatively constant from the fourth trial forward, indicating that a constant amount of enzyme was recycled into extraction. For that reason, only creams obtained from extraction trials 5–11 were considered to be at steady-state (seven replications). Mean oil and protein contents of 42.4 ± 2.9% and 4.4 ± 0.2%, respectively, were achieved for cream fractions from the 5th to the 11th extraction trials. We recently reported mean oil and protein contents of 25.4 ± 2.4% and 5.0 ± 0.2%, respectively, for cream fractions obtained at laboratory-scale when following the same enzyme recycling strategy [10]. The higher oil content in the cream fraction obtained at pilot-plant scale resulted from better separation of the cream from the skim by using a continuous, three-phase, horizontal, decanter centrifuge instead

Table 3 Cream composition before enzymatic demulsification and corresponding free oil yields obtained during integrated, countercurrent, two-stage, enzyme-assisted aqueous extraction of soybeans

Extraction trial	Cream composition (%, as is)		Demulsification yield of free oil (%)
	Oil	Protein	
1 (pre-steady-state)	11.4	5.4	89.2
2 (pre-steady-state)	12.2	5.8	79.8
3 (pre-steady-state)	33.8	4.9	85.0
4 (pre-steady-state)	38.4	4.6	96.3
5 (steady-state)	38.7	4.9	97.8
6 (steady-state)	43.6	4.3	89.9
7 (steady-state)	41.3	4.4	88.4
8 (steady-state)	43.8	4.4	90.7
9 (steady-state)	45.1	4.3	91.3
10 (steady-state)	45.6	4.1	89.3
11 (steady-state)	38.4	4.5	93.7
Mean of trials 5–11	42.4 ± 2.9	4.4 ± 0.2	91.6 ± 3.2

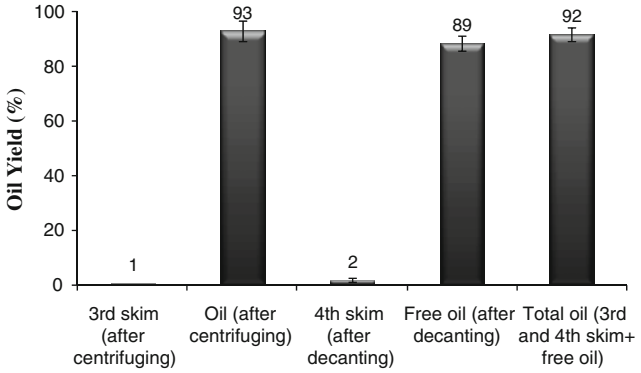

Fig. 5 Oil (crude free fat) distribution among the fractions after centrifuging the demulsified cream and decanting the free oil (in relation to the initial oil in the cream fraction). Mean and standard deviations are for seven replications at steady-state extraction

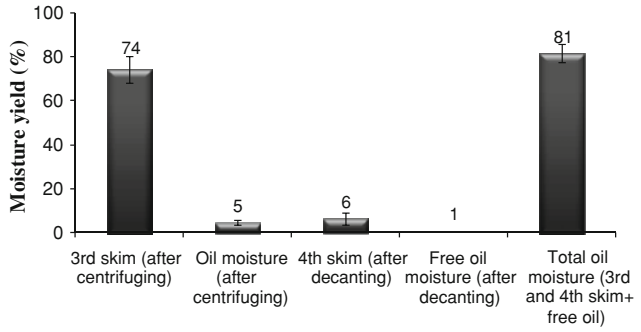

Fig. 6 Moisture distribution among the fractions after centrifuging the demulsified cream and decanting the free oil (in relation to the initial moisture in the cream fraction). Mean and standard deviations are for seven replications at steady-state extraction

of using the laboratory decantation procedure, thereby, avoiding dilution of the cream fraction with skim. Cream demulsification efficiency of 91.6 ± 3.2% was achieved at pilot-plant scale. Demulsification efficiency was improved by 13% in the pilot plant compared to the laboratory [10]. Since the amount of enzyme recycled into extraction was the variable parameter in both experiments, reduced DH of protein achieved in the pilot plant may have reduced emulsion formation and cream stability.

Lipid and moisture distributions in the oil fractions recovered after centrifuging the demulsified cream and settling/decanting to separate free oil from the fourth skim are shown in Figs. 5 and 6. The free oil fraction obtained after two-phase centrifuging retained 5.0% of the water and 93% of the oil in the original cream. The third and fourth skims, which were separated after centrifuging and decanting/settling steps, accounted for <1 and <2% of the oil in the cream prior to demulsification (Fig. 5). Although 91.6 ± 3.2% cream demulsification efficiency was achieved, final free oil recovery was 92.9 ± 2.7% because the residual undemulsified cream contained in the fourth skim (<2% of the oil in the cream) was recycled back into the demulsification step. Considering that only the third skim fraction left the demulsification process (<1% of cream oil) and that approximately 93% of the cream oil was recovered as free oil, the total oil loss for both centrifugation and decantation steps was about 6% of the initial amount of oil in the cream fraction. This oil was probably retained in the centrifuge and decanter and would not be an issue in industry practice. The final free oil contained approximately 1% moisture.

Despite achieving 98% oil extraction with the fully integrated extraction and demulsification process, the 2% of unextracted soybean oil present in the insolubles and the 19% of the oil that ends up in the skim fraction reduce overall free oil recovery (relative to the initial amount of oil in the extruded FFSF) to approximately 79%. Overall free oil recovery was higher when conducted in the pilot plant than in the laboratory (64 vs. 79%), being favored by better fraction separation and reflecting lower oil yield in the skim fraction (19 vs. 32%) and higher cream demulsification efficiency (81.2 vs. 91.6%) [10]. When comparing overall free oil recovery in the pilot plant with enzyme recycling (Fig. 4) to oil recovery obtained in the laboratory without enzyme recycling (Table 2), the increased oil concentration in the skim fraction due to higher cream concentration achieved with a continuous, three-phase, horizontal, decanter centrifuge (12 vs. 19%) slightly reduced overall free oil recovery from 83% (considering that unbroken cream is recycled into extraction) [6] to 79%. The economic viability of the EAEP of soybeans is highly dependent on maximizing the use of all fractions produced, the skim (protein- and sugar-rich fraction), insoluble (fiber-rich fraction), and the oil-rich fraction (cream). Since most of the cream oil is recovered by enzymatic demulsification, the main challenge in the process is finding high-value uses for the other two fractions, skim and insolubles. Since the skim fraction is rich in highly soluble hydrolyzed peptides, recovery from protein skim by membrane filtration or by combining isoelectric precipitation and whey nanofiltration could produce protein concentrates suitable for food applications adding value to this fraction. When aiming to capture skim protein to food purposes, the presence of flatus-producing oligosaccharides in the skim may require a α-galactosidase treatment of the skim before membrane filtration. In addition to capturing skim protein for food purposes, the presence of soluble carbohydrates in the skim may enable its utilization to slurry corn ethanol fermentation, improving ethanol yields and nutritional quality of distiller's dry grains with soluble (DDGS). The insoluble fraction (fiber-rich fraction) could be used as renewable source of fermentable sugars to produce bioethanol and other chemicals [3, 7–9, 11, 19–21] (Table 3).

Conclusions

Proof-of-concept for integrating cream demulsification with countercurrent, two-stage, EAEP using active enzyme in both stages was successfully demonstrated at pilot-plant scale (480 kg of slurry) using equipment typical of industry practice. Approximately 98.0% of oil and 96.5% of protein were extracted. The use of a continuous, three-phase, horizontal, decanter centrifuge to separate insoluble, skim, and cream fractions reduced the amount of cream produced, thereby reducing the amount of enzyme needed to demulsify the cream and consequently the amount of enzyme recycled into extraction. The amount of enzyme used in the entire pilot-plant process was reduced by 35% by not adding fresh enzyme in the extraction. The reduced amount of enzyme in extraction reduced the extent of protein hydrolysis, which made cream separation from skim easier. Cream demulsification efficiency of 91.6% and free oil recovery of 93.0% were achieved when recycling residual un-demulsified cream into the next demulsification, with an overall free oil recovery of 79% being achieved by the integrated process. Improvements achieved for the integrated process at pilot-plant scale represent a significant achievement and foretell probable industrial adoption of the EAEP of soybeans if the amount of oil in skim can be further reduced or high-value uses for skim are identified.

Acknowledgment This work was supported by funds provided by the U.S. Department of Agriculture, Cooperative State Research, Education, and Extension Service, Grant #2009-34432-20057.

References

1. 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. Enzym Microb Technol 28:499–509
2. 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
3. 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
4. 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
5. de Moura JMLN, de Almeida NM, Johnson LA (2009) Scale-up of enzyme-assisted aqueous extraction processing of soybeans. J Am Oil Chem Soc 86:809–815
6. de Moura JMLN, de Almeida NM, Jung S, Johnson LA (2010) Flaking as a pretreatment for enzyme-assisted aqueous extraction processing of soybeans. J Am Oil Chem Soc 87:1507–1515
7. de Moura JMLN, Campbell K, de Almeida NM, Glatz CE, Johnson LA (2011) Protein extraction and recovery in enzyme-assisted aqueous extraction processing of soybeans. J Am Oil Chem Soc. doi:10.1007/s11746-010-1737-0
8. de Almeida, NM, de Moura JMLN, Johnson LA (2010) Functional properties of protein produced by two-stage aqueous countercurrent enzyme-assisted aqueous extraction, 101st American Oil Chemists' Society annual meeting abstracts, May 16–19, Phoenix, AZ, p 130
9. de Moura JMLN, Campbell K, de Almeida NM, Glatz CE, Johnson LA (2011) Protein recovery in enzyme-assisted aqueous extraction processing of soybeans using isoelectric precipitation and ultrafiltration. J Am Oil Chem Soc (accepted)
10. de Moura JMLN, Maurer D, Jung S, Johnson LA (2011) Integration of extraction and cream de-emulsification in the two-stage enzyme-assisted aqueous extraction of soybeans. J Am Oil Chem Soc (in press)
11. Campbell K, Glatz CE, Johnson LA, Jung S, de Moura JMLN, Kapchie V, Murphy PA (2010) Advances in Aqueous Extraction Processing of Soybeans. J Am Oil Chem Soc. doi:10.1007/s11746-010-1724-5
12. Woerfel JB (1995) Extraction. In: Erickson DR (ed) Practical handbook of soybean processing, utilization. AOCS Press, Urbana
13. Jung S, Maurer D, Johnson LA (2009) Factors affecting emulsion stability and quality of oil recovered from enzyme-assisted aqueous extraction of soybeans. Bioresour Technol 100:5340–5347
14. AOCS (1992) Official methods of analysis, 15th edn. Association of Official Analytical Chemists, Washington, DC
15. Jung S, Rickert DA, Deak NA, Aldin ED, Recknor J, Johnson LA, Murphy PA (2003) Comparison of Kjeldahl and Dumas methods for determining protein contents of soybean products. J Am Oil Chem Soc 80:1169–1173
16. AACC (1983) Approved methods of the American Association of Cereal Chemists, 8th edn. St. Paul, MN, USA
17. Adler-Nissen J (1986) Enzymatic hydrolysis of food proteins. Elsevier Applied Science Publishers, New York, pp 110–169
18. Qi M, Hettiarachchy NS, Kalapathy U (1997) Solubility and emulsifying properties of soy protein isolates modified by pancreatin. J Food Sci 62:1110–1115
19. Jung S, de Moura JMLN, Campbell KC, Johnson LA (2011) Enzyme-assisted aqueous extraction of oilseeds. In: Lebovka N, Vorobiev E, Chemat F (eds) Enhancing extraction processes in the food industry. Food Engineering Series, Taylor & Francis LLC (In press)
20. Karki B, Maurer D, Kim TH, Jung S (2011) Comparison and optimization of enzymatic saccharification of soybean fibers recovered from aqueous extractions. Bioresour Technol 102:1228–1233
21. Karki B, Maurer D, Jung S (2011) Efficiency of pretreatments for optimal enzymatic saccharification of soybean fibers. Bioresour Technol (in press)