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Moisture Removal Substantially Improves the Efficiency of in Situ Biodiesel Production from Soybeans

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Abstract In an effort to reduce the production cost of biodiesel, we previously described an approach termed "in situ transesterification" wherein the transesterification of a vegetable oil occurred directly in its raw agricultural material. In that method, substantial quantities of reagents were required to achieve high-efficiency transesterification. Here we report that by drying the substrate a marked reduction in the reagent requirements was achieved. Reaction conditions for maximum fatty acid methyl ester (FAME) production were identified using statistical experimental design methods. In 16-h reactions with 5 g of flakes (2.6% moisture) and 18 mL of 0.10 N NaOH in methanol, 97% theoretical maximum transesterification was achieved. For dry flakes, optimal transesterification was achieved in 10 h in reactions containing 5 g of flakes and 12 mL of 0.10 N NaOH in methanol. This represented a 60% reduction in methanol and a 56% reduction in NaOH use compared with the transesterification of full-moisture flakes. Under these conditions the degree of transesterification was 100% of the theoretical maximum. The transesterification of 20 kg of flakes with a moisture content of 0.8% was conducted under optimal reaction conditions. Both triacylglycerols (TAG) and phosphoacylglycerols (PL) were transesterified to high degrees, with an overall efficiency of 97.3% of the maximum theoretical efficiency. PL were not detected in the flakes following transesterification, and the amount of remaining unreacted TAG was only 1.0% of that input. Following washes with water, 0.5 M NaCl, and dilute NaOH, the recovered FAME met the ASTM specifications for biodiesel. The tocopherol levels of the FAME exceeded by 76% those of a representative commercial biodiesel. Soy flakes retained 97% of their protein following in situ transesterification.

Keywords Biodiesel · Fats and oils utilization · Fatty acid ester · In situ transesterification · Transesterification

Introduction

"Biodiesel," a renewable, low-emissions diesel fuel derived from fats and oils and consisting of the simple alkyl esters of fatty acids (FA) is presently making a global transition from a research and demonstration item to an accepted transportation fuel. Among the challenges to widespread adoption of biodiesel is economic noncompetitiveness, compared with petroleum diesel fuel. The relatively high price of biodiesel arises from the high costs of the refined edible oils that are the predominant feedstocks for fuel production [1]. These biodiesel feedstocks are isolated from oil-bearing raw materials and refined prior to conversion to biodiesel.

In addition to efforts to improve the economic competitiveness of biodiesel by developing methods to

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use less expensive lipids as feedstocks, reducing costs by simplifying the production technology to synthesize biodiesel from first-use fats and oils is needed. In previous reports [2, 3] we described a method, termed ''in situ transesterification,'' in which an oilseed was treated directly with alkaline methanol at ambient temperature and pressure to achieve transesterification of its acylglycerols (AG). That is, the oil in the oilseed was not isolated prior to transesterification to fatty acid methyl esters (FAME). The initial work used flaked soybeans, since soybeans are the predominant source of oil for biodiesel production in the USA. In situ transesterification of soy flakes under optimum reaction conditions resulted in both high efficiency (95%) of lipid removal from the raw material and efficient transesterification to methyl esters [2]. Since this approach to FAME synthesis eliminates the need for isolation, and possibly refining, of the oilseed lipid, the process could reduce biodiesel production costs.

To achieve maximum FAME yields in the in situ transesterification of flaked soybeans, however, 543-fold molar excess of methanol over the amount of AG in the substrate was required [2]. Removal and recovery of the unreacted methanol remaining after transesterification of the AG-linked FA requires substantial investment in equipment and energy, possibly jeopardizing any cost savings. Therefore, we have sought means to reduce the alcohol requirement for high-efficiency in situ transesterification. In the present work we report on the effects on the alcohol requirement of the prior removal of endogenous water from the oilseed substrate, as well as the compositional examination of FAME produced by the in situ transesterification of dry, flaked soybeans, and its analysis as per the ASTM standard specification for biodiesel fuel [4].

Experimental Procedures

Chemicals

Flaked soybeans, produced for hexane extraction in a commercial edible oil plant, were obtained from Perdue Farms (Salisbury, MD, USA) within 5 h of flaking, and were stored in sealed plastic bags at 4 °C.

SoyGold biodiesel, produced from refined soybean oil, was the product of Ag Environmental Products (Lenexa, KS, USA). Soybean oil triacylglycerols (TAG), free fatty acids (FFA), phosphoacylglycerols (PL), and α-tocopherol for use as chromatography standards were obtained from Sigma-Aldrich (St. Louis, MO, USA). Palmitic, stearic, oleic, linoleic, and linolenic acids were mixed in amounts proportional to their mass abundance in soybean oil [5] for use as the FFA standard. A mixture of FAME whose composition reflected the FA content of soy oil (RM-1) was the product of Matreya (Pleasant Gap, PA, USA). Organic solvents were B&J Brand® high-purity grade (Burdick & Jackson, Muskegon, MI, USA). Other reagents were analytical reagent grade or better.

Drying the Feedstock and Moisture Determination

All determinations were conducted in at least duplicate, with the means being reported. In all cases, individual determinations varied by no more than 5% from the mean. The moisture content of the soy flakes was determined by lyophilization to constant mass. For water removal for in situ transesterification, flakes were dried in a gravity convection oven at 77 °C in shallow pans until the desired residual moisture content, determined gravimetrically, was reached. The relatively low drying temperature was chosen to avoid damage to the flakes or the lipid in them. Higher temperatures and associated shorter drying times may also be acceptable. For the work described here, batches of flakes with either 2.6 or 0 wt% water were prepared.

Determination of Lipid Content of Flaked Soybeans

Flakes were completely dried by lyophilization to constant mass (to determine the lipid levels in as-received flakes) or by air and gravity convection oven drying (posttransesterification flakes), and a known mass (approximately 20 g) was extracted with hexane for 4.5 h in a Soxhlet apparatus. Hexane was removed with a vacuum and the weight of the recovered oil was determined. The TAG content of the oil was determined by high-performance liquid chromatography (HPLC), allowing calculation of TAG recovery by Soxhlet extraction and the oil content of the flakes.

Following the recommendations of Khor and Chan [6], PL were extracted from soy flakes with chloroform–methanol according to the method of Folch et al. [7]. Flakes were milled to the consistency of baking flour prior to extraction.

Conduct and Optimization of in Situ Transesterification: Small-Scale Reactions

Flaked soybeans (5.00 g unless otherwise stated) were mixed with methanol in which sodium hydroxide had been previously dissolved (alkaline alcohol) in screw-capped bottles of capacity at least 5 times the reaction volume. The contents of the bottles were mixed by orbital shaking at sufficient speed to keep the flakes well suspended. Reactions were conducted at room temperature.

Central composite response surface design methods [8] were employed to coordinately investigate the effects and interactions of the amount of alkaline methanol, NaOH concentration, and reaction time on the yields of FAME, FFA, and unreacted TAGs in the liquid phase.

Preliminary studies (data not shown) were conducted to focus the statistically designed work in the region of variable space giving the greatest FAME production. For final optimization of reaction conditions using 2.6 wt% moisture flakes, the amounts of alkaline methanol tested were 10.00, 12.00, 15.00, 18.00, and 20.00 mL; NaOH concentrations were 0.07, 0.08, 0.10, 0.12, and 0.13 N; and reaction times were 4.0, 6.0, 10.0, 14.0, and 16.0 h. The analogous values for fully dried flakes were 7.00, 9.00, 13.00, 17.00, and 19.00 mL methanol; 0.04, 0.05, 0.07, 0.09, and 0.10 N NaOH; and 4.0-, 5.0-, 7.0-, 9.0-, and 10.0-h reaction time. Each series involved 19 reactions at various combinations of these levels.

Following reaction, the reaction bottles were allowed to sit for 15 min at room temperature to allow the spent flakes to settle. The liquid phase was removed, the spent flakes were washed twice by resuspending them in 10 mL methanol, the washes were pooled with the reaction liquid, and the mixture was analyzed by HPLC to determine the contents of FAME, TAG, and FFA.

Best-fit equations correlating these data with the reaction compositions were constructed using SAS/STAT software [9]. Numerical analysis of these equations and examination of the corresponding three-dimensional surfaces allowed identification of the conditions predicted to give maximum FAME yield with minimum contaminating FFA and TAG.

Conduct and Optimization of in Situ Transesterification: Small Pilot-Scale Reaction

Soy flakes were oven-dried in several batches, producing 20.036 kg of dry flakes with 0.8% average moisture content. The goal in drying the flakes was to achieve less than 1% final moisture content, since data indicated that the optimum reaction conditions for transesterification of water-free flakes also gave highly efficient reactions below this value.

Reactions were conducted in nine batches over a period of 2 weeks. Typically, 5.75 L of 0.1 N NaOH in methanol was added to 2,250 g of flakes in a polyethylene screw-capped jug (20 L). This represents the ratio of reactants predicted to give optimal transesterification. The mixture was agitated for 5.5 h on a bottle roller, and the liquid phase was recovered by vacuum filtration through Whatman no. 1 paper. The flakes were returned to the reaction vessel and agitated for 10 min each with 2 × 3.2 L of methanol. After each wash the liquid phase was recovered by filtration and added to the main reaction liquid. The final pooled liquids were adjusted to pH 7-7.5, as measured with pH paper, by adding concentrated HCl (typically 15 mL), and were stored at 4 °C until all batch reactions had been completed.

Cleanup of the Crude FAME

Methanol was removed from the post transesterification liquid under vacuum at 35–55 °C. The resulting crude ester preparation was filtered through Whatman no. 1 paper on a Buchner funnel and the residue was washed by gently agitating it at room temperature for 5 min with 5 vol of warm tap water. The FAME layer recovered after centrifugation (5,900*g*, 15 min) was washed with 0.33 vol of 0.5 M NaCl. The mixture was again centrifuged, and the FAME layer was dried with 5×10^{-4} vol of anhydrous Na_2SO_4. To further reduce the acid number, the sample was then sequentially washed with 0.33 vol of 0.5 M NaCl, 0.03 N NaOH, and 0.1 vol of 0.5 M NaCl, and dried again with Na_2SO_4.

Characterization of Posttransesterification Products

Following in situ transesterification and washing with methanol, the flakes were dried in air and then in a 77 °C convection oven. The residual TAG contents of duplicate samples (20 g) were determined by Soxhlet extraction with hexane, as described above for determining the lipid content of unreacted flakes. The contents of PL in posttransesterification flakes were determined as described above for unreacted flakes.

To determine the presence of PL in the glycerol phase generated by transesterification, the methanol was removed with a vacuum from a sample of posttransesterification liquid phase. To the resulting cloudy brown syrup was added chloroform, methanol, and water (3.4:1.7:1 by volume). After mixing, the phases were separated by centrifugation and the organic layer was analyzed for PL by HPLC.

Analytical Methods

Following transesterification, the combined reaction solution and methanol flake washes were centrifuged

(5,900*g*, 15 min) and the resulting supernatant removed. Following dilution to 45 mL with methanol, 1 mL was mixed with 1 mL of 0.1 N acetic acid in methanol. The methanol was removed under nitrogen, and 10 mL of HPLC mobile phase (hexane, acetic acid, 2-propanol, 98.9:0.1:1.0 vol%) was added. An aliquot of this solution was diluted with twice its volume of mobile phase and analyzed by HPLC to quantify FAME, FFA, and TAG levels. Samples were injected onto a diol-modified silica column (LiChrosorb 5 Diol, Varian, Walnut Creek, CA, USA), the contents of which were eluted with the mobile phase at a flow rate of 0.5 mL/min. Peaks were detected by evaporative light scattering and quantified by reference to standard curves constructed with known pure compounds. This was a short-duration chromatography method and was not suitable for determining the amounts of diacylglycerols (DAG) or monoacylglycerols (MAG). Our previous work had indicated that these did not appear in substantial amounts in FAME generated by in situ transesterification [2].

Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) in extracts of soy flakes and of the product glycerol phase were determined by HPLC using a silica column (Lichrosorb Si 60-5, 3 mm × 100 mm, Varian) with detection by evaporative light scattering [10]. The amounts of phospholipids present were calculated by reference to appropriate standards.

Identification of the FA in the FAME product was achieved by gas chromatography (GC) with flame ionization detection. The column was a Hewlett-Packard Innowax fused-silica capillary, 30 m × 0.53 mm (internal diameter), run as previously described [11]. FAME assignments were made by comparison with known FAME.

FAME produced by the small pilot-scale transesterification of fully dried soy flakes was analyzed according to the ASTM standard specification for biodiesel fuel (B100), designation D 6751-02 [4]. The analyses were conducted by Magellan Midstream Partners (Kansas City, KS, USA).

Soy flake nitrogen contents (weight percent) were determined using a LECO FP2000 nitrogen analyzer (LECO, St. Joseph, MI, USA). The nitrogen content was multiplied by 5.71, as recommended [12] for the derivation of soy protein contents based on nitrogen analysis data.

The tocopherol content of the FAME product was determined by HPLC on a diol-modified silica column with elution by a concentration gradient of 2-propanol in a background of methyl *t*-butyl ether in ether [13]. Analyte detection was by fluorescence (excitation 294 nm, emission 326 nm), with quantification by reference to a response curve generated using α-tocopherol standard solutions at known concentrations. Peak identities were confirmed by mass spectrometry.

Results and Discussion

The oil content of the soy flakes as received was 20.5 wt% (22.6 wt% dry basis). The moisture content, determined by lyophilization to constant mass, was 9.40% (mass basis). These values are typical for the oil and water contents of commercial flaked soybeans [14]. The PC and PE contents of the flakes were 0.48 ± 0.03 and 0.17 ± 0.02 wt% (dry basis).

Optimization of the in Situ Transesterification of Low-Moisture Soy Flakes

Since water inhibits transesterification, we postulated that the large methanol requirement for optimum transesterification of full-moisture flakes [2] was the result of the need to dilute the water in the system to a sufficiently low concentration to support transesterification. Trial studies demonstrated that at intermediate moisture levels (e.g., 5 wt%) the amount of methanol required was still unacceptably large. Soy flakes were, therefore, prepared that had reduced water contents (2.6 or 0%), and central composite experimental design methods were employed to determine the optimum conditions for in situ transesterification of the oil in the flakes. Relatively short reaction times were chosen to reduce batch cycle times. Equations 1 and 2, derived from our results, describe the relationship of FAME yield to the amounts of methanol and NaOH in reactions using 5 g of flakes with 2.5 and 0 wt% moisture, respectively:

$$\begin{aligned}\text{FAME} = {} & 26.15 + 56.73\,V + 4154\,N \\ & + 23.11\,T - 112.5\,VN + 0.647\,VT \\ & - 42.19\,NT - 1.265\,V^2 - 5633\,N^2 - 0.853\,T^2, \\ R^2 = {} & 0.92 \end{aligned} \tag{1}$$

and

$$\begin{aligned}\text{FAME} = {} & -185.0 + 22.92\,V + 2461\,N \\ & - 3.434\,T - 112.2\,VN + 0.0813\,VT \\ & + 20.63\,NT - 0.4525\,V^2 - 4544\,N^2 + 0.1678\,T^2, \\ R^2 = {} & 0.98 \end{aligned} \tag{2}$$

where FAME is the predicted FAME yield (mg), *V* is the volume of volume (mL), *N* is the normality of NaOH, and *T* is the reaction time (h).

Figure 1 shows the response surface predicting FAME yields following 10-h transesterification of completely dry soy flakes, as functions of the amount of methanol in the reaction and the concentration of NaOH in the methanol. Analysis of Eq. 2, Fig. 1, and analogous data obtained for partially dry flakes (2.6 wt% moisture) allowed identification of reaction conditions predicted to achieve maximum FAME production from these substrates (Table 1). To confirm our predictions, reactions were conducted using these reaction conditions. FAME yields between 97 and 100% of the theoretical maximum were obtained (Table 1).

Drying the substrate caused a substantial reduction in the reagent requirements for high-efficiency transesterification; thus, compared with full-moisture flakes, transesterification of flakes with 2.6 wt% water required 40% less methanol and 33% less NaOH, while dry flakes were effectively transesterified with 60% less methanol and 56% less NaOH (Table 1). These reductions in reagent usage were achieved with no sacrifice of transesterification efficiency. In fact, the 97–100% of the theoretical maximum conversion observed was greater than that observed previously for full-moisture flakes (Table 1).

Despite the reduction in methanol when using dry substrate, the molar ratios of methanol to TAG (227:1 for completely dry flakes) remained substantially higher than the 6:1 values common in the alkaline transesterification of refined oils [15]. Some of this was probably required to provide access of alcohol and alkaline catalyst to the substrate: approximately 7.5 mL of liquid was necessary simply to cover the 5 g of flakes in these reactions with liquid. In addition, the required methanol and NaOH may play other roles in facilitating reaction, perhaps altering the permeability of the solid substrate, allowing access of the reactant and the catalyst to the TAG.

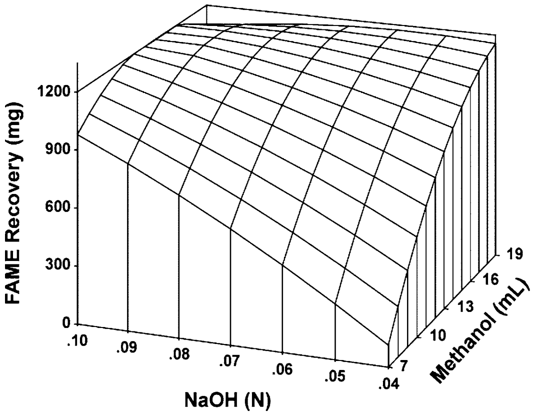

Fig. 1 Reaction surface showing predicted fatty acid methyl ester (*FAME*) yields for the transesterification of 5 g soy flakes as a function of the amount of methanol and its NaOH concentration (room-temperature reaction, 10-h incubation)

Small Pilot-Scale in Situ Transesterification

A series of pilot-scale reactions were conducted, using dried soy flakes and the reaction conditions predicted to give maximum FAME production. The primary goal was the production of sufficient FAME to conduct an analysis as per ASTM specifications [4]. Rather than the 10-h reaction time called for by the optimized reaction conditions (Table 1), 5.5-h reactions were conducted to allow the complete protocol to be completed in the course of a normal workday. Preliminary data (not shown) had demonstrated that reasonably high degrees of transesterification could be achieved within this reaction time. Yield data are presented for these reactions, but it is notable that the reactions were conducted primarily to produce FAME, not to optimize ester production and recovery.

In soybean flakes the primary source of FA for transesterification to FAME is TAG. It is conceivable that phospholipids could also be transesterified during the in situ reaction; therefore, phospholipid levels in the flakes were determined before and after in situ transesterification. Following transesterification, PL levels were below the detection limits of 0.05 and 0.09 wt% for PC and PE, respectively. No PL was detected in the glycerol phase generated during transesterification. We concluded that the in situ method achieved essentially complete transesterification of the phospholipids present in the feedstock, though it cannot be ruled out that PL are hydrolyzed, contributing to the low level of FFA appearing in the FAME. In the production of biodiesel by conventional methods, PL are usually removed as a "gums" fraction during preparation of a crude oil for transesterification, and can present a disposal challenge. Alternatively, if present during transesterification, they can cause difficulties due to emulsion formation during water washing the FAME product. The conversion of phospholipid FA to FAME during in situ transesterification would not only eliminate these difficulties, but also very slightly augment the yield of FAME from the process.

From the total amount of soy flakes employed in the pilot-scale reactions, their TAG and phosphoacylglyceride contents noted above, and the proportions of

Table 1 In situ transesterification: effect of substrate moisture content on reaction conditions predicted for maximum fatty acid methyl ester (*FAME*) yield, and actual observed yields (based on 5 g soy flakes per reaction)

Moisture content of flakes (wt%)	Methanol (mL)	NaOH (N)	Reaction time (h)	Maximum FAME yield (mg)	FAME yield observed (% max. theoretical)
7.4[a]	30.0	0.09	8.0	981	93[b]
2.6	18.0	0.10	16.0	1,096	97
0.0	12.0	0.10	10.0	1,125	100

[a] Data for full-moisture flakes are from Haas et al. [2]

[b] Value differs from the originally reported overall efficiency of 80%, owing to a prior error in calculation of the triacylglycerol content of the substrate

FA in these species, a maximum theoretical FAME yield of 4.55 kg (5.20 L) can be calculated. HPLC analysis of the crude FAME product from four of the individual pilot-scale reactions indicated 97.3 ± 2.9% of the maximum theoretical transesterification efficiency.

The crude FAME was quite pure, with TAG levels below 0.6 wt% and FFA levels of 0.18–0.9 wt% in the individual pilot-scale reactions. This was consistent with our previous observation [2] of high transesterification efficiencies and low contaminant levels with the in situ process.

Following washing of the FAME combined from all the small pilot-scale reactions, the product was subjected to HPLC to determine the ester content. The amount of FAME recovered after washing was 3,809 g (4.35 L), 83.7% of the maximum theoretical yield. Both chemical transesterification efficiency and the degree of recovery of FAME after the washing protocol impacted the total recovery value. Given the high transesterification efficiency of the in situ reaction, as evidenced by the high FAME content of the crude ester preparation, increased yields should be attainable with improvements in washing and recovery.

Compositional and Quality Traits of the FAME Product

Analysis of the FAME product of the small pilot-scale reactions by GC indicated that the five prevalent FAME were those of linoleic (55.6 wt%), oleic (18.8 wt%), palmitic (11.3 wt%), linolenic (9.0 wt%), and stearic (3.6 wt%) acids. This compared favorably with the FA composition of commercial soybean oil based biodiesel (53.6, 21.0, 10.7, 7.6, and 4.6%, respectively), and indicated that the in situ transesterification reaction lacked discrimination with regard to FA. Analysis of the washed pooled FAME product of the small pilot-scale reactions indicated that in most regards the material met the ASTM D 6751-02 standard specification for biodiesel fuel (Table 2).

The cetane value of the FAME preparation was not determined. Since the cetane value is a function, among other things, of the FA profile [16] and since the FA profile of the ester product closely matched that of commercial soybean oil based methyl esters (see before), it is probable this product would exhibit a cetane value of 49–56, i.e., in the range typically reported for soy-based FAME [17], and meet the ASTM minimum specification of at least 47 [4].

Initially the crude FAME sample was washed only with water and with 0.5 M NaCl. The acid number of the resulting product exceeded the ASTM maximum value by 12% (Table 2). Washing the FAME with dilute alkali brought its acid number to within the specifications (Table 2). Further refinement of the production method may eliminate the need for this alkaline treatment.

Contaminating unreacted AG in the FAME product, analyzed in the course of determining total glycerin content (Table 2), were notably low. The concentrations of TAG, DAG, and MAG were 0.004, 0.008, and 0.267 wt%, respectively. These low levels were consistent with the high degree of transesterification observed. Thus, little of the input lipid remained as undesirable AG in FAME prepared by in situ transesterification.

Given the relatively high degree of unsaturation in soybean FA, there may be concern for the oxidative stability of biodiesel made from soybean oil. The endogenous antioxidants of a feedstock, if they transfer to the resulting biodiesel, may stabilize the fuel. It was therefore of interest to determine the levels of tocopherols, the primary antioxidants in soybean, in FAME produced by in situ transesterification. Table 3 indicates that the tocopherol levels of soy-based FAME produced by in situ transesterification exceed by 76% those of a representative commercial biodiesel produced by the transesterification of refined soy oil. Accordingly, biodiesel produced by in situ transesterification should have oxidative stability similar to, or greater than, biodiesel from refined soy oil.

Table 2 Properties of FAME synthesized from soy flakes by in situ transesterification (Determined by Magellan Midstream Partners, Kansas City, KS, USA)

Property	Assay method (ASTM)	Soy flake methyl ester	Biodiesel standard[a] (maximum allowed, unless stated)
Flash point (°C)	D 93	160	130 minimum
Water and sediment (vol%)	D 2709	0	0.05
Carbon residue (wt%)	D 524	<0.010	0.05
Sulfated ash (mass%)	D 874	0.000	0.020
Kinematic viscosity (cSt, at 40°C)	D 445	4.017	1.9–6.0
Sulfur (wt%)	D 5453	0.00035	0.05
Cloud point (°C)	D 2500	0.0	Report
Copper corrosion	D 130	1a	3
Acid number (mg KOH/g)	D 664	0.9 (0.04[b])	0.80 mg KOH/g
Free glycerin (wt%)	D 6584	0.000	0.02
Total glycerin (wt%)	D 6584	0.071	0.240
Phosphorus (wt%)	D 4951	0.0000	0.001
Reduced pressure distillation (temperature at 90% recovery, °C)	D 1160	350	360

[a] American Society for Testing and Materials, Philadelphia, PA, USA. Standard designation D 6751

[b] Repeat determination after additional washing with 0.33 vol of 0.5 M NaCl, 0.03 N NaOH, and 0.1 vol of 0.5 M NaCl

Table 3 Tocopherol contents ($\times 10^{-4}$ g/mL) of commercial soy-based biodiesel and FAME produced by the in situ transesterification of soy flakes

Tocopherol	Commercial biodiesel[a,b]	In situ FAME[b]
α	2.8	0.3
γ	7.6	11.4
δ	6.0	17.2
Total	16.4	28.9

[a] SoyGold, Ag Environmental Products, Lenexa, KS, USA

[b] Means of triplicate determinations. Maximum standard deviations, 3% of mean

Characterization of Spent Flakes

After completion of the reaction, a sample of spent flakes from one of the small pilot-scale reactions was dried and extracted with hexane in a Soxhlet apparatus. The mass of the recovered oil phase was determined, and its composition was analyzed by HPLC. TAG equivalent to only 1.0% of the original lipid content of the flakes was recovered, indicating a TAG extraction efficiency of 99.0%. High TAG extraction efficiency is desirable, because low efficiency would severely restrict the overall FAME yield. This result compared favorably with the results of smaller reactions reported above and previously [2].

Hexane extraction and HPLC also determined that the spent flakes contained FAME equivalent to 3.8% of the theoretical maximum ester yield. In small-scale reactions, two methanol washes removed virtually all the FAME from posttransesterification flakes. This approach, however, was not as effective at pilot scale.

If the FAME contents on one batch of posttransesterification flakes are regarded as representative, then the sum of the FAME recovered in the liquid phase after transesterification/aqueous washing and that remaining on the flakes following methanol washing accounted for 87.5% of the maximum theoretical FAME production. A small part of this theoretical yield (1.0% in the one pilot-scale batch measured) remained as unreacted TAG in the flakes following transesterification. From the extracted TAG, approximately 99.0% of the theoretical ester yield could have been produced. Since an average of 97.3% of the maximum theoretical FAME yield was achieved in the immediate posttransesterification crude ester product, the transesterification of extracted TAG operated at an efficiency exceeding 95%.

From the TAG that were extracted, 3,996 g (4.567 L) of FAME was recovered after transesterification and washing, or can be accounted for as remaining bound to the washed flakes after reaction. This was 87.8% of the maximum theoretical FAME yield. The sum of (1) the potential FAME lost owing to unreacted, unextracted TAG, (2) the amount of recovered, washed, FAME, and (3) the FAME bound to the washed flakes after the reaction accounts for approximately 86% of the maximum theoretical ester yield. The balance, roughly 14% of the maximum attainable yield, was presumably lost to interfaces during washing. We have not optimized the wash protocol. Advances in this area would substantially increase net FAME recovery.

On a dry weight basis, unreacted soy flakes contained 39.24% protein. Following removal of lipid from the flakes by in situ transesterification, the dry weight protein content was 48.3%. Correcting for lipid loss upon transesterification, this corresponds to the retention of 97.0% of the protein by the flakes. Protein retention is desirable, since the current major use of extracted soy meal is in animal feeds, where the value of a meal is primarily a function of its protein content.

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