In situ Alkaline Transesterification: An Effective Method for the Production of Fatty Acid Esters from Vegetable Oils

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ABSTRACT: The production of simple alkyl FA esters by direct alkali-catalyzed *in situ* transesterification of the acylglycerols (AG) in soybeans was examined. Initial experiments demonstrated that the lipid in commercially produced soy flakes was readily transesterified during agitation at 60°C in sealed containers of alcoholic NaOH. Methyl, ethyl, and isopropyl alcohols readily participated in the reaction, suggesting that the phenomenon is a general one. Statistical experimental design methods and response surface regression analysis were used to optimize reaction conditions, using methanol as alcohol. At 60°C, the highest yields of methyl ester with minimal contamination by FFA and AG were predicted at a molar ratio of methanol/AG/NaOH of 226:1:1.6 with an approximately 8-h incubation. An increase in the amount of methanol, coupled with a reduced alkali concentration, also gave high ester yields with low FFA and AG contamination. The reaction also proceeded well at 23°C (room temperature), giving higher predicted ester yields than at 60°C. At room temperature, maximal esterification was predicted at a molar ratio of 543:1:2.0 for methanol/AG/NaOH, again in 8 h. Of the lipid in soy flakes, 95% was removed under such conditions. The amount of FAME recovered after *in situ* transesterification corresponded to 84% of this solubilized lipid. Given the 95% removal of lipid from the soy flakes and an 84% efficiency of conversion of this solubilized lipid to FAME, one calculates an overall transesterification efficiency of 80%. The FAME fraction contained only 0.72% (mass basis) FFA and no AG. Of the glycerol released by transesterification, 93% was located in the alcoholic ester phase and 7% was on the post-transesterification flakes.

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The simple alkyl esters of FA have numerous established uses in the food, textile, cosmetic, rubber and metal processing, and synthetic lubricant industries. FAME are the predominant esters consumed, with a year 2000 global consumption of 1 million metric tons (MMT) (1). In addition, FAME are the favored starting material for the production of fatty amides, more complex esters, ester sulfonates, and fatty alcohols. Fatty alcohols alone were consumed at a world rate of 6.2 MMT in 2000 (1). Production and consumption of the methyl esters of FA are rapidly increasing due to their growing use as biodiesel, a renewable replacement for petroleum-based diesel engine fuel.

Contemporary industrial technology for the synthesis of fatty acyl esters of vegetable oils involves isolation of oilseed acylglycerides by extrusion or solvent extraction, degumming and refining of the oil, and its alkali-catalyzed transesterification. Hexane extraction is the main technology for oil recovery in the United States. Extraction plants achieve high levels of solvent recovery, with a typical soybean processing plant losing less than 1.25 L of solvent per metric ton of input (2). Nonetheless, with a typical daily operating throughput of 3,000 tons of oilseed, this could result in the release to the atmosphere of nearly 3,800 L of hexane per day per facility. Hexane contributes to the production of atmospheric smog and to global warming and is classified as a hazardous air pollutant (3). Because of this, and since the replacement of lost solvent represents a significant cost to the extraction facility, interest exists in further reducing or eliminating the use of hexane in oilseed extraction.

A recent and fast-growing use of simple alkyl FA esters, especially methyl and ethyl esters, is as biodiesel, a diesel fuel replacement and additive. Perhaps the largest single impediment to wider adoption of biodiesel is its cost. When produced from refined oils, feedstock cost contributes more than 70% to the cost of the ester product (4). Thus, there is interest in reducing feedstock cost in biodiesel production. Simplification of the oil production or esterification processes could lower the cost of this attractive biobased fuel.

Consideration of the current route from oilseed to ester caused us to inquire whether isolation of the oil from the seed, and its refining, were necessary. In contrast, transesterification reagents might be able to access acylglycerides resident in oilseeds and achieve their transesterification directly, *in situ*. Such a route to fatty acyl esters could eliminate the expense associated with solvent extraction and oil cleanup, and simplify the steps in ester production. This could result in a decrease in the cost of the product. We therefore investigated the effectiveness of *in situ* alkaline transesterification in the synthesis of simple FA esters.

While this work was underway, we became aware of prior work in this area. Using sulfuric acid catalysis under reflux conditions, Harrington and D'Arcy-Evans (5,6) first explored the feasibility of *in situ* transesterification, using homogenized whole sunflower seeds as substrate. Using reflux conditions, a 560-fold molar excess of methanol and a 12-fold molar excess of sulfuric acid relative to the number of moles of TAG present, these authors observed ester production, with yields up to 20% greater than in the transesterification of preextracted oil. They

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attributed this to an effect of the water content of the seeds, to an increased extractability of some seed lipids under acidic conditions, and to the transesterification of seed-hull lipids.

Siler-Marinkovic and Tomasevic (7) studied a range of acid and methanol levels in the *in situ* transesterification of homogenized sunflower seeds. They reported ester yields as high as 98% theoretical, based on the oil content of the substrate, although these figures were based on the mass of crude, not refined, ester recovered. The best yields were obtained with reaction conditions of either 1 h at 64.5°C or 4 h at 30°C using an alcohol/sulfuric acid/oil molar ratio of 300:9:1. This involved use of an amount of sulfuric acid equal to the weight of oil present.

Ozgul-Yucel and Turkay (9,10) applied *in situ* acid-catalyzed transesterification to rice bran, which can have high FFA levels. Using methanol as reactant and reaction conditions similar to those of Harrington and D'Arcy-Evans (5,6), they observed efficient esterification of the FFA but found that AG were poorly transesterified and remained in the bran. With ethanol as reactant, the extraction and transesterification of AG were greater than 90% theoretical, although unreacted FFA were as high as 10%.

Turkay's group (11) also applied acid-catalyzed *in situ* transesterification to ground soybeans. Reaction occurred with all primary alcohols examined: methyl, ethyl, propyl, and butyl. With methanol, only 20 to 40% of the oil was removed from the substrate, and of this only 55% was transesterified. The product also contained 3% FFA. Calculating recovery on the basis of the amount of mass lost from the substrate, the higher alcohols extracted up to 99% of the oil present, with up to 85% of it undergoing transesterification. The FFA content of these products was between 1 and 8%.

Alkaline catalysis is known to achieve the transesterification of AG with high speed and efficiency, and to be more effective than acid catalysis in this capacity (12). We therefore investigated and identified optimal conditions for the *in situ* transesterification of soy oil by this method.

EXPERIMENTAL PROCEDURES

Chemicals. Flaked soybeans, prepared for hexane extraction in a commercial oil plant, had a thickness of 0.28 to 0.35 mm. The oil content of the flakes, determined by extraction with hexane for 4.5 h in a Soxhlet apparatus, was 23.9% (mass basis). Their moisture content, determined by overnight lyophilization, was 7.4% (mass basis). These values are typical for the oil and water contents of commercial flaked soybeans (13). The flakes have a somewhat higher oil content than that of whole soybeans, which typically consist of 19% oil, because the hulls are removed prior to flaking. Since the oil content of hulls is virtually zero, the effect of their removal is to elevate the oil content of the remaining material, which becomes flakes. Flakes were stored under nitrogen at −20°C.

Lipid standards were obtained from Sigma-Aldrich (St. Louis, MO). Palmitic, stearic, oleic, linoleic, and linolenic acids, mixed in amounts proportional to their mass abundance in soybean oil (14), served as the FFA standard. A mixture of FAME with a composition reflecting the FA content of soy oil (RM-1) was the product of Matreya, Inc. (Pleasant Gap, PA). Necessary reagents for the determination of glycerol were obtained as components of a TG assay kit (Sigma-Aldrich). Organic solvents were B&J Brand high-purity grade (Burdick & Jackson, Inc., Muskegon, MI). Sulfuric acid (96.3%) was the product of Mallinckrodt Baker (Paris, KY). Other reagents were analytical-reagent grade quality or better.

Conduct and optimization of in situ *transesterification.* Flaked soybeans (5.00 g unless otherwise stated) were mixed with methanol in which sodium hydroxide had been dissolved ("alkaline alcohol") in screw-capped bottles of capacity at least five times the reaction volume. These were mixed by orbital shaking at a speed sufficient to keep the flakes well-suspended. Following the reaction, bottles were allowed to sit for 15 min at room temperature to allow the flakes to settle and the reaction to cool. The liquid phase was removed and, for qualitative analysis, directly analyzed by TLC. For quantitative analysis, the spent flakes were washed twice by resuspension in 10 mL of methanol, and the washes were pooled with the reaction liquid. The combined methanol layers were centrifuged (15 min, 5900 × *g*), and the resulting supernatant was removed. Following dilution to 40 mL with methanol, 1 mL of supernatant was mixed with 10 mL of 2 M KCl-HCl buffer, pH 1.0, and extracted with 10 mL of hexane. The organic layer was recovered and its lipid components analyzed by HPLC.

Focusing on the reaction with methanol, Central Composite Response Surface design methods (15) were employed to coordinately investigate the effects and interactions of the amount of alkaline methanol, the NaOH concentration, and reaction time on the yields of FAME, FFA, and unreacted AG 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 highest FAME production.

Two temperatures were investigated: 60 and 23°C (room temperature). For the 60°C reaction, the amounts of alkaline methanol tested were 7.5 (the minimum to cover 5 g of flakes), 12.1, 18.7, 25.4, and 30.0 mL; the NaOH concentrations tested were 0.05, 0.14, 0.275, 0.41, and 0.5 N; and reaction times were 0.25, 1.8, 4.00, 6.2, and 7.8 h. For reactions at room temperature, the amounts of alkaline methanol tested were 14.2, 18.7, 25.4, 32.1, and 36.7 mL; NaOH concentrations were 0.02, 0.052, 0.10, 0.148, and 0.18 N; and reaction times were 2.5, 4.0, 6.2, 8.5, and 10.0 h. Each experimental series involved 20 reactions at various combinations of these levels.

FAME, FFA, and AG levels were quantitated by HPLC following sample preparation as previously described in this section. Best-fit equations correlating these data with the composition of the reactions were constructed using SAS/STAT software (16). Numerical analysis of these equations and examination of the corresponding 3-D surfaces allowed identification of the conditions predicted to give maximal FAME yield with minimal contaminating FFA and AG.

Determination of transesterification efficiency (room temperature). Soy flakes (100 g, conducted in duplicate) were subjected to *in situ* transesterification at room temperature under identified optimal reaction conditions (680 mL of 0.1 N NaOH in methanol, 7.75-h incubation). After settling of the flakes, the liquid phase was recovered by filtration. The flakes were washed three times by resuspension in 150 mL of methanol for 10 min each, and the washes were pooled with the reaction liquid. The extracted flakes were air-dried, lyophilized to dryness, and their mass determined.

To determine the efficiency of lipid removal from the flakes during *in situ* transesterification, 20 g of the dried, post-reaction flakes was extracted for 4 h with 150 mL of hexane in a Soxhlet apparatus. The liquid phase was recovered, its hexane removed under vacuum, and the AG content of the extract was determined by HPLC.

The transesterification reaction liquid phase and the liquid from the post-transesterification washes of the flakes were pooled, adjusted to pH 3 with concentrated HCl, and the methanol removed under vacuum. The resulting viscous liquid was resuspended in 150 mL of water and extracted five times with 300 mL of hexane. The combined organic phases were dried over sodium sulfate, recovered, and hexane removed under vacuum. The mass of the resulting liquid was determined, and its FAME and FFA contents measured by HPLC.

Determination of the fate of glycerol. Samples (28–30 g, conducted in duplicate) of the dried post-transesterification flakes generated in the preceding section were washed by swirling for 30 min each in 2 × 300 mL of water. The washes were recovered by filtration, pooled, and adjusted to neutrality with HCl. Glycerol contents of this spent-flake wash, and of the water-soluble portion of the original reaction liquid, prepared as described in the preceding section, were determined by an enzymatic assay linking the glycerol kinase-catalyzed phosphorylation of glycerol, *via* the intermediate actions of pyruvate kinase and lactate dyhydrogenase, to the oxidation of NADH (17). Solutions of glycerol of known concentration served as reference standards.

TLC. TLC was performed on 250-μm Silica G plates (Analtech, Newark, DE). The developing solvent was hexane/diethylether/acetic acid (80:20:1, by vol). Spots were visualized by spraying with sulfuric acid and charring on a hotplate.

HPLC. The presence and amounts of FAME, FFA, and AG were determined by HPLC on a silica column (18). Peaks were eluted with gradients of isopropanol and water in hexane/0.6% acetic acid (vol/vol), detected by evaporative light scattering and quantitated by reference to standard curves constructed with known pure compounds. Minimal detectable levels of lipid species per reaction conducted as described in the *Conduct and optimization of* in situ *transesterification* section were: FAME: 60 mg; FFA: 1.1 mg; TAG, DAG, MAG: 1.8 μg; phosphoacylglycerols: 2.7 μg.

RESULTS AND DISCUSSION

Preliminary investigations demonstrated that even brief incubations of soy flakes in alkaline solutions of simple alcohols at 60°C resulted in the production of FA alkyl esters (Fig. 1). This occurred with methanol, ethanol, and isopropanol, suggesting that the effect was a general one. Similar results were reported for acid-catalyzed *in situ* transesterification (10). In that work, low oil extraction (20–40%) and low transesterification of that oil (40–55%) were observed in methanol. Here, under alkaline conditions, ester production with methanol appeared as strong as with less polar alcohols (Fig. 1). FFA were produced during alkaline *in situ* transesterification (Fig. 1), a phenomenon also noted with acid catalysis (10).

Optimization of reaction. Optimization of reaction conditions has the potential to reduce reagent consumption, increase yields, and decrease contamination by FFA and AG. Owing to the industrial importance of the methyl esters of FA, we focused on optimizing conditions for *in situ* transesterification with this alcohol.

Two reaction temperatures were investigated: (i) 60°C, which suffices to achieve rapid reaction yet is below the boiling point of the system, eliminating the need for pressurized equipment, and (ii) 23°C (room temperature), at which heat-

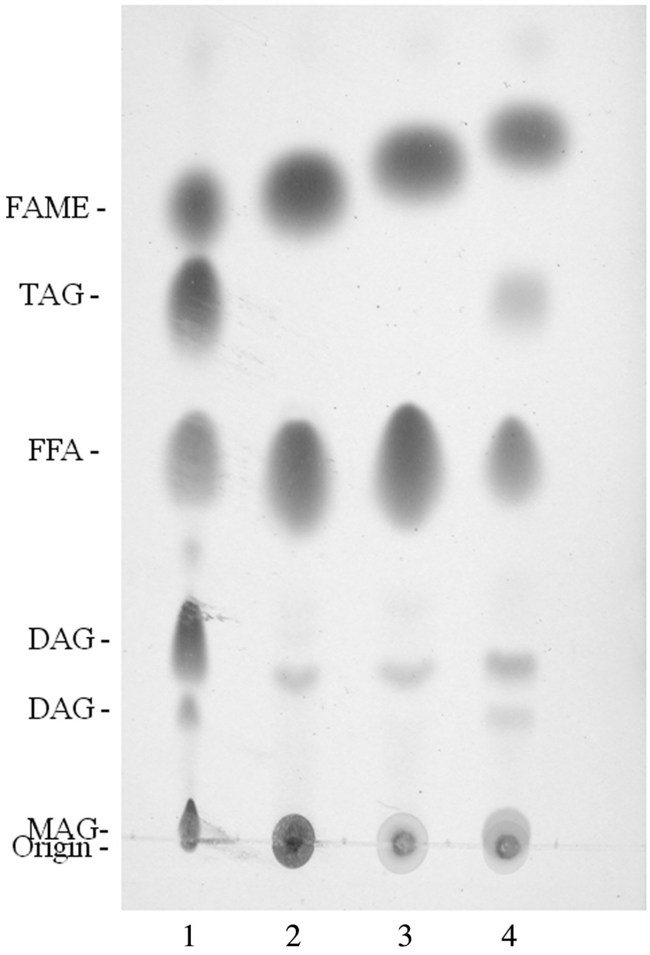

FIG 1. Thin-layer chromatogram of 10 μL of the products of incubation for 2.5 h at 60°C of 5 g of soy flakes in 15 mL of 0.33 N NaOH in various alcohols. Lane (1) Standards: 125 μg each of soy methyl esters (FAME), soy oil TAG, soy FFA, 1,3- diolein (DAG), 1-monoolein (MAG); (2) alcohol: methanol; (3) alcohol: ethanol; (4) alcohol: isopropanol.

ing of the reaction is not required and at which the reduced volatility of the alcohol component eases vapor containment and reduces the need for solvent replacement. Reaction conditions yielding high degrees of transesterification with low levels of FFA and free AG were sought. A low content of FFA is desirable because these represent lost potential FAME and because FFA negatively impact fuel properties. Also, low FFA levels are specified for FAME preparations intended for use as biodiesel (19), which necessitates additional cleanup steps for high-FFA preparations.

The best-fit second-order response surfaces to describe the production of FAME, FFA, and TAG in reactions conducted at 60°C are given in Equations 1–3:

$$\begin{aligned}\text{FAME} = &-1280 + 138\,T + 93.7\,V + 6160\,B - 0.464\,T^2 \\ &- 2.89\,TV - 275\,TB - 1.26\,V^2 - 137\,VB + 6010\,B^2 \qquad [1]\end{aligned}$$

$$\begin{aligned}\text{FFA} = &-184 + 34.4\,T + 5.16\,V + 771\,B - 8.22\,T^2 + 2.02\,TV \\ &+ 96.2\,TB - 0.347\,V^2 + 75.8\,VB + 1140\,B^2 \qquad [2]\end{aligned}$$

$$\begin{aligned}\text{TAG} = &\ 4.62 + 0.956\,T + 0.253\,V - 54.2\,B - 0.0661\,T^2 \\ &- 0.0117\,TV - 0.587\,TB - 0.00735\,V^2 + 0.196\,VB \\ &+ 78.4\,B^2 \qquad [3]\end{aligned}$$

where FAME, FFA, and TAG are expressed as mg/reaction, T = incubation time (h), V = volume of alkaline alcohol (mL), and B = alkali concentration (normality) in the alcohol. These equations gave acceptable fits to the experimental data, with R^2 values of 0.864 for FAME, 0.975 for FFA, and 0.640 for TAG. Di-, mono-, and phospho-AG were not detected in FAME samples prepared at 60°C.

Equations 1–3 allowed construction of surfaces describing the levels of TAG, FAME, and FFA in the reaction liquid as a function of its composition during *in situ* transesterification at 60°C (Figs. 2,3). After 2 h, FAME production was nearly completed; additional incubation, to 6 h total, only slightly increased the yield. In fact, transesterification proceeded rapidly, with some reactions producing 80% of the FAME yield seen at 6 h within 15 min. Incubation beyond 6 h did not further increase yield. The level of unreacted oilseed TAG, extracted from the seeds but not transesterified, was low over virtually the entire coordinate space examined (Figs. 2, 3). FFA levels were also low in reactions containing low alkali concentrations and low to moderate amounts of alcohol (Figs. 2,3). Numerical optimization, and examination of Figs. 1–3, indicated that at 60°C the conditions resulting in high FAME production with low contamination by FFA and TAG from 5 g soy flakes were 12 to 25 mL of methanol, a NaOH concentration between 0.1 and 0.2 N, and a reaction time of approximately 6 h. The greater the alcohol volume, the lower the alkali concentration required to give good yields of FAME with low FFA and AG contamination. Using 22.5 mL of 0.1 N NaOH, the predicted amounts of FAME, FFA, and TAG were 762, 62, and 3 mg, respectively. Upon reducing the methanol to 12.5 mL and increasing NaOH to 0.18 N the predicted product composition after 7.7 h of reaction was 675 mg of FAME, less than 1 mg of FFA, and no TAG. These latter conditions correspond to a molar ratio of 226:1:1.6 for methanol/TAG/alkali. By comparison, optimal conditions for the conventional alkali-catalyzed transesterification of refined soy oil at 60°C are molar ratios on the order of 6:1:0.22 for methanol/TAG/NaOH (12). Thus, in the present configuration, the *in situ* method employs about 38 times more alcohol and 7 times more alkali than does the conventional method. The excess reagents could be recovered for reuse if desired.

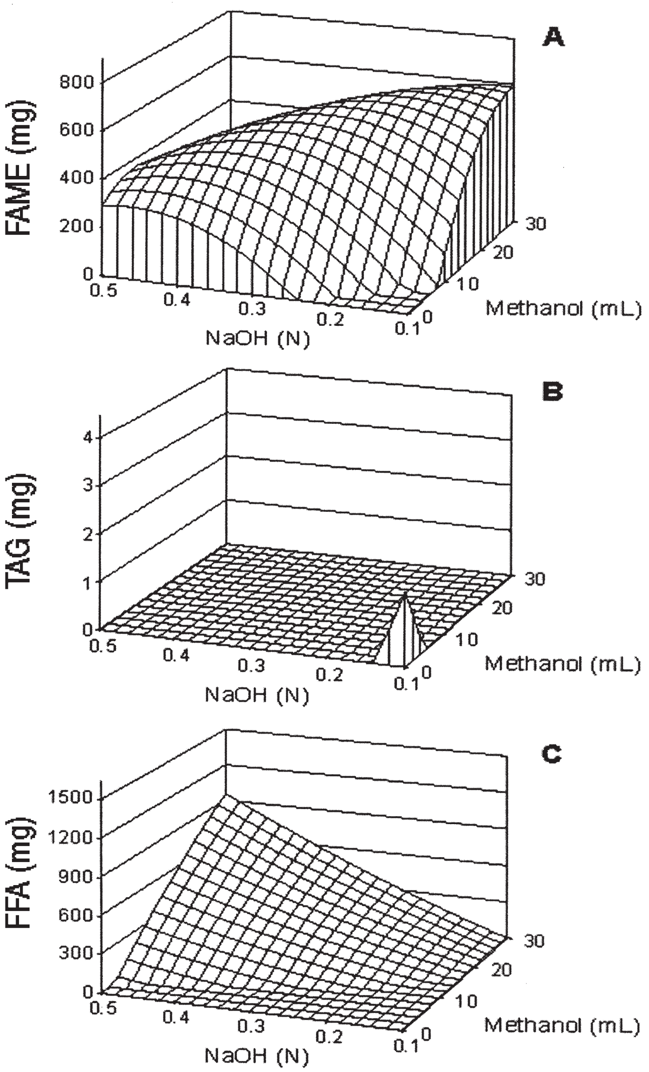

FIG. 2. Predicted response surfaces, calculated from Equations 1–3, for the product composition after 2 h of *in situ* transesterification of 5.00 g of soy flakes at 60°C, as a function of the amount of alcohol and the concentration of sodium hydroxide. (A) FAME; (B) TAG; (C) FFA.

Extraction/transesterification also proceeded well at room temperature. Although elevated temperatures are sometimes used, vegetable-oil transesterification at ambient temperature is not novel, having been described by Freedman *et al.* (12), who also cited similar observations dating back to the 1940s.

When conducted at room temperature, no tri-, di-, mono-, or phospho-AG were detected in the liquid phase following transesterification. The best-fit second-order response sur-

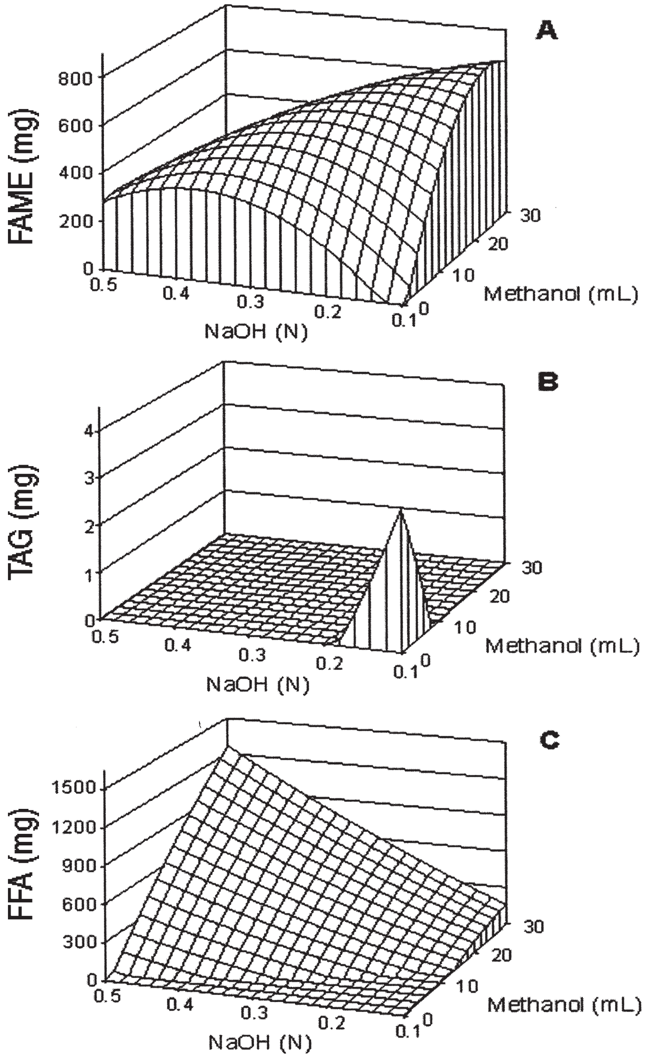

FIG. 3. Predicted response surfaces, calculated from Equations 1–3, for the product composition after 6 h *in situ* transesterification of 5.00 g of soy flakes at 60°C, as a function of the amount of alcohol and the concentration of sodium hydroxide. (A) FAME; (B) TAG; (C) FFA.

faces to describe the FAME and FFA levels as a function of the composition of the reaction were:

$$\begin{aligned}\text{FAME} = {} & -1355 + 129.2\,T + 63.22\,V + 13710\,B - 8.214\,T^2 \\ & - 0.2204\,TV - 147.0\,TB - 0.7243\,V^2 - 143.8\,VB \\ & - 33360\,B^2\end{aligned} \qquad [4]$$

$$\begin{aligned}\text{FFA} = {} & -21.78 - 9.141\,T + 2.050\,V - 733.0\,B - 1.005\,T^2 \\ & + 0.0570\,TV + 41.72\,TB - 0.0580\,V^2 + 14.22\,VB \\ & + 2393\,B^2\end{aligned} \qquad [5]$$

The R^2 values for the fits of these equations to the data were 0.937 for FAME and 0.986 for FFA, indicating that the data were well-modeled by the equations.

Using Equations 4 and 5, predictive surfaces were constructed to describe the composition of the reaction products as a function of alkali concentration, amount of methanol, and reaction time at room temperature (Fig. 4). Maximal FAME production was achieved after about 8 h of reaction, with 90% of maximum occurring by 2 h (data not shown). For reactions of about 8-h duration, the best yields of FAME and lowest levels of contamination by FFA were predicted for reactions containing 5 g of flakes and 30 mL or more of methanol (minimal molar ratio of methanol/TAG = 543) with an NaOH concentration of 0.09 N (molar ratio of NaOH/TAG = 2.0). Predicted FAME and FFA levels under these conditions were on the order of 940 and 35 mg, respectively. This is a higher FAME yield and lower FFA level than predicted for reactions under optimal conditions at 60°C. As at 60°C, the molar reagent requirements at room temperature are substantially greater than those for alkaline transesterification of refined oil (12): 90 times more methanol and 9 times more NaOH. The methanol requirement at room temperature was also approximately 2.4 times that at 60°C (above), but the additional expense of this increase may be compensated for by the reduced costs of room-temperature operation.

Transesterification efficiency. The FAME fraction recovered after *in situ* transesterification of 100 g of soy flakes at

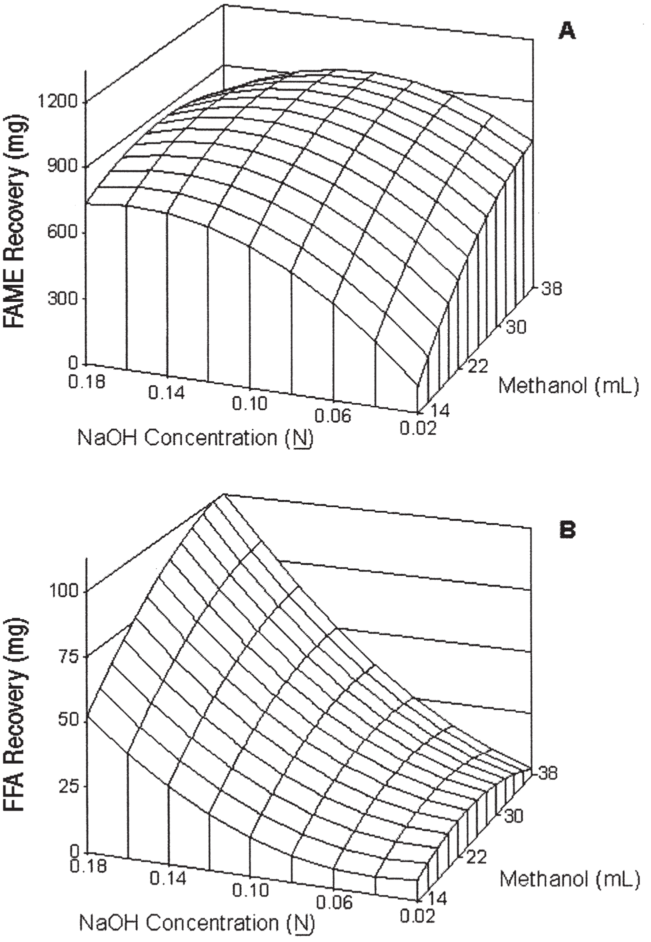

FIG. 4. Predicted response surfaces, calculated from Equations 4 and 5, for the product composition after 8 h of *in situ* transesterification of 5.00 g of soy flakes at 23°C, as a function of the amount of alcohol and the concentration of sodium hydroxide. (A) FAME; (B) FFA.

room temperature for 7.75 h under optimal conditions (680 mL of 0.1 N NaOH in methanol) weighed 19.5 g and was determined by HPLC to contain 18.9 g (97 wt%) of FAME and 0.14 g (0.72 wt%) of FFA (all data are means of replicate reactions; individual values differed from the means by no more than 4%). Given an initial TAG content of 23.9% in the flakes, the theoretical maximal FAME recovery was 23.8 g. Overall FAME recovery was thus 79.4% theoretical. No AG were detected in the FAME product.

Soy flakes lost 31.9% of their mass during *in situ* transesterification at room temperature. This exceeds the total lipid content of the flakes (23.9%) but is consistent with a high degree of removal of water (original content: 7.4%) as well as lipid during transesterification. Hexane extraction of dried post-transesterification flakes removed 1.3 g of material. HPLC analysis indicated that TAG made up 83% of this material. Thus, approximately 1.1 g, 5% of the lipid content of the flakes, was neither extracted nor transesterified during the *in situ* reaction. This would contribute to the less-than-quantitative recovery of FAME that was observed.

Using acidic methanol under reflux, Kildiran *et al.* (11) observed a maximal extraction of 40% of the oil from finely ground soybeans, with only 55% transesterification of this extracted oil, giving an overall FAME yield of 22%. As opposed to acid catalysis, the alkaline room-temperature reaction conducted here achieved a much greater removal of oil from the substrate (95%) and more effective transesterification of the extracted oil (84%). Some of the unrecovered lipid and ester may have been lost to a small emulsion layer that formed during extraction of the samples with water and hexane during analysis. The greater ester yield with alkaline vs. acid catalysis is consistent with the known greater effectiveness of alkaline, compared with acidic, catalysis in the transesterification of AG (12), and may also indicate a better access of transesterification reagents to the oil component of the seeds under alkaline conditions.

Given a 19% oil content for soybeans, a 97% efficiency of oil recovery by commercial hexane extraction (13), 2.5% neutral oil losses during refining, bleaching, and deodorizing (R.L. Stroup, personal communication), and a 97% transesterification efficiency (12), an overall efficiency of 92% can be calculated for the production of biodiesel by use of the current standard process. With an overall FAME recovery of approximately 79% of maximal theoretical, alkaline *in situ* transesterification does not compare favorably with this process. In addition, the ester product is diluted by a great excess of unreacted methanol. This will have to be removed for the esters to be employed as biodiesel, since residual methanol will lower the flashpoint below acceptable minima (19). Further refinement of the *in situ* process would best include yield improvement and a reduction in the amount of methanol required for a high-efficiency reaction.

Fate of glycerol. Glycerol is a coproduct of the transesterification process. There was interest in determining the fate of glycerol in the *in situ* process, since current biodiesel specifications (19) limit the amount allowed and since its recovery could give rise to another product stream. Also, since a typical use of solvent-extracted oilseed flakes is as an animal feed, there was interest in determining the degree to which glycerol might be bound to the flake fraction, where it might affect nutritional performance of the flakes.

Aqueous extraction was used to recover glycerol from the FAME and the spent-flake fractions of the 100-g reactions described above. Enzymatic assay determined that recovered glycerol was located predominantly (93%) in the liquid fraction following transesterification: Its contents in the FAME and spent-flake fractions were 1.9 and 0.14 g, respectively. The sum of these values accounts for approximately 84% of maximal theoretical glycerol recovery. Some of the remainder can be attributed to the 5% of the oil fraction that was not extracted from the flakes.

Overview. As demonstrated previously (10), methanol itself is a poor vegetable-oil extractant. We detected only negligible amounts of ester following a 4-h extraction of soy flakes with methanolic NaOH in a Soxhlet extractor (unpublished data). Presumably, this is because the flake bed is exposed only to the methanol component under Soxhlet conditions. As shown here, however, incubation of soy flakes with alkaline methanol results in the recovery of substantial amounts of FA ester. Conceivably, alkaline alcohol destroys intracellular compartmentalization in the oilseed, allowing solubilization and subsequent transesterification of the AG. Alternatively, fatty acyl esters are expected to be efficient at extracting acylglycerides from oilseeds. The initial and continued production of these during the *in situ* reaction may render the liquid phase of the system an increasingly more effective extracting agent, resulting in higher efficiencies of glyceride extraction and subsequent transesterification.

Compared with acid-catalyzed *in situ* transesterification (10), the alkali-catalyzed process offers the advantages of (i) efficient operation using soy flakes prepared by current industrial technology rather than requiring completely pulverized beans, (ii) use of less reagents and milder reaction conditions, and, most importantly, (iii) substantially higher ester yields. Although we have investigated only soy flakes as a substrate here, general principles and prior reports using *in situ* acid catalysis (5–9) suggest that the technique will be applicable to other oilseeds as well. In addition, this process should lend itself to continuous operation, a desirable format that also may increase ester yields.

Hexane extraction to recover TG from crushed soybeans is only economical when conducted at substantial scales (>2400 tons per day crush capacity; R.L. Stroup, personal communication). This can retard the installation of smaller facilities, e.g., in geographic areas that lack the potential to support such capacity. Direct *in situ* transesterification to produce FA esters for uses such as biodiesel may be a useful method whereby smaller scale operations can generate a marketable product from the oil portion of an oilseed crop.

Since the esters produced here were derived from soybean oil *via* mild conditions and by methods similar to those used in conventional biodiesel production, it is expected that they should serve as an acceptable biodiesel without a great deal of further treatment. TAG, an item subject to maximal allowed levels in biodiesel, were not detected in the product generated by *in situ* transesterification at room temperature. FFA and glycerol are also subject to maximal allowable limits in biodiesel. Although present in the ester produced here, they are routine contaminants of crude biodiesel produced from traditional feedstocks, and relatively straightforward methods for their removal are employed in industry. Final determination of the suitability of the esters produced by the method described here awaits full analysis in light of the accepted specifications for biodiesel (19), and engine testing.

Use of the oil-free meal as animal feed is an essential component of the economic viability of an oilseed-processing operation. In the case of soy, the meal value of the bean exceeds the value of its oil. Thus, the economic viability of the method described here could depend on whether the extracted flakes generated by this process are nutritionally and organoleptically suitable for use as animal feed. No publications to date have addressed this topic, which remains to be investigated.

A U.S. patent application has been filed in regard to the alkaline *in situ* transesterification process (20).

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