

Simple, High-Efficiency Synthesis of Fatty Acid Methyl Esters from Soapstock

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ABSTRACT: We report a simple method that efficiently esterifies the fatty acids in soapstock, an inexpensive, lipid-rich by-product of edible oil production. The process involves (i) alkaline hydrolysis of all lipid-linked fatty acid ester bonds and (ii) acid-catalyzed esterification of the resulting fatty acid sodium salts. Step (i) completely saponified all glycerides and phosphoglycerides in the soapstock. Following water removal, the resulting free fatty acid sodium salts were rapidly and quantitatively converted to fatty acid methyl esters (FAME) by incubation with methanol and sulfuric acid at 35°C and ambient pressure. Minimum molar reactant ratios for full esterification were fatty acids/methanol/sulfuric acid of 1:30:5. The esterification reaction was substantially complete within 10 min and was not inhibited by residual water contents up to *ca.* 10% in the saponified soapstock. The product FAME contained >99% fatty acid esters, 0% triglycerides, <0.05% diglycerides, <0.1% monoglycerides, and <0.8% free fatty acids. Free fatty acid levels were further reduced by washing with dilute sodium hydroxide. Free and total glycerol were <0.01 and <0.015%, respectively. The water content was <0.04%. These values meet the current specifications for biodiesel, a renewable substitute for petroleum-derived diesel fuel. The identities and proportions of fatty acid esters in the FAME reflected the fatty acid content of soybean lipids. Solids formed during the reaction contained 69.1% ash and 0.8% protein. Their sodium content indicated that sodium sulfate was the prime inorganic component. Carbohydrate was the predominant organic constituent of the solid.

Paper no. J9282 in *JAOCS* 77, 373–379 (April 2000).

KEY WORDS: Biodiesel, esterification, fats and oils utilization, fatty acid, fatty acid ester, soapstock.

There is continued and growing interest in the use of renewable resources as feedstocks for replacements for petroleum-derived chemicals. It has long been known that diesel engines can use the triglycerides in fats and oils as fuels. However, their use eventually results in engine failure (1–6). This problem is alleviated by conversion of the fatty acids found in natural lipids into their simple esters, usually methyl or ethyl esters.

An increasing body of evidence indicates that these esters perform well in essentially unmodified diesel engines and that, relative to petroleum-diesel fuel, they can reduce the output of particulate and hydrocarbon pollutants (7–12). The term biodiesel is applied to these esters, which are also being explored as replacements for nonrenewable chemicals in other applications, including cleaning agents, fuel additives, and substitutes for organic solvents.

Refined triglyceride oils have been the predominant feedstocks for the synthesis of biodiesel to date. However, such triglycerides are relatively high in cost and result in a product that cannot compete economically with petroleum-derived diesel fuel (13,14), fostering investigation of the use of lower-value lipids, primarily animal fats and waste greases (15–18), as feedstocks. Soapstock (SS), a lipid-rich by-product of vegetable oil refining, is another relatively inexpensive source of fatty acids. It contains substantial amounts of glycerides, phosphoglycerides, and free fatty acids (FFA), the latter as their sodium or, less commonly, potassium salts. From soybeans, the predominant source of edible oil in the United States, SS is generated at a rate of about 6% of the volume of crude oil produced (19), amounting to as much as *ca.* one billion pounds of SS annually. Compared to refined oils, SS represents an inexpensive source of fatty acids, with its price being as low as one-tenth that of refined vegetable oil. To the producer this represents a low financial return, and there is interest in the oilseeds industry in the development of higher-value products from SS.

Without meaning to imply that there should be only one feedstock for biodiesel production, it is notable that, relative to the use of waste grease as a feedstock, SS is a potentially attractive source of simple fatty acid esters. This is because (i) its production is relatively centralized, eliminating the need for a collection infrastructure, and (ii) SS is not routinely subjected to the potentially damaging extended, repeated high-temperature regimes typical of waste greases.

Some methods for the production of fatty acid methyl esters (FAME) from SS have been reported (20–22). However, the adoption of these may be limited by their use of elevated temperatures and pressures, incomplete esterification of all fatty acids in the starting material, and/or relatively long incubation times. In a previous report we described a two-step method for the production of biodiesel from SS (23). However,

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this scheme achieved esterification of only 81% of the total fatty acids and involved the use of lipase catalysis. It is possible that the cost and operational requirements associated with enzymatic catalysis might retard adoption of the process.

We report here the results of an investigation into the possibility of using inexpensive reagents and simple technologies to produce fatty acid esters from SS, both to address the economic challenges facing biodiesel and to provide new routes for SS utilization.

EXPERIMENTAL PROCEDURES

Chemicals. SS was produced at an industrial-scale facility by alkali refining of crude soybean oil using sodium hydroxide. Triolein, 1,3-diolein, 1-monoolein, free fatty acids, L- α -phosphatidylinositol, L- α -phosphatidylethanolamine, and L- α -phosphatidylglycerol for use as reference standards in high-performance liquid chromatography (HPLC) were obtained from Sigma Chemical Co. (St. Louis, MO). Reference L- α -phosphatidylcholine was from Avanti Polar Lipids (Alabaster, AL). A mixture of FAME whose composition reflected the fatty acid content of soy oil (RM-1; Matreya, Inc., Pleasant Gap, PA) was used as a standard for calibrating detector response in HPLC. A reference standard for FFA was prepared by mixing palmitic, stearic, oleic, linoleic, and linolenic acids (Sigma Chemical Co.) in amounts proportional to their mass abundance in soybean oil (24). Organic solvents were B&J Brand™ High Purity Grade (Burdick & Jackson, Inc., Muskegon, MI). Methanol was dried over sodium sulfate before use. Sulfuric acid (96.3%) was the product of Mallinckrodt Baker (Paris, KY).

Saponification of SS. Various proportions of sodium hydroxide solution (50 wt%) were added to SS, and the mixtures were incubated at atmospheric pressure and selected temperatures with agitation. Samples were removed periodically, and their contents of residual glycerides and phosphoglycerides were determined by HPLC, as described below in the analytical methods section.

Prior to esterification, water was removed from the saponified SS by lyophilization.

Optimization of the esterification reaction. A Central Composite Response Surface design (25) was employed to coordinately investigate the effects and interactions of methanol and sulfuric acid concentrations, and residual water in the saponified SS, on the efficiency of esterification. Each reaction contained 2.85 g (dry weight equivalents) of saponified SS. Incubation was for 2 h at 35°C with shaking in 20 mm (diam.) \times 150 mm screw-capped tubes. The amounts of FAME and of residual unesterified FFA were then determined by HPLC of hexane extracts. Preliminary studies identified the values of the experimental variables that gave high and low degrees of esterification, and the settings of these variables in the statistically designed experiment were then chosen to give substantial degrees of conversion. These settings were: methanol: 3, 4.8, 7.5, 10.2, 12 mL; sulfuric acid: 0.2, 0.4, 0.7, 1.0, 1.2 mL; water content of saponified SS (mass basis): 0, 1.1, 2.5, 3.9, 4.6%.

Production of FAME at optimal conditions. To determine the

time course of the esterification reaction using optimal ratios of reactants, a series of 20 \times 150 mm screw-cap tubes containing 2.85 g (dry wt) of saponified SS, 7.0 mL methanol, and 0.68 mL sulfuric acid were incubated with shaking at 35°C. At selected times, tubes were withdrawn, extracted with hexane, and the FAME and FFA contents of the extracts determined by HPLC.

Scaled-up esterification reactions were conducted to produce FAME for analysis and to validate the predictions of the statistically designed experiment. Fully dried saponified SS was the feedstock. The amounts of SS, methanol, and sulfuric acid were chosen on the basis of Equation 1 (see Results section) to give high degrees of esterification. The steps in a typical reaction were: (i) Saponified SS was dehydrated by lyophilization and passed through a 1-mm stainless steel mesh screen. The resulting small particle size of the SS aided solubilization in the reaction medium. (ii) Dried, sieved, saponified SS (149 g) was added to a solution of methanol (350 mL) and sulfuric acid (34 mL) containing *ca.* 35 glass beads (5 mm; Thomas Scientific, Swedesboro, NJ) and incubated with shaking for 2 h at 35°C.

Isolation of FAME, determination of its distribution in the reaction by-products. All steps were performed at 40–45°C. (i) Following the esterification incubation, the reaction mixture was centrifuged 10 min at 4,600 \times *g*. The resulting upper liquid layer (FAME) was removed, and the lower, methanol/sulfuric acid layer was poured off a solid pellet. (ii) The FAME fraction was washed twice by gentle mixing with 28% of its volume of water, followed by centrifugation (40 min, 4600 \times *g*). (iii) The pellet generated during the esterification reaction was washed twice by resuspension in 175 mL methanol, recovered by centrifugation (10 min, 4,600 \times *g*), dried under a stream of nitrogen, and stored over a desiccant.

For determination of their compositions by HPLC, the FAME fraction was diluted in hexane, the methanol/sulfuric acid fraction was extracted with hexane, and other fractions were extracted with hexane/0.6% acetic acid (vol:vol).

Analytical methods. The water content of alkali-saponified SS was determined by difference following lyophilization of 100-g samples to constant weight. HPLC was conducted on a silica column to determine the contents of glycerides, phosphoglycerides, lysophosphoglycerides, and FFA (23). 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 (23).

The presence and relative proportions of fatty acid esters in the FAME product were determined by gas chromatography (GC) using a Hewlett-Packard (HP; Wilmington, DE) model 5890 gas chromatograph with flame-ionization detector, equipped with an HP-Innowax fused-silica capillary column, 30 m by 0.53 mm (internal diameter) (26). FAME assignments were made by comparison with standards (Nu-Chek-Prep, Elysian, MN).

Analysis of the product FAME to determine water content, free and total glycerin, and acid value was performed by Williams Pipeline Company (Kansas City, KS).

Nuclear magnetic resonance (NMR) analysis was conducted on both aqueous solutions (in deuterium oxide) and organic solvent extracts (deuteriochloroform) of the solid material remaining after the esterification reaction, using a Varian (Palo Alto, CA) Gemini 200 MHz spectrometer equipped with a 5 mm H1/C13 computer-switchable probe operating at 25°C. For carbon spectra, between 19,000 and 50,000 transients were collected, with a total recycle time of 4 s and a spectral width of 13,000 Hz. Samples were processed with a line-broadening factor of 10 Hz. Proton data were collected using 128 transients, with a total recycle time of 2.6 s and a spectral width of 4,000 Hz.

The protein content of the solid remaining after esterification was determined, following its dissolution in water, using the Bio-Rad Protein Assay. Bovine serum albumin was used as the reference protein (Bio-Rad Laboratories, Richmond, CA). The ash content of the solid was determined gravimetrically as the residual mass following heating for 15 h at 600°C in a Thermolyne 1500 furnace (Thermolyne Corp., Dubuque, IA). The sodium content of the solid was determined using a PerkinElmer 1100B (Norwalk, CT) atomic absorption spectrophotometer.

Percentages are expressed on a mass basis unless indicated otherwise. Data are the averages of at least two independent runs. The results of all such replicate determinations differed by less than 5%.

RESULTS

SS saponification. In attempts to achieve full saponification at near-ambient temperatures, it was found that the addition of NaOH to hot SS to a final concentration of 6.3% (wt of NaOH/wt of SS) achieved quantitative hydrolysis of all lipid-linked fatty acid ester bonds in SS over the course of a 3-d incubation at 40°C. Complete saponification was more quickly achieved at elevated temperatures. At 60°C, with a minimum NaOH addition of 14.6%, full saponification occurred in 5 h. At 100°C, a minimum of 4.2% NaOH achieved full saponification in 2 to 4 h. The 100°C incubation may be preferable, since it achieves full hydrolysis more quickly, requires less alkali, will consequently require less acid in subsequent steps, and will generate lower amounts of sodium sulfate by-product.

SS saponified under the conditions described for a 40°C incubation contained 51.1% water and 26.6% FFA, and had a pH of 13.5. HPLC analysis indicated that SS saponified under any of the conditions described above lacked triglycerides, partial glycerides, phosphoglycerides, and lysophosphoglycerides (minimum detection limits: glycerides: <0.04%, phosphoglycerides and lysophosphoglycerides: <0.06%).

Optimization of the esterification reaction. Preliminary studies indicated that high degrees of fatty acid esterification could be achieved by incubation of fully saponified SS in acidic alcohol solutions at mild temperatures and ambient pressure. Statistical experimental design techniques were employed to determine the effects of the amounts of saponified SS, methanol and sulfuric acid, and of residual water in the SS,

on the efficiency of esterification. The resulting data were analyzed to determine the best-fit second-order response surface to describe the relationship between these variables and the percentage of remaining unreacted FFA. That surface is described by Equation 1:

$$\text{FFA} = 87.05 - 3.95 W - 7.11 M - 121.72 S + 0.245 WM - 0.64 WS + 0.5476 MS + 0.579 W^2 + 0.361 M^2 + 63.667 S^2 \quad [1]$$

where FFA = free fatty acids remaining after the reaction, as a percentage of their initial content; W = water content of the saponified SS (% mass basis); M = amount of methanol in the reaction (mL) per 2.85 g of dry SS; and S = amount of sulfuric acid in the reaction (mL) per 2.85 g of dry SS.

A repeat of this experiment yielded essentially identical results. The R^2 value of the equation was 0.96, indicating that it represented a good fit to the data. The relatively high values of the coefficients of most terms involving sulfuric acid indicate that variations in acid content had the greatest impact on the degree of esterification. From Equation 1 it can be calculated that the minimum reactant concentrations consistent with full esterification correspond to substrate molar ratios (fatty acid/methanol/sulfuric acid) of 1:30:5.

Figure 1 shows the predicted degrees of esterification, calculated from Equation 1, as a function of the amounts of methanol and sulfuric acid in the reaction and with water contents of 0 and 4.6% in the saponified SS reactant. These surfaces encompass and identify reaction conditions that are predicted to result in nearly quantitative esterification. It is notable that the reaction proceeds even when some water is present in the SS feedstock. This could be an economically attractive feature of this process, since it implies that it is not necessary to go to rigorous extremes to dry all components of the reaction or to exclude water during its conduct.

Figure 2 illustrates the extremely rapid rate and high efficiency of this reaction at optimal substrate ratios. In this case, fully dehydrated saponified SS was employed, and reactant concentrations were those predicted by the response surface analysis to yield high degrees of conversion. Within the first minute of incubation, the reaction achieved 90% of theoretical maximal ester production, with a concomitant decline in the concentration of FFA. Greater than 99% theoretical yield of ester was achieved in the first 10 min of incubation.

The esterification reaction also proceeded rapidly when the saponified SS contained some residual water: in using a sample containing 4.6% water, the degree of esterification exceeded 99% after 10 min of incubation. Up to a water content of about 10% in the saponified SS, essentially quantitative esterification occurred in 2 h of incubation in reactions containing the optimal proportions of reactants.

Production of FAME under optimal conditions. To verify the predictions of the statistically designed experiment regarding optimal ratios of reactants for full esterification and to assess the quality of the fatty acid ester product, the esterification was conducted on a somewhat larger scale (149 g lyophilized, saponified SS). Following incubation, an amount of FAME

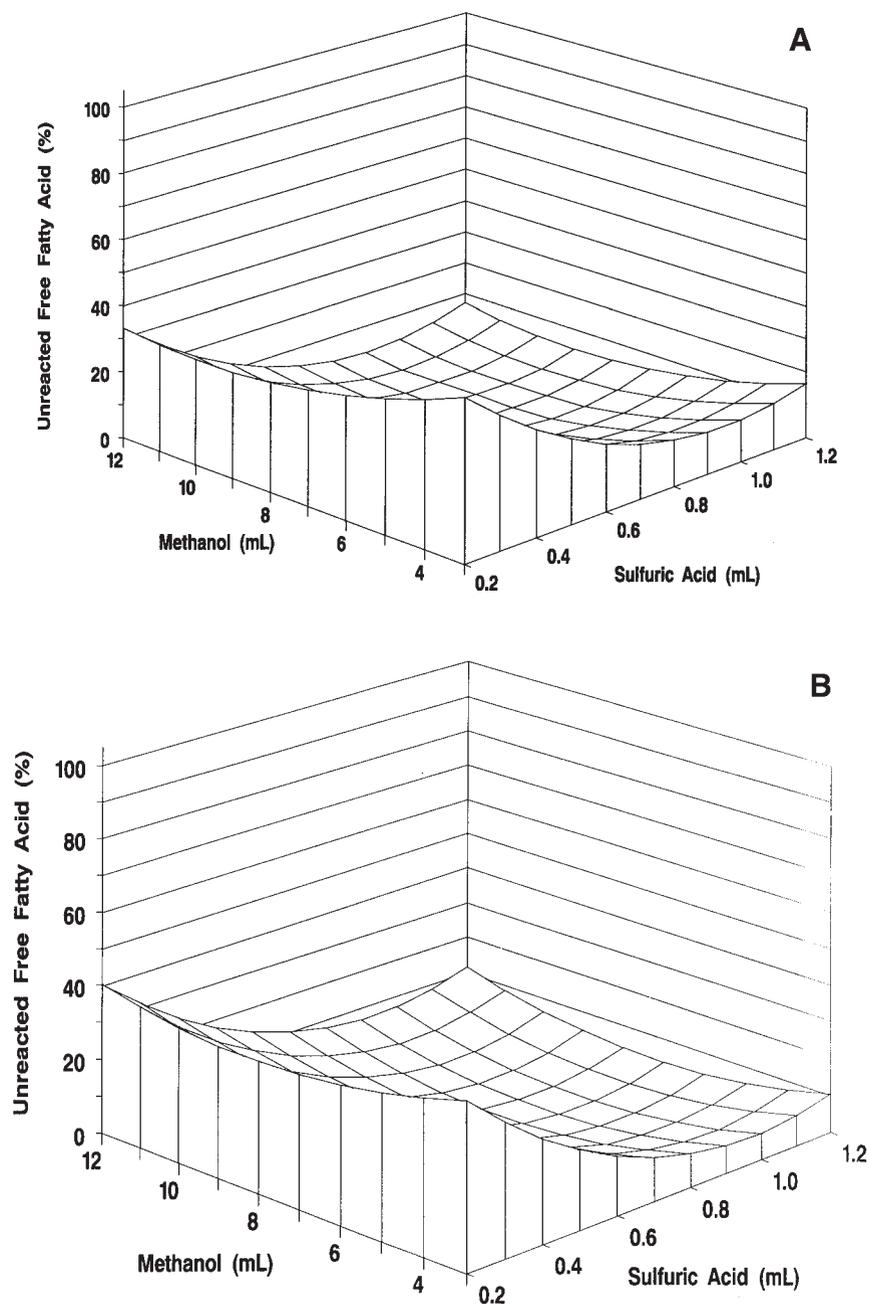


FIG. 1. Predicted response surfaces for the production of fatty acid methyl esters from saponified soy soapstock (2.85 g dry wt) at 35°C and ambient pressure as a function of the amounts of methanol and sulfuric acid. Results are expressed as the percentage of free fatty acids that remained unesterified at the end of a 2-h incubation. Residual water contents of the soapstock: (A) 0%, (B) 4.6%.

equal to the theoretical maximal yield, and barely detectable traces of FFA, were present in the reaction mixture. No glycerides, phosphoglycerides, or lysophosphoglycerides were detected.

Crude FAME (66 mL) were recovered directly from this reaction mixture after incubation. Following water washing, 56 mL of product was recovered, with a FAME content, deter-

mined by HPLC, of 99.1 vol%. This represents recovery of 60% of the theoretical maximal yield of ester. The amounts of residual glycerides, glycerin, and water met the specifications for biodiesel (Table 1). In some cases, the amount of residual FFA in the FAME product was as high as 0.7 wt%. This was sufficient to exceed the acid number specification for biodiesel (0.80 mg KOH/g). However, a wash with dilute NaOH reduced

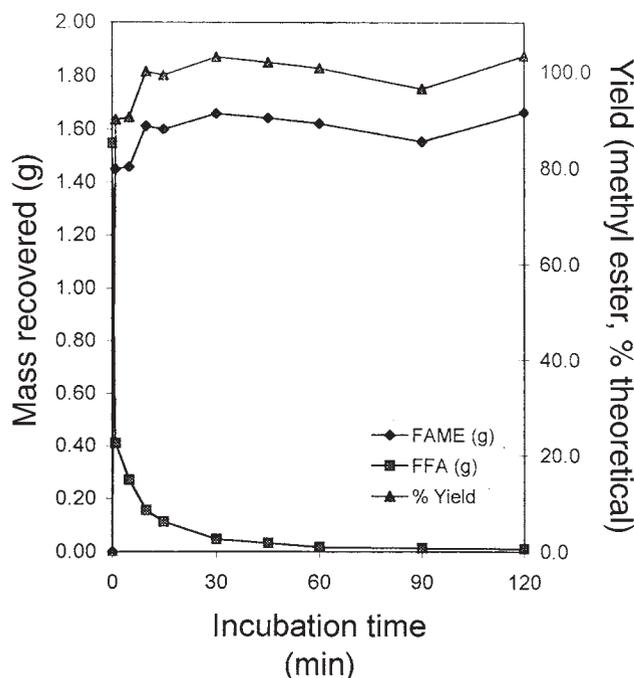


FIG. 2. Time course of the production of fatty acid esters, and loss of free fatty acids (FFA), during the incubation of 2.85 g dry saponified soapstock with 7.0 mL methanol and 0.68 mL sulfuric acid at 35°C. Points are the averages of two independent determinations; individual values differed from the average by less than 5%. FAME, fatty acid methyl esters.

the acid value to zero. These observations strongly suggest the potential suitability of this material for use as a renewable fuel and in other applications requiring high-purity fatty acid esters.

Five major peaks, constituting 98.9% of the total peak area, were detected by GC analysis of the water-washed FAME. The retention times of these peaks were identical to those of the methyl esters of the five most common fatty acids in soy lipids: palmitic, stearic, oleic, linoleic, and linolenic acids. The relative mass proportions of these esters, as indicated by GC, were 15.5, 4.4, 14.9, 56.6, and 7.7, respectively, which agrees well with the proportions of these fatty acids in soybean oil (24) and phospholipids (27). This indicates that the process described here successfully esterifies all fatty acid types found in soybean soapstock, consistent with the very high overall degree of fatty acid esterification achieved by the reaction.

Of the 40% of product FAME that did not spontaneously separate from the esterification reaction, HPLC analysis indicated that 11% (expressed as percentage of total FAME yield) was located in the acidic methanol layer, and 25% was located in the solid material present in the reaction mixture after esterification. Virtually all of the FAME in the solids fraction was released by washing with methanol. Of the theoretical total FAME yield, 19% was located in the first, and 4% in the second, wash. The methanol-washed solids retained only 2% of the total FAME.

Characterization of the solid material formed during esterification. The solid white material formed during esterification

TABLE 1
Composition of Fatty Acid Methyl Esters Synthesized from Soy Soapstock, and Corresponding Proposed Specifications for Biodiesel^a

Property	Content	
	Methyl ester product	ASTM proposed
Fatty acid methyl ester (vol%)	99.1	97.0 min.
Total glycerides (wt%)	<0.15	0.2 max.
Free glycerin (wt%)	<0.01	0.02 max.
Total glycerin (wt%)	<0.015	0.24 max.
Water (wt%)	<0.04	0.05 max.

^aS.A. Howell, of MARC-IV (Kearney, MO): Provisional standards for biodiesel, American Society for Testing and Materials. Personal communication (1999).

was equal in mass to 31% of the amount of dry SS in the reaction. It was largely water soluble and had an inorganic content, determined by ashing, of 69.1%. The sodium content of the solid was determined by atomic absorption spectrophotometry to be 22.6%. This equals the content predicted by assuming that its inorganic portion was sodium sulfate formed on addition of sulfuric acid to the highly alkaline SS. The protein content of the solid material was negligible: 0.8%. ¹³C NMR spectra of aqueous solutions showed signals at 17 (-CH₃), 25–38 (-CH, -CH₂), 58–80 (complex, multiple peaks, -CHOH), 100–104 (-CO,OH, anomeric), and 128–132 (alkene) ppm. The strong signals at 58–80 and 100–104 ppm indicate that carbohydrate was a predominant organic component of the solid. This could have originated from glycolipid in the SS. Proton NMR spectra of this sample were too indistinct for interpretation. ¹³C NMR analysis of chloroform extracts of the solid showed strong signals at 15 (terminal methyl), 23–36 (-CH, -CH₂), 52 (-OCH₃), 128–132 (alkene), and 175 (carbonyl) ppm, consistent with the presence of FAME. Proton NMR of the chloroform-soluble sample supported this conclusion, with peaks at 0.8 to 1 (terminal methyl), 1.2–2.9 (-CH, -CH₂), 3.7 (-OCH₃), and 5.4 (alkene) ppm.

DISCUSSION

SS contains both free- and ester-linked fatty acids, the former as the sodium or, less often, potassium salts. The esterified fatty acids can be in the form of either acylglycerides or phosphoglycerides. The chemical reactivities of these classes of fatty acids differ. A successful route for efficient FAME synthesis from SS must esterify them all. The use of alkali to hydrolyze lipids probably predates recorded history. Acid-catalyzed esterification of FFA is also an established technology (22). However, we are unaware of previous reports describing the sequential application of saponification and esterification to produce simple alkyl fatty acid esters from SS. The simplicity, speed, and high efficiency of both of these reactions result in an attractive overall process.

In initial investigations we explored the use of two consecutive reactions, alkaline transesterification and acid-catalyzed esterification, to convert the ester-linked and FFA of SS, respectively, to FAME (Haas, M.J., S. Bloomer, and K. Scott,

unpublished data). High degrees of overall conversion were obtained. However, it was subsequently found that SS saponifies very readily, with essentially quantitative conversion of all ester-linked fatty acids to free acids. These can then be readily esterified by alcohol in the presence of mineral acid. The resulting scheme involves fewer steps and/or less expensive reagents than those we previously reported and studied (23; and Haas, M.J., S. Bloomer, and K. Scott, unpublished observations) and achieves essentially quantitative esterification.

Soapstock is normally quite alkaline, with pH values between 10 and 11. Relatively little additional alkali is required to achieve full saponification of its fatty acid esters. The reaction proceeds essentially spontaneously. It is necessary to subsequently remove much of the water from the saponified SS in order to conduct acid-catalyzed esterification. However, water removal is inexpensive and readily achieved.

Under the optimal conditions described here, methanol was present in a 30-fold molar excess over the concentration of fatty acids. This excess fostered complete and rapid reaction, even in the presence of moderate levels of water. The excess unreacted methanol can be recovered and used in subsequent reactions, increasing the efficiency of the process. The requirement for a fivefold molar excess of sulfuric acid to achieve complete reaction is most likely due to the need to also neutralize the substantial alkaline content of the saponified SS before conditions sufficiently acidic to catalyze esterification are achieved.

The sodium sulfate, as well as some materials present in the saponified SS, partitions into the solid fraction that forms during the reaction, and should not retard use of the product FAME as biodiesel or in other applications.

We focused on developing a rapid, simple, efficient process for the production of FAME from saponified SS. The method described here appears to be simpler and more efficient than others described for the production of simple esters from SS (20–23). The direct recovery of FAME as a separate layer following the esterification reaction typically yielded 60% of the theoretical amount of product. The remaining FAME were located in other liquid and solid fractions resulting from the reaction, particularly the solid portion. We did not explore the recovery of these FAME, other than to demonstrate they were readily released by washing with methanol. The ease of removal of methanol under reduced pressure suggests a relatively simple means of recovering this FAME fraction, although other methods of recovery may also be suitable. Additionally, the use of optimized methods for the water-washing of FAME (28) may decrease ester loss during recovery and cleanup.

Soy SS was used as the starting material because soybeans are the predominant oilseed processed in the United States, making soybean SS the predominant soapstock. However, the reaction scheme described here is not feedstock-limited and should achieve highly efficient FAME synthesis using SS derived from any oilseed. Waste greases may also be suitable feedstocks for this reaction. Our choice of methanol as the co-reactant alcohol was based on its affordability and the fact that

the majority of investigations of the suitability of fatty acid esters as diesel fuels have involved methyl esters. There is no obvious reason to suspect that the reactions we have described will not also work efficiently with other alcohols. The solid by-product generated during the esterification reaction was composed largely of sodium sulfate. There is interest in the use of potassium hydroxide, rather than sodium hydroxide, in the production of SS, because wastes from the process have potential use as fertilizer (29,30). It is conceivable that potassium sulfate generated during the synthesis of FAME from such SS might have a similar potential.

ACKNOWLEDGMENTS

The skillful assistance of John Phillips in statistical design and analysis, Thomas Foglia in GC analysis, Janine Brouillette in NMR analysis, and Michael Kurantz in atomic absorption spectrophotometry is appreciated.

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[Received June 10, 1999; accepted December 10, 1999]