Production of Alkyl Ester as Biodiesel from Fractionated Lard and Restaurant Grease

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ABSTRACT: Methyl or ethyl esters were produced from lard and restaurant grease by lipase- or base-catalyzed reactions. Before esterifying, some renewable substrates (lard and restaurant grease) should be manipulated through acetone fractionation or on a chromatography column packed with an adsorbent to obtain maximal reaction rate. Because lipase activity was hindered by excess amounts (more than 1 mol) of methanol, each 1 M methanol was added sequentially after 24 h of reaction. Through a three-step reaction, 74% conversion to tallow-methyl ester was obtained. However, a porous substance, such as silica gel, improved the conversion when more than 1 M methanol was used as reaction substrate. When a 1:3 (fractionated lard/methanol, mole ratio) substrate was used, the conversion rates (i.e., extent of conversion) were 2.7 (24 h) and 2.8% (48 h). However, with 10% silica gel in the reaction mixture, the conversion rates increased to 25 and 58%, respectively. Regenerated restaurant grease (FFA removed through column chromatography) was further converted to esters by alkali-catalyzed methanolysis. After 24 h of reaction, 96% conversion was obtained, while only 25% conversion was observed from crude grease. Alkyl esters produced in this study could be used for fuels, potentially as biodiesel.

Paper no. J10012 in *JAOCS 79,* 191–195 (February 2002).

KEY WORDS: Alkyl ester, biodiesel, fractionation, grease, lard.

In the early 1930s, vegetable oils were tested on diesel engines as fuel. However, the high viscosity of vegetable oil, compared to petroleum-derived fuels, caused the engines to malperform, impeding their application as engine fuel. Nevertheless, concerns about petroleum shortages and environmental issues have continuously promoted the development of "alternative fuels" from vegetable oils, animal fats, and even restaurant grease (1–3).

Monoalkyl esters of fats and oils are known as biodiesel (BD), which can be used as a blend with conventional petroleum fuels. Oils are esterified with alcohols (alcoholysis) to overcome the high-viscosity problem as engine fuel. In diesel engine performance and emission tests, a 20% blend of alkyl ester with No. 2 diesel fuel (BD-20) reduced emissions of particulate matter (–26.8%), carbon monoxide (–72.8%), and total unburned hydrocarbons (–73.2%) (4). Therefore, BD is one of the alternative fuels regarded as environmentally friendly.

Lard is an inexpensive co-product of the meat-packing industry that could be further converted to value-added products such as BD. However, the relatively high contents of saturated fatty acids (SFA) in lard result in the poor cold-temperature properties of its alkyl ester. To improve cold-temperature properties, the content of SFA in lard could be reduced through a fractionation process before esterification. As a consequence of this procedure, BD from fractionated lard resists forming crystals at low temperatures, thus improving cold-temperature properties. Restaurant grease would be another renewable source for BD production. During cooking, especially deep-frying, oils are hydrolyzed into FFA and degraded by complex chemical reactions. As a result, abused cooking oils contain compounds such as polymers, volatiles, FFA, and other degradation products. Among them, FFA hydrolyzed from TAG impede alcoholysis reaction for alkyl ester production (5). Thus, FFA would need to be removed prior to alcoholysis.

In this study, fractionated lard having a low SFA content was converted to monoalkyl esters by lipase- or base-catalyzed alcoholysis to produce BD. In the lipase reaction, the effect of silica gel was studied. In addition, restaurant grease was regenerated through filtration and column chromatography to remove FFA and was then further esterified for BD production.

MATERIALS AND METHODS

Materials. Chirazyme L-2 was provided by Amano Enzyme Inc. (Troy, VA). Acetone, methanol, and ethanol (HPLC analytical grade) were obtained from Burdick & Jackson Inc. (Muskegon, MI). Lard was donated by Hatfield Quality Meats Inc. (Hatfield, PA). Grease was obtained from a local restaurant (Philadelphia, PA). Magnesium silicate (100–200 mesh) and 14% boron trifluoride (BF_3) in methanol solution were purchased from Sigma Chemical (St. Louis, MO). Aluminum oxide (basic, 150 mesh) and THF were provided by Aldrich Chemical (Milwaukee, WI). Bis-trimethylsilyl-trifluoroacetamide (BSTFA) was purchased from Pierce Chemical (Rockford, IL).

Fractionation. Lard was fractionated at 24, 27, and 33°C in the absence of solvent (dry fractionation). In addition, acetone fractionation (wet fractionation) was performed with three solute/solvent ratios (1:5, 1:10, and 1:20; lard/acetone, wt/vol), each at three temperatures (–38, –18, and 0°C). The procedure was described previously (6).

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Enzymatic alcoholysis. The liquid fraction of lard (MW 872.9) 2 g was mixed with methanol (MW 32.04), 7 mg (1:1 molar ratio), and alcoholysis was performed in the presence of immobilized lipase Chirazyme L-2 (lipase from *Candida antarctica;* 10% w/w of total substrate) at 30°C. Reactions were performed in screw-cap vials with magnetic stirring at 200 rpm. After 24 h of reaction, methanol in the amount of one to three molar equivalents was added to the reaction mixture, which was further incubated for 24 h. Meanwhile, Chirazyme L-2 catalyzed the reaction in a 1:3 molar ratio (fractionated lard, 2 g/methanol, 0.22 g) mixture for 24 h and was then recovered. An additional 24 h of reaction was conducted with recovered Chirazyme L-2 in substrate mixture (1:1 molar ratio) to study whether inactivation of immobilized lipase was irreversible by high amounts of methanol used as substrate.

For the three-step reaction, 1:1 molar substrate was first reacted for 24 h. Then an additional 1 M methanol or ethanol was added to the mixture and further reacted for another 24 h. Thereafter, 1 M methanol or ethanol was mixed and reacted for 24 h again. To study the effects of silica gel on the reaction, 10% (wt%) silica gel (based on the weight of total substrate) was mixed with the substrate mixture at 1:3 molar ratio, and the conversion rate was studied after 24 and 48 h of reaction. Other reaction conditions were the same as described above. After lipase-catalyzed alcoholysis, the reaction products were isolated from immobilized enzyme or silica gel by twice filtering and washing with excess distilled water. The upper phase containing methyl or ethyl esters was passed through a sodium sulfate column and analyzed.

Adsorbent column for restaurant grease. Restaurant grease (20 g; 15% FFA content acid value) was mixed with hexane (7 mL) and filtered by Whatman filter paper (#1) and passed through an adsorbent column. The column was prepared with 50% magnesium silicate + 50% aluminum oxide (basic). After column chromatography, the hexane was evaporated, and the regenerated grease was recovered. The regenerated grease, from which FFA were removed, was used for base-catalyzed alcoholysis.

Base-catalyzed alcoholysis. Each liquid part of fractionated lard, crude, or regenerated grease (2 g each) was mixed with 6 M methanol (0.42–0.45 g) with or without anhydrous THF (0.41 mL, 1:1 vol/vol methanol) as cosolvent. The reaction was catalyzed by NaOH (4 mg, 2 wt% based on the used grease). The alcoholysis was performed at room temperature (24 ± 1°C) with magnetic stirring (400 rpm) for 1 h. After alcoholysis, 0.5 N HCl was added to the reactants until pH test paper indicated neutral, and excess distilled water was added twice for washing. The recovered ester layers were passed through the sodium sulfate column and analyzed.

Analysis. FFA, TAG, DAG, and MAG were identified by HPLC. An elution gradient using methyl-*t*-butyl ether and hexane as solvent, described by Foglia and Jones (7), was used. Fatty acid composition was determined by GC after conversion to FAME with 14% BF_3 in methanol. The analysis conditions were described previously (6). Percentages of glycerol, alkyl ester, MAG, DAG, and TAG were obtained by high-temperature GC. For high-temperature GC, 100-mg samples were mixed with 100 μL of BSTFA. The mixture was then placed at room temperature (24 ± 1°C) for 20 min. The trimethylsilyl derivatives were then diluted in 10 mL hexane and analyzed by using high-temperature GC. A Hewlett-Packard Model 6890 GC (Palo Alto, CA) equipped with a DB1-HT methyl-silicone capillary column (15 m × 0.32 mm i.d., film thickness 0.1 μm; J&W Scientific, Folsom, CA) was used along with an on-column capillary automatic injector and an FID. Injector and detector temperatures were 380ºC. The carrier gas was helium at a flow rate of 3 mL/min. The column conditions were initial temperature 50ºC (hold 1 min); 15ºC/min to 180ºC (rate 1); 7ºC/min to 230ºC (rate 2); and finally 30ºC/min to 380ºC (hold 10 min). From the chromatographs, the alkyl ester conversion rate (area percentage of produced esters) was calculated.

Water content. Karl Fischer volumetric methods were used for measuring water content (8). A DL37 KF coulometer (Mettler Toledo, Hightstown, NJ) was used.

RESULTS AND DISCUSSION

Table 1 shows the fatty acid compositions of dry-fractionated (without solvent) lard at 24, 27, and 33ºC. By dry-fractionation, the unsaturated fatty acid (UFA) content increased only 1.9 to 4.6% from that of neat lard. Alkyl ester as BD needs stable performance at cold temperatures. Thus, solvent fractionation was conducted to reduce the content of SFA from lard owing to their relatively high m.p.. When −38ºC was used for fractionation, the content of UFA in the liquid fractions significantly increased ($P \leq 0.05$). From 65% UFA of the neat lard, the UFA contents increased from 83 to 86% depending on the solute-to-solvent ratio used (Table 2). Consequently, the SFA content was decreased to 14.4%. The lower the temperature used, the more the SFA content decreased. In addition, when the same fractionation temperature was applied, varying the solute-to-solvent ratio (1:5, 1:10, and 1:20) did not significantly decrease the SFA content ($P \leq 0.05$). Thus, liquid fractions at −38ºC were prepared and used for lipase-catalyzed transesterification reaction.

For lipase-catalyzed transesterification (alcoholysis), methanol and ethanol were used. Several studies have been conducted using lipase-catalyzed reactions for producing alkyl esters (9,10). Shimada *et al.* (11) suggested that three stepwise reactions be conducted for maximal conversion in methanolysis because lipases tend to be inactivated by excess methanol. Methanolysis decreased after more than 1.5 molar equivalent methanol was used. With a 1:3 (oil/methanol) mole ratio, only 10% conversion occurred. This phenomenon was also observed in our study. After 24 h of transesterification, 24% methyl esters were produced with 1:1 molar ratio (lard/methanol). An additional 24-h reaction (second step) with 1 mol of fresh methanol resulted in 56% conversion. When 2 M methanol was added to the reaction mixture, however, the additional 24-h reaction (second step) resulted in 35% conversion, which further decreased to 25% with 3 M

TABLE 1
FA Composition (area %) of Fractionated Lard Without Solvent at 24, 27, and 33°C

FA	14:0	16:0	16:1	18:0	18:1	18:2	UFA[a]	SFA[a]	Yield[b]
Lard	1.7	23.2^{d}	2.7^{g}	10.4^{f}	$42.8^{e,f}$	$19.1^{d,e,f}$	$64.7^{e,f}$	35.3^{e}	—
24L[c]	1.9	22.0^{e}	$2.9^{e,f,g}$	8.4^{h}	45.3^{d}	$19.5^{d,e,f}$	67.7^{d}	32.3^{g}	26.7
27L	1.9	22.4^{e}	$3.0^{d,e,f}$	8.7^{h}	43.5^{e}	20.6^{d}	67.0^{d}	$33.0^{f,g}$	57.9
33L	1.8	22.7^{e}	$2.8^{f,g}$	9.6^{g}	43.1^{e}	$20.0^{d,e}$	65.9^{e}	34.1^{f}	78.1
24S	1.8	23.8^{d}	$2.7^{f,g}$	11.1^{e}	$41.8^{f,g}$	$18.8^{e,f}$	$63.3^{f,g}$	36.7^{d}	73.3
27S	1.8	23.6^{d}	$3.0^{d,e}$	12.1^{d}	40.3^{g}	$19.2^{d,e,f}$	62.5^{g}	37.5^{d}	42.1
33S	1.9	23.4^{d}	3.1^{d}	12.1^{d}	$41.5^{f,g}$	18.0^{f}	62.6^{g}	37.4^{d}	21.9

[a] UFA is sum of unsaturated FA; SFA is sum of saturated FA.
[b] Recovery of liquid and solid fractions (wt%) at each 24, 27, and 33°C.
[c] 24 L is liquid fraction recovered 24ºC. Other recovered fractions similarly designated. Mean values within the same column having a similar roman superscript letter (d–h) do not differ significantly ($P \leq 0.05$).

methanol (Fig. 1). Therefore, the results strongly indicated that lipase was inactivated by excess amounts of methanol during the reaction.

Figure 2 shows the methyl and ethyl ester production during three stepwise transesterification reactions. High conversion to its alkyl ester by lipase-catalyzed reaction was obtained using methanol. After the third reaction, conversion to lard-methyl and -ethyl ester reached 74 and 43%, respectively.

Porous materials, such as silica, act as a polar substrate reservoir to improve the transesterification reaction when hydrophilic substrates are used. Berger *et al.* (12) and Castillo *et al.* (13) suggested that reaction yields would increase if adsorption of hydrophilic substrates onto solid supports having high internal surface was used prior to esterification. In the presence of organic solvent as the reaction medium, this phenomenon is accelerated with solvent (log $P > 2$). Log P value can be measured by taking the partition coefficient of a solvent between octanol and water as a quantitative measure of polarity. Solvents with log $P > 2$ are usually considered to be nonpolar. Mangos *et al.* (14) proposed that hydrophilic substrates would be adsorbed onto the porous materials, preventing blockage between lipase and substrate by excess alcohol. Indeed, addition of silica improves the transesterification reaction with triacetin (polar substrate) and hydrogenated soybean oil.

In this study, transesterification with a 1:3 (fractionated lard/methanol) molar ratio was performed with or without

TABLE 2
FA Composition (area %) of Lard Fractions After Solvent Fractionation at Low Temperature from Acetone[a]

FA	14:0	16:0	16:1	18:0	18:1	18:2	UFA[b]	SFA[b]	Yield[c]
Lard	$1.7^{f,g}$	23.2^{i}	2.7^{h}	10.4^{k}	42.8^{h}	19.1^{i}	64.7^{j}	35.3^{j}	—
−38L5[d]	$2.1^{e,f}$	11.8^{k}	4.6^{e}	2.9^{p}	48.9^{f}	$29.6^{e,f}$	$83.2^{e,f}$	$16.8^{n,o}$	17.5
−38L10	2.2^{e}	9.4^{l}	4.8^{e}	2.8^{p}	50.9^{e}	30.0^{e}	85.6^{e}	14.4^{p}	21.3
−38L20	$2.1^{e,f}$	11.2^{k}	4.6^{e}	2.9^{p}	50.7^{e}	28.5^{f}	$83.8^{e,f}$	16.2^{o}	31.0
−18L5	2.2^{e}	12.4^{k}	4.6^{e}	3.3^{o}	47.9^{g}	$29.6^{e,f}$	$82.1^{f,g}$	$17.9^{m,n}$	20.5
−18L10	2.2^{e}	12.1^{k}	$4.4^{e,f}$	3.4^{o}	$49.2^{e,f}$	28.8^{f}	81.3^{g}	18.7^{n}	27.9
−18L20	$1.9^{e,f}$	12.4^{k}	4.8^{e}	3.4^{o}	$49.3^{e,f}$	28.2^{f}	76.3^{h}	$17.7^{n,o}$	26.2
0L5	$1.8^{e,f}$	19.4^{j}	3.9^{f}	4.7^{m}	48.8^{f}	21.5^{g}	$74.1^{h,i}$	25.9^{l}	61.5
0L10	$1.8^{e,f}$	20.0^{j}	3.2^{g}	$5.3^{l,m}$	48.5^{f}	$21.2^{g,h}$	72.9^{i}	27.1^{k}	69.8
0L20	$1.8^{e,f}$	20.5^{j}	3.2^{g}	5.8^{l}	47.9^{g}	20.9^{h}	72.4^{i}	27.6^{k}	73.9
−38S5	$1.8^{e,f}$	25.9^{h}	2.3^{h}	12.0^{j}	$41.5^{i,j}$	16.4^{j}	60.3^{k}	39.7^{i}	82.5
−38S10	$1.8^{e,f}$	28.1^{g}	$2.1^{h,i}$	$13.4^{h,i}$	$40.5^{i,j}$	14.0^{k}	56.7^{l}	43.3^{h}	78.7
−38S20	$1.6^{f,g}$	$28.8^{f,g}$	$2.1^{h,i}$	13.2^{i}	$39.6^{j,k}$	14.7^{k}	56.4^{l}	43.6^{h}	69.0
−18S5	$1.7^{f,g}$	25.5^{h}	2.4^{h}	$12.7^{i,j}$	$40.9^{i,j}$	16.8^{j}	60.1^{k}	39.9^{i}	79.5
−18S10	1.5^{g}	$28.6^{f,g}$	1.9^{i}	14.1^{h}	$39.8^{j,k}$	14.0^{k}	55.8^{l}	44.2^{h}	72.1
−18S20	$1.7^{f,g}$	25.8^{h}	2.4^{h}	12.1^{j}	$42.0^{h,i}$	16.0^{j}	60.4^{k}	39.6^{i}	73.8
0S5	$1.7^{f,g}$	29.2^{f}	1.9^{i}	19.4^{g}	33.7^{l}	14.1^{k}	49.7^{m}	50.3^{g}	38.5
0S10	$1.6^{f,g}$	32.1^{e}	$1.5^{i,j}$	25.1^{f}	28.6^{m}	11.1^{i}	41.2^{n}	58.8^{f}	30.2
0S20	1.4^{g}	32.1^{e}	1.3^{j}	26.9^{e}	27.3^{m}	11.0^{i}	39.6^{o}	60.4^{e}	26.1

[a] Fractionation temperatures were −38, −18, and 0°C; solvent ratios used were 1:5; 1:10; and 1:20 (wt/vol).
[b] UFA is the sum of unsaturated FA; SFA is the sum of saturated FA.
[c] Recovery of liquid and solid fractions (wt%) at the same temperature and solvent ratio (e.g., −38L5 + −38S5 is equal to 100%).
[d] −38L5 is liquid fraction recovered by solvent crystallization of lard from acetone (1:5, wt/vol) at −38ºC. Other recovered fractions are similarly designated. Mean values within the same column having a similar roman superscript letter (e–p) do not differ significantly ($P \leq 0.05$).

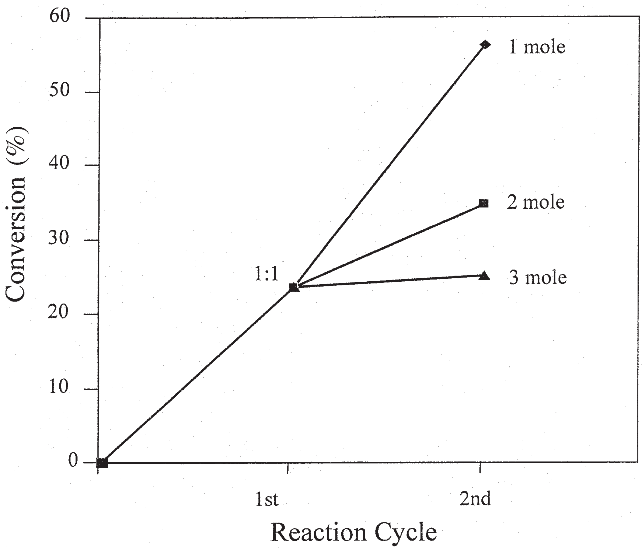

FIG. 1. Methyl ester conversion (%) vs. reaction cycle for 1:1 and 1:3 molar ratio substrates (fractionated lard/methanol). After the first cycle reaction, fresh 1, 2, and 3 mole equivalents of methanol were added to the 1:1 substrate mixture. After fresh methanol was added, the second cycle reaction further occurred for 24 h. From the chromatographs, ester conversion (area % of produced esters) was calculated.

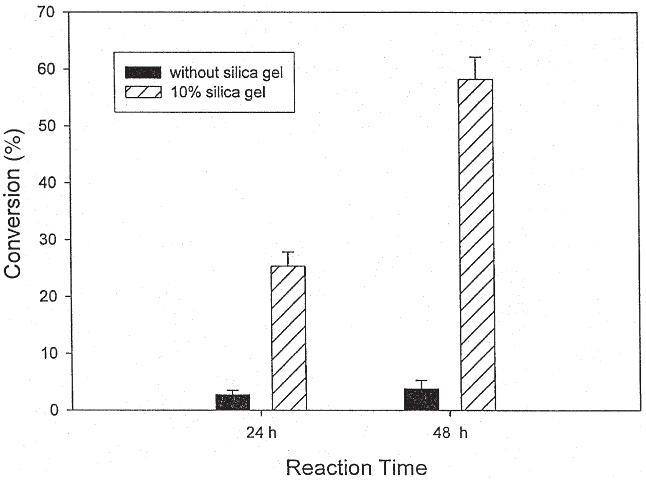

FIG. 3. Methyl ester conversion (%) with or without silica gel. Lipase-catalyzed reaction with 1:3 (fractionated lard/methanol) molar ratio substrates was conducted for 24 and 48 h.

silica gel. After 24 and 48 h of reaction, conversion to methyl ester was 2.7 and 2.8%, respectively, when the reaction was performed without silica. However, when methanol was mixed with silica (10% of total substrate weight) prior to the reaction, the conversion rates after 24 and 48 h were 25 and 58%, respectively (Fig. 3). From these results, we postulated that excess methanol forms a barrier around the lipase structure including its active sites. Therefore, lipase was hindered with respect to contact with the acyl donor and was inactivated. Furthermore, inactivation seemed to be irreversible because recovered IM60 from a 1:3 molar reaction did not improve the conversion with the freshly prepared 1:1 molar substrate as Shimada *et al.* (11) observed previously. However, addition of silica gel increased the reaction rate because silica acts as a methanol pool that prevents the exposure of lipase to a high amount of methanol.

Alkali-catalyzed methanolysis was further conducted on fractionated lard with or without THF as cosolvent (15). Because the optimal molar ratio was studied (1:6 oil/methanol), this ratio was employed with 2% NaOH at room temperature (24 ± 1°C) (5). During methanolysis under these study conditions, the reaction reached equilibrium within 10 min (Fig. 4). When THF (1:1, THF/methanol, volume ratio) was used, the ester yield increased more than 10% after 24 h (Fig. 4). High conversion within a short time led us to conduct alkali-catalyzed reactions for BD production from grease. Crude

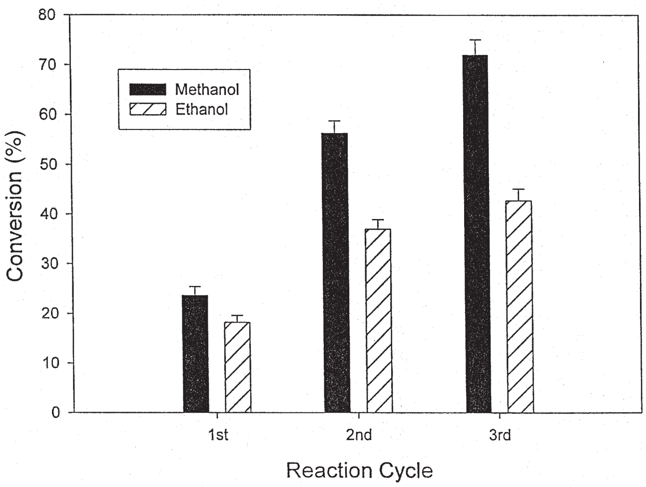

FIG. 2. Methyl and ethyl ester conversion (%) vs. reaction cycle for three times. Reaction conditions are described in the Materials and Methods section.

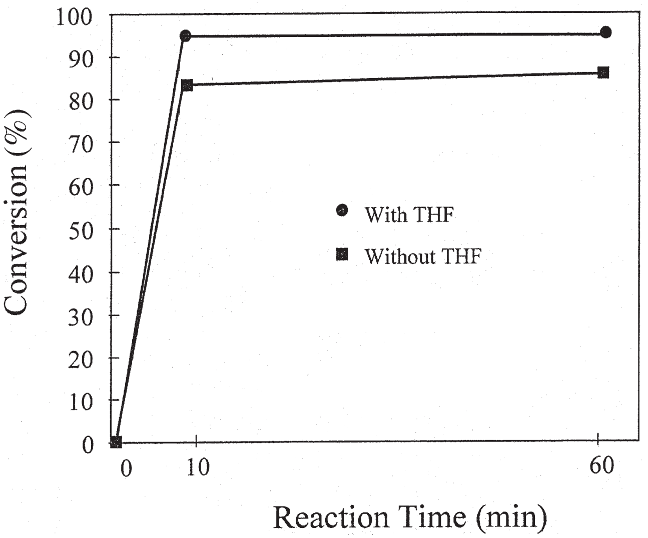

FIG. 4. Methyl ester conversion (%) with or without THF. Base-catalyzed reaction with 1:6 (fractionated lard/methanol) molar ratio substrate was conducted for 60 min.

or regenerated restaurant grease was transesterified with methanol in the presence of NaOH as an alkaline catalyst. In an alkali-catalyzed reaction, maximal conversion could be obtained in the nearly anhydrous and free of FA state because moisture and FFA in reaction system retard the reaction rate. Merely 0.3% water content in the reaction mixture significantly reduces the reaction rate and less than 0.5% FA content is needed to obtain maximal reaction (5).

Locally obtained restaurant grease contained 10.6% FFA and 0.2% water. To reduce FFA and water contents, a mixture of magnesium silicate and aluminum oxide (basic) was used to absorb such polar compounds (16). After column chromatography, the FFA and water contents decreased to 0.23 and 0.02%, respectively. Crude and regenerated restaurant greases were further transesterified using the reaction conditions described before. The conversion rate of crude grease (before column chromatography) was 25% after 24 h of reaction, which increased by 96% with regenerated grease (after column chromatography). Therefore, prior removal of polar compounds, including FFA and moisture, successfully maximized transesterification when restaurant grease was converted to BD.

In this study, lard was fractionated with acetone to reduce the SFA content in the recovered fractions that were further used for cold-temperature-resistant BD production. Thereafter, lipase-catalyzed transesterification was conducted between selected alcohols (methanol and ethanol) and lard fractions through a three-step reaction to maximize ester conversion. Porous materials, such as silica, were necessary to keep the lipase active during the reaction when excess methanol was used. Restaurant grease was converted to methyl ester by alkali catalyst. Because restaurant grease contains polar compounds that retard the reaction rate, they should be removed before the reaction. For that purpose, an absorbent mixture consisting of magnesium silicate and aluminum oxide (basic) successfully reduced the polar compound content. By using regenerated restaurant grease, 96% methanolysis was completed after 24 h of reaction. Esters from renewable fats/oils through enzymatic or chemical reaction have potential usage as BD.

ACKNOWLEDGMENT

This research was partly supported by the International Cooperation Research Program of the Ministry of Science and Technology, Republic of Korea (Project code: 01-H02-00-029-30).

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[Received June 25, 2001; accepted November 12, 2001]