Quantitation in the Analysis of Transesterified Soybean Oil by Capillary Gas Chromatography[1]

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A rapid quantitative capillary gas chromatographic method has been developed for studying transesterification of soybean oil (SBO) to fatty esters. Standard solutions containing methyl linoleate, mono-, di- and trilinolein were analyzed with a 1.8 m × 0.32 mm SE-30 fused silica column. The effect of carrier gas flow on reproducibility was determined. Prior to analysis, mono- (MG) and diglycerides (DG) were silylated with N,O-bis(trimethylsilyl) trifluoroacetamide.Tridecanoin was used as an internal standard. From plots of area and weight relationships, slopes and intercepts for all four compound classes were determined. Agreement between the measured and calculated compositions of the standard solutions was good; the overall standard deviation was 0.4. Slopes and intercepts also were determined for SBO and its methyl and butyl esters. Complete separation of ester, MG, DG and triglyceride was obtained in 12 min by temperature programming from 160 to 350 C. This method of analysis gave excellent results when used in a kinetic study of SBO transesterification.

Recently there has been a stong interest in simple fatty esters as an alternative to #2 diesel oil as a fuel for farm tractors. These esters are obtained from vegetable oils by transesterification. Previously we have studied variables affecting ester yields (1,2) with analyses by thin layer chromatography (TLC) using flame ionization detection (FID) (3), as well as transesterification kinetics of soybean oil (SBO) (4). The analytical method used to support this kinetic study was capillary gas chromatography (CGC) described in this paper. This method was chosen because it provided the accuracy and precision required.

Capillary columns offer a number of advantages over packed columns: they are more efficient, provide greater accuracy and precision, increase resolution and reduce analysis time (5-8). Nohl (9) noted that short capillary columns, 2-4 m in length, give the same resolution as packed columns. D'Alonzo et al. (10) successfully used a 6 m glass capillary column to separate a mixture of fatty acids, monoglycerides (MG), diglycerides (DG) and triglycerides (TG). Their separation required only 10 min compared to 35 min for the same separation on a 1.5 ft packed column. For our kinetic study, we required quantitation of classes of compounds, i.e. ester, MG, DG and TG, but not of compounds within a class. Short capillary columns provided short run times and offered adequate separation of these classes. Thus, CGC met our major objectives to quickly analyze diverse and numerous samples accurately and precisely.

EXPERIMENTAL

Materials. Reference standards used in this study, methyl linoleate (MeL), monolinolein (ML), dilinolein (DL), trilinolein (TL) and tridecanoin (TD), were purchased from Nu-Chek-Prep, Inc. (Elysian, Minnesota) and were chromatographically pure (>99%). Refined

TABLE 1

Effects of Helium Flow Rate on A_c/A_s Reproducibility of Standard Solutions

Solution number	Flow rate (in ml/min)	n[a]	Methyl linoleate		Monolinolein		Dilinolein		Trilinolein	
			$\bar{x}$ A_c/A_s	RSD (%)	$\bar{x}$ A_c/A_s	RSD (%)	$\bar{x}$ A_c/A_s	RSD (%)	$\bar{x}$ A_c/A_s	RSD (%)
1	50	2	10.88	2.8	.12	7.2	.18	4.7	.07	1.0
	200	4	7.96	2.1	.11	3.3	.16	0.9	.06	4.2
	350	4	8.01	4.5	.11	2.1	.16	2.2	.06	5.7
3	50	4	.12	43.1	.21	11.4	.15	17.3	8.54	5.5
	200	4	.07	1.3	.18	1.7	.12	17.3	9.29	3.8
	350	3	.10	11.1	.20	3.7	.15	14.3	8.28	1.6
5	50	3	4.31	3.0	1.22	1.7	2.21	1.2	2.70	2.5
	200	5	3.29	1.9	1.09	1.7	2.36	2.9	2.94	2.3
	350	3	3.61	2.3	1.15	0.8	2.31	1.5	2.72	2.8

[a]n, number of replicates.
[b]$\bar{x}$ A_c/A_s, mean of area of compound (A_c)/area of internal standard (A_s).
[c]RSD (%), % relative standard deviation.

[1]Presented at the AOCS meeting in Philadelphia in May 1985.
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SBO was obtained from Central Soya (Chattanooga, Tennessee). We prepared the soybean methyl esters (MeSBO) and soybean butyl esters (BuSBO) by transesterification (1). These esters were purified by distillation under reduced pressure. N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) was purchased form Pierce Chemical Company (Rockford, Illinois). Acetone was MCB Omnisolv (spectrograde).

Instrumentation and operating conditions. Analyses were performed with a Spectra-Physics SP7100 gas chromatograph equipped with a flame ionization detector and a 1.8 m × 0.32 mm bonded SE-30 fused silica capillary column. Injector and detector temperatures were 350 C. The injector was used in the split mode with a 50-100:1 split ratio. The oven temperature was programmed from 160 to 350 C at 30 C/min and then was held at 350 C for 6 min. Either helium or hydrogen was used as a carrier gas with identical results at a set flow of 200 ml/min and a back pressure of 5-7 kPa. Under these conditions the measured flow through the column was 2-4 ml/min. The detector range was 1×10^{-11} amp/mV. Run time was 12 min with 6 min cooling. Samples of 1-12 μl were injected either manually or by use of a Spectra-Physics Autosampler.

Standard solutions. Three sets of standard solutions were prepared each at a concentration of 20 or 40 mg/ml in acetone. The first set, standard solutions 1-5, contained MeL, ML, DL and TL. The second set, standard solutions 7-11, contained BuSBO, ML, DL and SBO. In the third set, MeSBO replaced BuSBO. The latter two compositions closely resembled transesterification reaction mixtures. Each solution also contained a known amount of TD, the internal standard, as described below. The measured composition of each standard solution was determined by CGC. Data from four replicate CGC analyses of these solutions were used to determine the intercepts and slopes needed for quantitation.

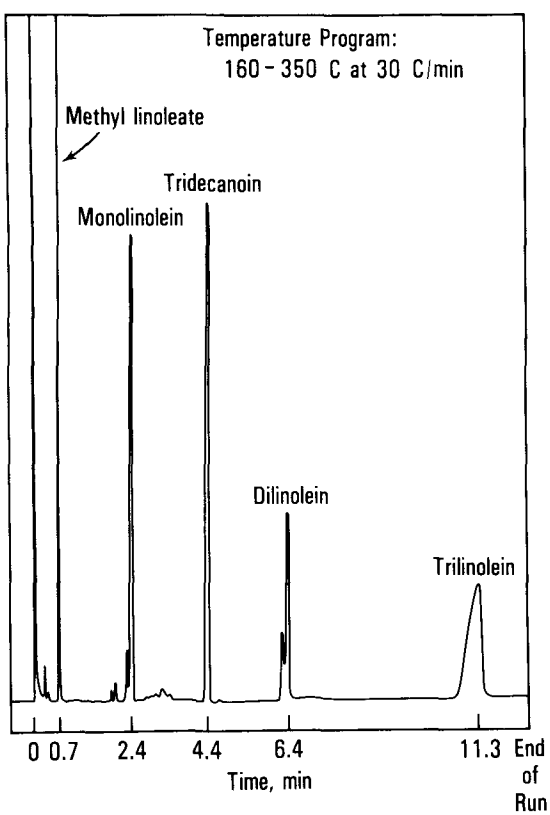

FIG. 1. Chromatogram of a mixture of ester, glycerides and internal standard.

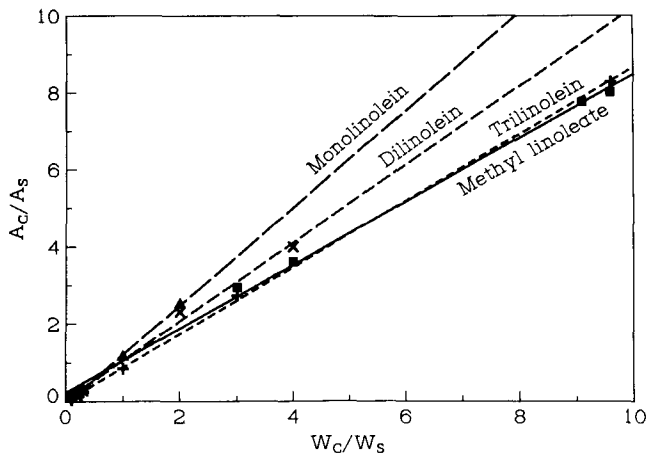

FIG. 2. Plots of A_c/A_s vs W_c/W_s for monolinolein, dilinolein, trilinolein and methyl linoleate.

The following description illustrates the procedures used for preparing standard solutions. Stock solutions of BuSBO, ML, DL and SBO were made up in acetone (40 mg/ml), and then each was used to prepare standard solutions 7-11. Although the proportions of these four components varied, the final concentrations of the components remained at 40 mg/ml. For example, standard solution 7 (SS7) was prepared by mixing 3.60 ml, 0.12 ml, 0.12 ml and 0.16 ml of stock solutions BuSBO, ML, DL and SBO in an 8-ml vial to give 160.0 mg of the four components in 4.00 ml of acetone. SS7 then had the composition: 90% BuSBO, 3% ML, 3% DL and 4% SBO. The contents of the vial were heated at 50 C, and acetone was removed under a stream of nitrogen.

Sufficient BSTFA, 0.600 ml, was then added to the vial to silylate ML and DL. This volume was adequate to silylate ML and DL in any of the standard solutions 7-11. The capped vial was heated in an aluminum block for 5 min at 100 C with brief shaking at 2.5 min and then was cooled to room temperature. To add 9.0 mg of TD to SS7, 0.225 ml of a TD stock solution, 40 mg/ml in acetone, was added to the vial. Acetone, 3.175 ml, was then added to bring the total volume to 4.0 ml (includes 0.6 ml BSTFA and 0.225 ml TD solution). Similar procedures were used to prepare other standard solutions.

Preparation of analytical solutions. To a vial containing 40-50 mg of oil layer from the transesterification of SBO with BuOH was added 0.182 ml BSTFA. This volume of BSTFA was in excess of the amount needed to silylate all ML and DL in the reaction mixtures. The vial was capped, heated to 100 C in a

TABLE 2

Statistical Data for Solutions 1-5[a]

	Methyl linoleate	Monolinolein	Dilinolein	Trilinolein
Y intercept	0.2323	−0.0584	0.0404	0.0070
Slope	0.8245	1.2660	1.0163	0.8654
Standard deviation	0.2082	0.0439	0.1597	0.0766
Correlation coefficient	0.9986	0.9993	0.9968	0.9998

[a]Four replicates were used for each compound.

TABLE 3

Composition of Standard Solutions 1-5 (wt % ± Standard Deviation)[a]

Solution number		Methyl linoleate	Monolinolein	Dilinolein	Trilinolein
1	Actual	96	1	2	1
	Measured	96.8 ± 0.3	0.9 ± 0.1	1.6 ± 0.2	0.7 ± 0.2
2	Actual	91	3	3	3
	Measured	91.6 ± 0.3	2.4 ± 0.03	3.0 ± 0.1	3.0 ± 0.2
3	Actual	1	2	1	96
	Measured	1.2 ± 0.2	1.4 ± 0.1	1.2 ± 0.3	96.2 ± 0.4
4	Actual	30	20	40	10
	Measured	33.9 ± 0.8	18.6 ± 0.2	37.8 ± 0.6	9.6 ± 0.3
5	Actual	40	10	20	30
	Measured	40.1 ± 1.4	8.4 ± 0.2	21.6 ± 1.0	29.9 ± 0.9

[a]Analyses based on 4 replicates per compound.

heating block for 5 min with brief shaking at 2.5 min, and then cooled to room temperature. To add 2.097 mg of TD to the sample, .1918 ml of a TD stock solution, 10.933 mg/ml in acetone, was added to the vial. Acetone, 0.8762 ml, was then added to bring the total volume to 1.25 ml (includes 0.182 ml BSTFA and 0.1918 ml TD solution) giving the solution a concentration of 40 mg/ml for a 50 mg sample.

RESULTS AND DISCUSSION

Flow optimization. Linear velocity, μ, in the 1.8 m capillary column was ca. 70-100 cm/sec using helium flows of 50-350 ml/min. This velocity range is considerably faster than the range of 25-40 cm/sec normally used with longer capillary columns. The effect of μ on the Height Equivalent to the Theoretical Plate (HETP) with various column lengths has been reported (9). HETP increases rapidly and therefore column efficiency decreases rapidly at μ above 40 cm/sec for columns 15-40 m in length; for 7.5 m or shorter columns HETP does not increase significantly with increasing μ. This observation suggests that the 1.8 m column may be operating efficiently at high velocities.

Another important consideration, particularly regarding quantitation, is which flow rate provides the best reproducibility. The basic unit chosen for our experimental measurement was the ratio between area of compound to area of internal standard, designated A_c/A_s. This A_c/A_s is an integral part of the quantitation, as discussed later. The relative standard deviation (RSD) of the A_c/A_s was therefore used to determine reproducibility. RSD was determined for data obtained at flow rates of 50, 200 and 350 ml/min using three standard solutions (Table 1). Inspection of the RSD values suggested that results from the 200 and 350 ml/min flow rates were more precise than results from the 50 ml/min flow rate. We used the 200 ml/min flow rate in subsequent analyses.

Silylation with BSTFA. For purposes of GC analyses, MG and DG have been derivatized with a variety of silylating agents. Among these are a mixture of trimethylchlorosilane, hexamethyldisilizane and pyridine (11), N-methyl-N-trimethylsilyltrifluoroacetamide (12), N-trimethylsilylimidazole (13), and BSTFA (8,10). One outstanding advantage of BSTFA compared to some silylating reagents is that both the reagent and reaction by-products are highly volatile and do not interfere with the analysis. Derivatization prevents rearrangement reactions and curtails chromatogram peak skewing (8,10). D'Alonzo et al. (8) used one ml of BSTFA to silylate a 40-mg mixture containing MG, DG and other fatty materials by heating the mixture to 100 C for 5 min. Our studies showed that 20, 40, 80 and 160 mg of a mixture of MG and DG were quantitatively silylated at 100 C for 5 min with one ml BSTFA as determined by TLC and TLC/FID analysis. This discovery led to more economical use of BSTFA. We used one ml BSTFA with 80 mg of MG and/or DG to be silylated. Presence of fatty esters and TG did not interfere with the silylation of MG and DG or with the subsequent analysis. These silylated mixtures remained stable for at least three mo when kept in a freezer.

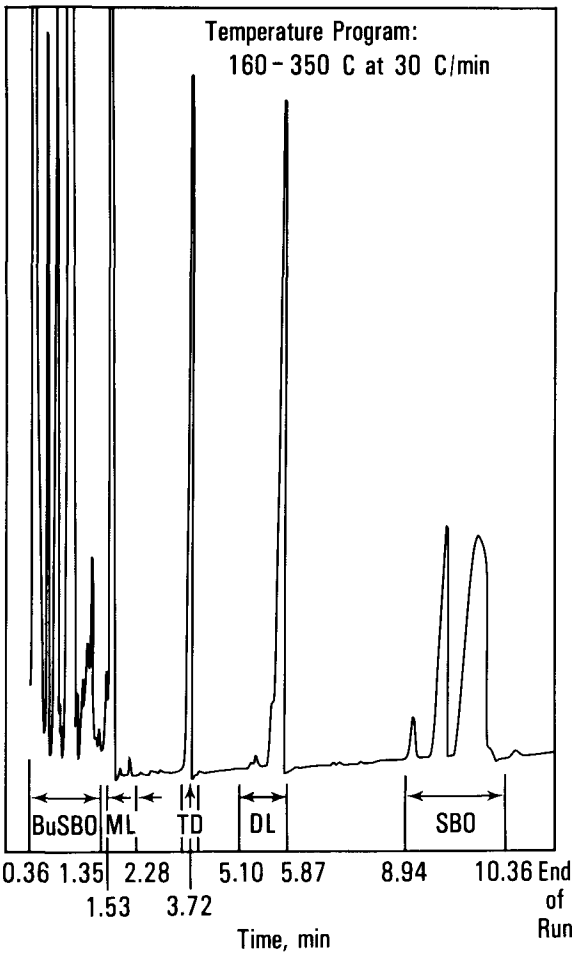

FIG. 3. Chromatogram of a standard containing soybean oil butyl esters, monolinolein, tridecanoin, dilinolein and soybean oil.

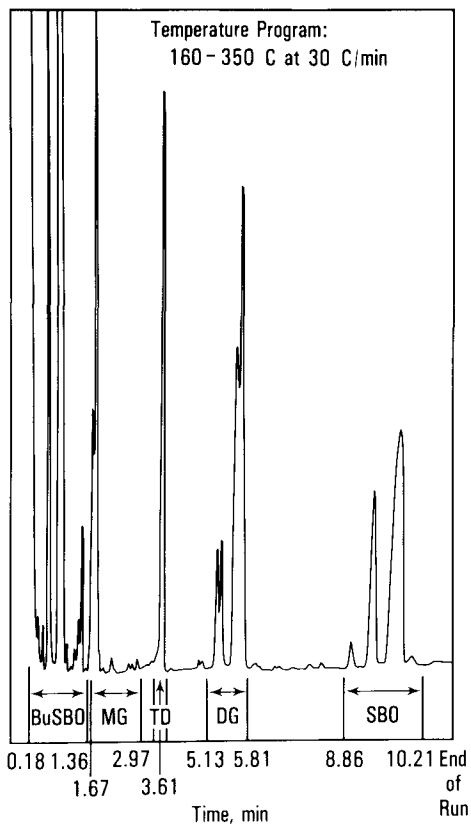

FIG. 4. Chromatogram of reaction products from transesterification of soybean oil with 1- butanol, plus tridecanoin.

Evaluation of the 1.8 m column. The question arose as to whether the relatively short column length could provide adequate separation of ester, glycerides and TD. We needed to separate compound classes but not compounds within a class. A chromatogram of a mixture containing MeL, ML, TD, DL and TL is shown in Figure 1. Not only were all classes of compounds well separated, but analysis time was less than 12 min. TD was a suitable internal standard meeting criteria previously noted (10,14). We thus concluded the 1.8 m column would provide adequate separation and permit a large sample throughput.

Quantitation. Our method for quantitating compounds of interest has been described previously (3). Standard solutions were prepared containing known weights of each component and internal standard. Each solution was analyzed by CGC to determine the A_c/A_s values. From plots of A_c/A_s vs weight of component (W_c)/weight of internal standard (W_s), intercepts and slopes were determined and used to quantify analytical solutions. Data from analyses of standard solutions 1-5 are plotted in Figure 2. The statistical data for standard solutions 1-5 (Table 2) show a high correlation coefficient for each component. These data indicated excellent linearity within the concentration ranges examined. The intercepts and slopes given in Table 2 were then used to measure composition on analyzing standard solutions 1-5 for a second time. A comparison of the measured and actual compositions (Table 3) generally showed good agreement. The measured value in most cases was within 1% of the actual value, with only a few values deviating by 3-4%. The overall SD was 0.4.

Standard solutions 1-5 contained only one ester, methyl linoleate, and one triglyceride, trilinolein. The transesterified SBO we needed to analyze contained a mixture of esters, either BuSBO or MeSBO, and SBO itself. Two additional sets of standard solutions were therefore prepared to more nearly approximate the composition of the transesterified SBO. The first of these, standard solutions 7-11, contained BuSBO, ML, DL and SBO. In the second set, MeSBO was substituted for BuSBO. A chromatogram of a standard solution containing BuSBO, ML, DL and SBO is shown in Figure 3. A chromatogram of a typical transesterified SBO reaction mixture (to which TD was added) is shown in Figure 4. A comparison of these two chromatograms reveals that the standard solution closely approximates the general characteristics seen in the transesterified mixture. Standard solutions containing MeSBO in place of BuSBO gave chromatograms similar to that in Figure 3.

After standard solutions 7-11 were analyzed by CGC, plots were made of A_c/A_s vs W_c/W_s (Fig. 5). The resulting plots indicated good linearity for all components. This was confirmed by the low values for standard deviation and high correlation coefficients (Table 4). The inter-

cepts and slopes obtained by linear regression were then used to calculate the measured composition of standard solutions 7-11. These measured compositions were calculated using the original A_c/A_s values in contrast to A_c/A_s values from independent analyses of solutions 7-11. Agreement between the measured and actual compositions for solutions 7-11 would therefore be expected to be closer than for solutions 1-5. This expectation was realized by the results obtained for solutions 7-11 (Table 5). Differences between the measured and actual values generally were less than 1%. Approximately the same accuracy and precision were observed with standard solutions containing MeSBO in place of BuSBO. Because of the good agreement between measured and actual values for standard solutions 7-11 as well as for standard solutions 1-5, we felt our analytical method would provide reliable and accurate analyses of transesterified mixtures. This CGC method was used with excellent results to support our kinetic studies on the transesterification of SBO (4).

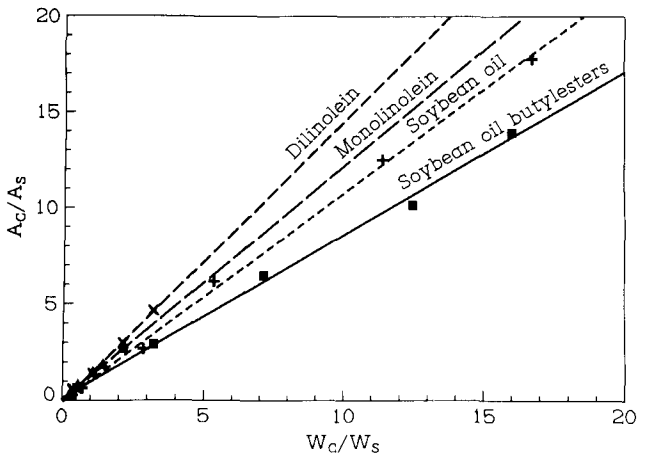

FIG. 5. Plots of A_c/A_s vs W_c/W_s for dilinolein, monolinolein, soybean oil and soybean oil butyl esters.

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TABLE 4

Statistical Data for Solutions 7-11[a]

	Soybean oil butyl esters	Monolinolein	Dilinolein	Soybean oil
Y intercept	0.1354	0.0616	−0.0792	−0.0399
Slope	0.8468	1.2127	1.4576	1.0816
Standard deviation	0.3936	0.0455	0.1270	0.3958
Correlation coefficient	0.9981	0.9990	0.9980	0.9988

[a]Four replicates were used for each compound.

TABLE 5

Composition of Standard Solutions 7-11 (wt % ± Standard Deviation)[a]

Solution number		SBO butyl esters	Monolinolein	Dilinolein	Soybean oil
7	Actual	90	3	3	4
	Measured	90.7 ± 0.2	3.1	2.7 ± 0.1	3.5 ± 0.2
8	Actual	70	8	6	16
	Measured	70.5 ± 0.3	8.5 ± 0.02	6.1 ± 0.2	14.9 ± 0.4
9	Actual	2	2	2	94
	Measured	1.3 ± 0.2	1.8 ± 0.2	2.7 ± 0.1	94.3 ± 0.5
10	Actual	40	12	18	30
	Measured	40.3 ± 0.8	11.4 ± 0.2	17.5 ± 0.4	30.9 ± 0.8
11	Actual	18	6	12	64
	Measured	18.0 ± 0.6	6.4 ± 0.7	11.5 ± 0.2	64.1 ± 0.8

[a]Analyses based on 4 replicates per compound.

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Transesterification Kinetics of Soybean Oil[1]

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Transesterification of soybean oil (SBO) and other triglycerides with alcohols, in the presence of a catalyst, yields fatty esters and glycerol. Di- and monoglycerides are intermediates. Reactions are consecutive and reversible. Rate constants have been determined for each reaction with a computerized kinetic program. The effects of the type of alcohol, 1-butanol or methanol (MeOH); molar ratio of alcohol to SBO; type and amount of catalyst; and reaction temperature on rate constants and kinetic order were examined. Forward reactions appear to be pseudo-first order or second order depending upon conditions used. Reverse reactions appear to be second order. At a molar ratio of MeOH/SBO of 6:1, a shunt reaction was observed. Energy of activation was determined for all forward and reverse reactions under a variety of experimental conditions from plots of log k vs 1/T. Values ranged from 8–20 kcal/mol.

Transesterification of vegetable oils with simple alcohols has long been a preferred method for preparing fatty esters. These esters have good potential as an alternative or emergency fuel to replace #2 diesel oil in farm tractors. Because of this potential, we have focused our attention on variables that affect the yield of these esters (1,2) as well as analysis of intermediates and products as determined by an Iatroscan analyzer (3). In the present paper we report on a study of the kinetics of the transesterification of soybean oil (SBO). A capillary gas chromatographic (CGC) method used to support this study has been described (4). One of our objectives was to determine how variations in type of alcohol, molar ratio of alcohol to SBO, catalyst type and reaction temperature affected kinetic order, reaction rates and energies of activation. Another objective was to gain a more fundamental understanding of the chemistry involved in the transesterification of vegetable oils.

Various mechanisms have been proposed for both acid- and alkaline-catalyzed transesterification (5-7). A number of kinetic studies have been reported for the transesterification of simple (non-fatty) esters with alcohols (6,8-10) and dimethylterephthalate with ethylene glycol (11,12). Only a few kinetic studies have dealt with the transesterification of vegetable oil or fatty esters. These include the conversion of castor oil to methyl ricinoleate (7), the glycerolysis of methyl oleate (13) and the transesterification of the esters of 9(10)-carboxystearic acid (14) and methyl 9(10)-carboxymethylstearate (15). The present study was undertaken with a commercially important vegetable oil, SBO, utilizing a computerized kinetic program (16) to shed additional light on the kinetics of transesterification reactions.

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[1]Presented at the AOCS meeting in Philadelphia, PA, May, 1985.
[2]Deceased.

EXPERIMENTAL

Materials. Refined SBO was obtained from Central Soya, Chattanooga, Tennessee. The molecular weight was assumed to be 872.4. Methanol (MeOH) and 1-butanol (BuOH) were MCB Omnisolv (spectrograde) and were stored over molecular sieves 4A. A solution of sodium butoxide (NaOBu) was prepared by the reaction of 240 mg of sodium with 10 ml of BuOH at 59-99 C with stirring. Sulfuric acid was purchased from B&A, Allied Chemical, Morristown, New Jersey. Sodium methoxide ($NaOCH_3$) was obtained from Aldrich Chemical Company, Milwaukee, Wisconsin.

Reaction conditions employed in kinetic studies. The scope of our kinetic studies is outlined in Table 1. Experiments were designed to determine kinetic order and rate constants using two simple alcohols, two molar ratios of alcohol to SBO, acidic vs alkaline catalysis, two weight percentages of NaOBu and two temperature ranges each containing five temperatures. All reactions studied were conducted at atmospheric pressure.

Transesterification reaction and sampling. The first two of five systems shown in Table 1 utilized a 250-ml round-bottomed four-necked flask, equipped with a mechanical stirrer, thermometer, condenser and/or drying tube and stopper (for sample removal). The remaining systems in Table 1 employed a 400-ml custom-made reaction flask with five necks. The additional neck was equipped with a thermocouple connected to a digital pyrometer. This pyrometer provided rapid temperature readings that were essential to follow the very fast reactions. The larger size flask enabled us to scale up the reaction and possibly permitted more vigorous agitation.

The reaction flask was immersed in a Polyscience Series 9000 refrigerated constant temperature bath and circulator. The operating range of this bath was −15 to +150 C, with a control accuracy of ±0.02 C. The temperature control of the reaction mixture was generally ca. 0.1 C.