Quality Control of Rapeseed Oil Methyl Esters by Determination of Acyl Conversion

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A simple procedure for the evaluation of vegetable oil conversion to methyl esters of fatty acids has been developed. These methyl esters, prepared by the transesterification of vegetable oil with methanol, are used as alternative fuel for diesel engines. A method of gas–liquid chromatography (GLC) on packed columns is used to determine the conversion of acyls bound in acylglycerols to methyl esters. This procedure is based on comparison of the peak areas of methyl esters in fuel samples before and after reaction with an effective transesterification reagent, which will transform unreacted acylglycerols to methyl esters. A correlation between the bound glycerol content, determined by the thin-layer chromatography/flame-ionization detector method, and the acyl conversion, determined by GLC, is given. In acyl conversions to methyl esters over 96.0%, the bound glycerol content is less than 0.25% by weight.

KEY WORDS: Diesel oil substitute, GLC on packed column, quality control, rapeseed oil methyl esters.

Methyl esters (ME) of higher fatty acids from vegetable oils are the starting material for oleochemistry, mainly for fatty alcohol production. An increased interest in ME in the recent decade is due to the fact that they can also be used as alternative fuel for diesel engines (1,2). This diesel fuel substitute is prepared from renewable sources; it solves problems of agricultural overproduction; it is miscible with fossil fuel in any ratio; direct-injection diesel engines can be used without adjustment while the engine gives practically the same power output; exhaust fumes contain smaller amounts of harmful substances; the fuel is biologically readily degradable; and during consumption there is no increase of the total amount of CO_2 in the atmosphere. In Europe, the most important vegetable oil for this purpose is rapeseed oil and, to a lesser extent, sunflower oil. ME from rapeseed oil (MERO) are suitably prepared by the transesterification of oil by methanol in the presence of an alkaline catalyst (3).

The evaluation of MERO properties as a diesel fuel is a major analytical problem. On the basis of engine tests, engine producers categorically required a high conversion of acylglycerols (AG) to ME because bound glycerol contents above 0.25 wt% cause the formation of engine deposits, carbonization of injection nozzles, pistons and valves, and formation of sludge in the lubrication system. The transesterification of triacylglycerols (TAG) by methanol to ME takes place gradually, which results in the presence of diacylglycerols (DAG) and monoacylglycerols (MAG) besides TAG in the reaction mixture. Also, free fatty acids (FFA) may occur, up to an acid number of 0.8 mg KOH/g, which comes from the original oil, or it is formed by the hydrolysis of AG through humidity. In terms of the amount of bound glycerol amount, the least favorable are MAG, which contain most of it.

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The enzymatic method (4) is recommended to determine bound glycerol in MERO. However, the method requires special and unstable reagents and a complicated sample treatment to remove interfering components that unfavorably affect accuracy. This procedure definitely does not meet the requirements of fast and reliable evaluation of fuel quality. In this respect, chromatographic methods are more suitable. In basic studies of vegetable oil transesterification by lower alcohols, Freedman *et al.* (3) used chromatography on a thin layer of SiO_2 evaluated by a flame-ionization detector (FID) (IATROSCAN; Iatron, Tokyo, Japan). The method of thin-layer chromatography (TLC)/FID enables determination of ME, TAG, DAG, MAG, FFA and minority components within the accuracy of ±0.1 wt% (5). The drawback of the method is the high cost of the device. Trathnigg and Mittelbach (6) used a gradient high-performance liquid chromatograph (HPLC) for MERO analysis with parallel determination of AG and ME in the fuel. This method has an accuracy of ±1 wt%, but it is equally inappropriate for current MERO analysis.

Gas–liquid chromatography (GLC) on capillary columns also enables quantitative determination of all groups of components present in MERO (7,8). The possibilities of GLC on packed columns are smaller for MERO analysis. However, packed columns are used to analyze FFA–ME mixtures after transesterification of AG. Depending on the type of the sample analyzed, a suitable polyester, cyanosilicone or polyethyleneglycol (Carbowax 20 M; Supelco S.A., Crans, Switzerland) stationary phase is selected (9). The method's reproducibility is about ±1%. There is an opportunity to exploit this simple GLC method with packed columns to evaluate MERO quality as a diesel fuel, provided that a changed approach is taken to the problem. The principal change consists of comparing the ME content of the original sample with that of the same sample after adjustment in a standard way, without the need to determine the contents of the individual compounds. Peaks of ME are evaluated in a MERO sample before and after reaction with an effective transesterification reactant that will safely transform the present unreacted AG to ME. From the peak areas before and after an additional transesterification, the conversion of acyls bound in AG to ME can be determined.

EXPERIMENTAL PROCEDURES

Materials. The standards triolein (glyceryl trioleate) and methyl oleate were purchased from Sigma (Deisenhofen, Germany) and were chromatographically pure (>99%). The solvents *n*-hexane, anhydrous methanol, benzene, toluene, acetone, ethyl acetate, chloroform, acetic acid, formic acid and diethyl ether were of analytical grade (Chemapol Prague, Czechoslovakia).

The MERO samples were taken from commercial production and were prepared from low-erucic RO, cold-pressed and filtered on a filter press without any further treatment. Anhydrous methanol and NaOH used in the production were of pure grade.

Preparation of the MERO samples. The MERO samples were prepared by a method used in the Slovak Republic

at five production plants, each with 500 t MERO capacity per year, by a two-stage low-temperature oil transesterification with methanolic NaOH at an alkali concentration of 4 wt%. In the first stage, the molar ratio of TAG/methanol is 1:3.50, and in the second stage, it is 1:0.95 (10). The glycerol sediment phase is removed from the ester phase after each stage. Raw MERO then passes through a finishing treatment in the so-called dry process. Its aim is to remove the unreacted methanol, to split the alkaline catalyst with a mineral acid, and finally, to bind FA liberated from alkaline soaps into ash-free salt. The methanol content in MERO is reduced to below 0.3 wt% by means of short-time air-stripping at an increased temperature, which corresponds to the MERO flashpoint above 56°C. By intense mixing of a small amount of phosphoric acid into MERO (less than 0.1 wt%), the alkaline components will change to phosphates. The amount of phosphoric acid is based on the ash content in raw MERO. The phosphoric acid surplus and the FFA from split soaps are then neutralized by ammonia, and the ammonia surplus is stripped by air (11). Eventually, the free glycerol, together with moisture, is adsorbed on the formed salts, and this mixture is trapped in a centrifuge or in a filter.

The MERO samples, prepared and modified by the above-described procedure, are then analyzed both by the TLC/FID method, without any further treatment, and by the GLC method, either without further treatment or after effective transesterification.

MERO sample preparation for GLC analysis. An amount of 0.30–0.35 g of MERO was weighed into a 25-mL volumetric flask and was diluted by *n*-hexane up to the mark. Three mL of the mixture was pipetted into two screw-cup vials fitted with septums. A transesterification reagent (0.5 mL) was added to the first test tube, which was shaken vigorously for 20 min. The transesterification reagent was prepared by dissolving 1.15 g of metallic Na or 2.05 g of K in a mixture of 20 mL benzene and 30 mL methanol with an addition of 7.5 mg phenolphthalein (12). After 20 min, a sufficient amount of methanolic HCl was added to the first test vial through a septum with a 500-μL Hamilton syringe to decolorize the mixture. The methanolic HCl was prepared by trapping HCl in undercooled methanol. Finally, the whole content of the test vial was homogenized after adding 2 mL acetone. NaCl or KCl, formed by neutralization, was left standing for 10 min to settle.

The second test vial served as a reference. To 3 mL of the MERO solution in *n*-hexane was added 0.5 mL of a mixed solvent of benzene–methanol (2:3 in volume), then a volume of methanol equal to the volume of the methanolic HCl used for neutralization in the first vial and finally 2 mL of acetone.

The contents of the ME of C_{16}, C_{18} and C_{20} acids in the original reference samples and in the additionally treated samples were determined by GLC with the same injection volume of both samples. The treated and reference samples were analyzed in turns.

GLC analysis. GLC was carried out on a Hewlett-Packard (Palo Alto, CA) 5830A chromatograph fitted with a dual FID. The glass column (1.8 × 4 mm i.d.) was packed with 10% SE 30 on Chromatone NAW dimethylchlorosilane 0.125–0.16 mm (Lachema, Brno, Czechoslovakia). Nitrogen was used as a carrier gas at 35 mL min^{-1}. Hydrogen and air were supplied to the detector at 43 and 256 mL min^{-1}, respectively. The oven temperature was isothermically held at 250°C, the injector and detector temperatures were 240 and 270°C, respectively. The samples were injected with a 10-μL Hamilton syringe at a 5 μL injection volume.

The GLC conditions were chosen to enable ME of C_{16}, C_{18} and C_{20} acids to elute in one peak. The acyl conversion of AG in MERO samples was then determined from the area ratio of the C_{18} ME, or from the ratio of the area sum of the C_{16}, C_{18} and C_{20} ME in the reference and in the additionally transesterified samples.

IATROSCAN TLC/FID analysis. The TLC/FID method was used as a reference method to provide information on the bound glycerol content in MERO samples. An IATROSCAN MK-5 (Iatron) was used for measurement. The 0.5-g MERO sample was diluted in a 25-mL volumetric flask with a $CHCl_3$ solution of an internal standard (paraffin oil PENCO), which was prepared by dissolving 5 g of the internal standard in 250 mL $CHCl_3$. The sample was spotted on a rod at a volume of 1 μL by a 5-μL micropipette. Analysis conditions were: layers of CHROMAROD S III (Iatron); elution system 1—benzene 96 mL, ethylacetate 3.8 mL, acetic acid 0.6 mL, water 0.2 mL; elution up to a height of 3.5 cm from the start. An elution system 2 of *n*-hexane 13 mL, diethyl ether 7 mL and formic acid 0.1 mL was used for elution for 20 min. The hydrogen flow rate was 160 mL/min, the air flow rate 2000 mL/min, and the scanning rate per rod was 30 s. The standard deviation in determining the AG content by the TLC/FID method was 0.1% which means that a standard deviation in the bound glycerol content for TAG was 0.010%, for DAG 0.015%, and for MAG 0.026% (5).

RESULTS AND DISCUSSION

The presented method of fuel quality control is based on the comparison of the ME content in a sample with the ME content in the same sample after an effective additional transesterification. Thus, selection of a proper transesterification system as well as selection of the reaction conditions for analytical requirements are important factors to avoid distorting the results. In the literature, a good number of such systems is mentioned. Bannon *et al.* (13) present a survey of such methods.

Because MERO is prepared from ROs with acidities below 2 mg KOH/g and because under the production conditions these FFA are not transformed to ME, it is necessary to carry out an additional transesterification for analytical purposes in such a way that during complete conversion of AG to ME, FFA remain in the nonesterified form. That is why, during the method development, we used a reaction system and a procedure recommended by Christoperson and Glass (12), who used as transesterification reagent Na methanolate in a mixture of benzene-methanol (2:3 in volume) and dissolved the sample in *n*-hexane. The reaction mixture consists of two layers: the upper layer contains ME while the lower one contains glycerol.

However, the existence of the two liquid phases is not favorable for a quantitative analysis because there exists a partial mutual solubility of the components in the phases according to their distribution coefficients. For this reason, we have modified the method to form a homo-

geneous phase by means of a proper solvent. We found that such a solvent is acetone. We also discovered that there is another efficient way of gaining a homogeneous system—substituting *n*-hexane with a mixed solvent, benzene–methanol (2:3 in volume), in the preparation of the sample solution. In this way, a one-phase system is attained, even in the course of the reaction. Due to the toxic nature of benzene, it was substituted in mixed solvent by toluene. The one-phase system remains preserved, and the results obtained with toluene are the same as with benzene.

Table 1 shows data acquired by GLC analysis of two standard mixtures and two samples from the first stage of an alternative diesel fuel production. The acyl conversion in the standards is in good agreement with the theoretical value. The standard deviation of measurements, estimated from five replicates, is comparable with currently given values. The difference in results for Na methanolate and K methanolate is practically indiscernible. The same conversion values are also ndependent of what is compared: the area of the common peak of ME of C_{18} acids in the reference sample and in the additionally transesterified sample, or sums of the peak areas of the ME of C_{16}, C_{18} and C_{20} acids in both samples. The use of a different solvent *n*-hexane, or a mixture of benzene-methanol does not influence the determination of the acyl conversion of AG to ME. For the mixed solvent, when additional acetone is not used as homogenizing agent, the system is homogeneous from the beginning of the reaction and requires less mixing. In the method development, the samples with lower conversion were specially selected for the effect of an additional transesterification to be substantially distinctive.

Table 2 presents the time needed in an additional sample transesterification for the reaction to completely take place. As the experimental results show, the reaction is practically over in 15–20 min. The system is slightly more reactive in a homogeneous reaction medium (solvent of benzene–methanol) in which the final conversion is reached in about 10 min. The decreasing conversion with time in Table 2 is formally linked with the way of its evaluation. If the conversion is defined as a ratio of the peak area of ME in an untreated reference sample and the peak area of ME in an additional transesterified sample, then the numerator value is constant and the denominator value is gradually increasing with time to reach its ultimate value. The proper acyl conversion of AG to ME in the sense of chemical kinetics is, of course, increasing in the course of the reaction.

Table 3 contains the summarized data of the composition of a series of samples as determined by the TLC/FID method with the IATROSCAN. The ME, TAG, DAG, MAG and FFA contents have been determined directly, the bound glycerol content has been obtained through calculation from all AG values. At the same time, Table 3 also shows the acyl conversion of AG to ME as determined by the method developed by us. From the statistical evaluation of bound glycerol dependence on acyl conversion by nonlinear regression, it follows that, at an acyl conversion over 96.0%, the bound glycerol content is less than 0.25 wt%. The MERO samples with a con-

TABLE 1

The Effect of Solvent and Transesterification Reagent on the Course of an Additional Sample Transesterification[a]

Sample	Acyl conversion (%)				Theory (%)
	With CH_3ONa		With CH_3OK		
	Peak C_{18}[b]	Peaks sum[c]	Peak C_{18}[b]	Peaks sum[c]	
Standard I in *n*-hexane[d]	81.1 ± 1.1	81.2 ± 1.1	81.2 ± 1.0	81.1 ± 1.0	81.16
Sample 1 in *n*-hexane[d]	82.2 ± 1.3	82.2 ± 1.2	82.3 ± 1.2	82.2 ± 1.2	—
Sample 2 in *n*-hexane[d]	77.6 ± 1.2	77.7 ± 1.2	77.6 ± 1.2	77.6 ± 1.2	—
Standard II benzene–methanol	90.4 ± 1.2	90.5 ± 1.1	90.5 ± 1.1	90.4 ± 1.1	90.45
Sample 1 in benzene–methanol	82.2 ± 1.2	82.3 ± 1.2	82.2 ± 1.2	82.2 ± 1.2	—
Sample 2 in benzene–methanol	77.7 ± 1.2	77.6 ± 1.1	77.6 ± 1.1	77.7 ± 1.1	—

[a]Conditions: ambient temperature; reaction time 20 min; stock solution of 0.32–0.35 g of sample in 25 mL *n*-hexane or a mixed solvent of benzene–methanol (2:3 in volume); transesterification reagent CH_3ONa or CH_3OK in a mixture of benzene and methanol (2:3); five replicates. Standard I: 0.2650 g methyl oleate and 0.0612 g triolein. Standard II: 0.3082 g methyl oleate and 0.0324 g triolein.
[b]Evaluated from the peak areas ratio of methyl esters of C_{18} acids in the reference and the additionally transesterified sample.
[c]Evaluated from the ratio of the sum of the peak areas of methyl esters of C_{16}, C_{18} and C_{20} acids in the reference and additionally transesterified sample.
[d]A two-phase system homogenized by acetone.

TABLE 2

The Influence of Reaction Time on the Course of an Additional Transesterification[a]

Sample	Reaction time (min)	Acyl conversion (%)	
		Peak C_{18}[b]	Peaks sum[c]
Sample 2 in *n*-hexane[d]	2	80.6 ± 1.2	80.7 ± 1.2
	4	79.4 ± 1.2	79.4 ± 1.2
	10	78.3 ± 1.1	78.3 ± 1.2
	15	77.6 ± 1.2	77.6 ± 1.2
	20	77.6 ± 1.2	77.7 ± 1.2
Sample 2 in benzene–methanol	1	79.7 ± 1.2	79.8 ± 1.2
	2	78.4 ± 1.1	78.4 ± 1.1
	5	77.7 ± 1.2	77.7 ± 1.2
	10	77.6 ± 1.1	77.6 ± 1.1
	20	77.6 ± 1.1	77.6 ± 1.1

[a]The transesterification reagent was CH_3OK in benzene–methanol (2:3 in volume), other conditions the same as in Table 1.
[b,c,d]See Table 1.

TABLE 3

The MERO Sample Compositions According to TLC/FID and Their Acyl Conversions According to GLC[a]

Sample	Composition (TLC/FID, wt %)						Acyl conversion GLC (%)
	ME	TAG	DAG	MAG	FFA	Bound glycerol	
3	78.9	17.6	2.2	1.1	0.2	2.45	82.0
4	81.6	14.7	2.0	0.6	1.1	1.98	84.0
5	86.5	9.9	2.0	0.6	1.0	1.48	87.5
6	96.5	2.1	0.5	0.8	0.1	0.50	93.4
7	98.2	0.6	0.3	0.7	0.2	0.29	94.2
8	97.8	1.1	0.3	0.4	0.4	0.26	94.9
9	98.3	0.9	0.3	0.3	0.2	0.22	95.9
10	98.8	0.6	0.2	0.3	0.1	0.17	96.0
11	98.8	0.7	0.1	0.3	0.1	0.17	96.6
12	97.9	0.4	0.2	0.6	0.9	0.23	97.5
13	98.4	0.5	0.2	0.6	0.3	0.24	98.3
14	98.0	0.8	0.5	0.3	0.4	0.23	99.0
15	98.8	0.4	0.3	0.4	0.1	0.19	99.2
16	99.1	0.1	0.2	0.4	0.2	0.14	99.7
17	98.7	0.0	0.5	0.6	0.2	0.22	99.8

[a]TLC/FID, thin-layer chromatography/flame-ionization detector; GLC, gas–liquid chromatography; ME, methyl esters; TAG, triacylglycerols; DAG, diacylglycerols; MAG, monoacylglycerols; FFA, free fatty acids.

version above 96.0% meet the quality parameter required for diesel fuel concerning the bound glycerol content.

From Table 3 it is evident that any connection between TAG, DAG and MAG contents is lacking in the individual samples.

The contribution of the developed method for evaluating the quality of ME from vegetable oils, used as diesel fuel substitute, is associated mainly with the fact that it is possible to use this method in commercial-scale chromatographs with available and cheap columns. The method is sufficiently accurate, it does not require special chemicals or standards, and the sample treatment before analysis is not complicated. The packed-column parameters remain intact for a long time and do not change even after five-hundred analyses.

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