Ethanolysis of Rapeseed Oil: Quantitation of Ethyl Esters, Mono-, Di-, and Triglycerides and Glycerol by High-Performance Size-Exclusion Chromatography

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ABSTRACT: High-performance size-exclusion chromatography (HPSEC) was used to evaluate the influence of different variables affecting the transesterification of rapeseed oil (RSO) with anhydrous ethanol and sodium ethoxide as catalyst. The effect of temperature, ethanol/RSO molar ratio, catalyst concentration, and time can be interpreted by observing the variations of the reaction medium composition. HPSEC has made the quantitation of ethyl esters, mono-, di-, and triglycerides and glycerol possible. The best results for laboratory-scale reactions were obtained at 80°C with a 6:1 molar ratio of EtOH/RSO and 1% of NaOEt by weight of RSO.

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KEY WORDS: Alcoholysis, ethanolysis, gel-permeation chromatography, high-performance size-exclusion chromatography, transesterification.

Considering the progressive decrease of the world's petroleum resources and the concerns expressed by farmers and ecologists, we are seeing a renewed interest in research and development of alternative fuels. Vegetable oils, and particularly their methyl esters, have been shown to be good alternative diesel fuels that do not require engine modifications. Chemical transformation of vegetable oil to fatty acid alkyl esters, called transesterification or alcoholysis, provides many advantages, such as viscosity reduction (roughly eight times less) and minimization of carbon deposits on injector nozzles (1–3).

The methanolysis reaction is well known because it has been used industrially for the past 50 years to simplify the manufacture of soaps and detergents (4). On the other hand, the ethanolysis reaction has rarely been studied (5,6), especially in comparison to the intensive studies undertaken by numerous researchers on the methanolysis reaction (7–12). Producing ethyl esters rather than methyl esters is of considerable interest because it allows production of an entirely agricultural fuel, and the extra carbon atom brought by the ethanol molecule slightly increases the heat content and the cetane number (13).

The aim of this study was to evaluate the different variables affecting the alkaline ethanolysis of rapeseed oil (RSO), i.e., time, temperature, ethanol/RSO molar ratio, and catalyst concentration. Sodium ethoxide was used as catalyst because of its high efficiency and its low tendency for soap formation.

To optimize the lab-scale reaction, it was necessary to quantitate the influence of each parameter affecting the reaction. These effects can be interpreted by observing the variations of the reaction medium composition—the ethyl esters formed, the freed glycerol, the unreacted triglycerides, and the intermediate mono- and diglycerides.

First we tried quantitation by thin-layer chromatography and densitometric evaluation, with unsuccessful results. This method has many disadvantages according to Freedman *et al.* (14) and Naudet *et al.* (15).

Gas chromatography is intensively used in the analysis of lipids, but it is always necessary to silylate the samples before injection (16–19); this is time-consuming and may also cause errors. For samples that contain different classes of lipids, the multitude of peaks on the chromatogram makes their attribution and identification difficult; there can be superposition of different peak groups, which makes quantitation even harder and less accurate (16).

Gel-permeation chromatography, also called high-performance size-exclusion chromatography (HPSEC), appeared to be an appropriate technique, considering its simplicity and its high reliability. HPSEC is based on the selective retention of molecules according to their size when they enter the pores of the polymer matrix (20). In the case of a transesterification medium, the average difference in molecular weight between glyceride classes is about 250, and their steric hindrance is also very different. These differences make HPSEC interesting because there is only one average peak for each lipid class. This technique is relatively new for lipid analysis because there has been considerable progress in column technology (21,22). We developed a simple and reproducible method that allowed us to perform accurate kinetics measurements and to know the exact lipid composition of the reaction

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medium at any time. The samples for injection need no preparation other than dilution in tetrahydrofuran, unlike for other analytical techniques.

EXPERIMENTAL PROCEDURES

Materials. The RSO was of edible grade. The ethanol was of rectapur grade (Prolabo, Gradignan, France) with an alcohol titre of 99.5% (by vol). Sodium ethoxide, as an anhydrous light-yellow powder (synthesis grade), was 95% pure and contained less than 1% free hydroxides (Merck Schuchardt, Nogent-sur-Marne, France).

Apparatus. We used a 500-mL three-necked reactor with a small drain cock on the bottom to recover the glycerine layer. This reactor was equipped with a mechanical stirrer, a water condenser, and a dropping funnel. The reactions were conducted in a Rhodorsil silicone oil bath (Rhône-Poulenc, St. Fons, France), thermostatted with a Julabo V.C. temperature regulator (Technalab, Toulouse, France) over an operating range of 20–200°C, with control accuracy of ±0.1°C. For temperatures lower than 20°C, we used an ethanol bath thermostatted with a Huber HS40 cryostat (Bioblock Scientific, Illkirch, France) over an operating range of –40–20°C.

Ethanolysis reaction procedures and sampling. After immersion of the reactor in the bath, the RSO was added and preheated to the desired temperature, and the agitator was started. The catalyst was prepared by dissolving the proper amount of the sodium ethoxide powder in the desired amount of ethanol for the reaction. This ethanolic solution was added to the RSO by means of the dropping funnel, thereby avoiding any evaporation of the alcohol. The timing was started at that moment.

At a desired time, a sample was prepared by diluting four drops of the reaction medium in *ca.* 5 mL of high-performance liquid chromatographic (HPLC)-grade tetrahydrofuran (Janssen Chimica, Noisy Le Grand, France). This large dilution readily stops the reaction course; no changes have been observed in the composition of a sample after 24 h. The diluted sample is then ready for injection.

Analytical procedures. The chromatographic system consisted of an isocratic pump P 1000 (Spectra Physics, San Jose, CA), a refractometer detector Shodex RI-71 (Showa Denko, Japan), and an oven for columns thermostatted at 40°C by a Croco.Cil temperature regulator. The samples were injected with a six-port Rheodyne 7125 syringe-loading injector valve with a 20-µL sample loop. The chromatograms were recorded, and the peaks were integrated by an SP 4270 integrator (Spectra Physics). Two HPSEC Phenogel analytical columns were used (Phenomenex, Torrance, CA), 300 × 7.8 mm, packed with spherical styrene divinylbenzene copolymer beads with an average particle size of 10 µm. We first placed a column with a pore size of 100 Å, corresponding to a molecular weight (MW) range of 50–1000. This was connected in series to a column with a pore size of 500 Å, corresponding to an MW resolving range of 500–10,000. The mobile phase was HPLC-grade tetrahydrofuran (Janssen Chimica), and the flow rate was 0.5 mL/min. The typical pressure at this flow rate was *ca.* 120 PSI.

The main difficulty was in obtaining pure standards to calculate the correction factors of ethyl esters, mono-, and diglycerides. It would not have been precise enough to reconstitute each lipid species mixture with individual standards, knowing the RSO fatty acid composition, so we tried to separate and purify these three components from the reaction medium. The ethyl esters were obtained by distilling the reaction medium under high vacuum (150–160°C, under 1 mbar). We controlled the ester fraction by gas chromatography. However, the mono- and diglycerides decomposed before evaporating, so we tried to separate them on a silica-gel column (Chromagel 60 ACC, particle size 20–200 µm; SDS, Valdonne, Peypin, France). The mobile phase was a mixture 1:1 in volume of normapur-grade *n*-hexane (Prolabo) and diethyl ether stabilized with 5 ppm of butylated hydroxytoluene (Janssen Chimica). This technique was not successful for the separation of monoglycerides, so an individual standard of 1-monooleyl-*rac*-glycerol (C18:1, [*cis*-9]), representing about 60% of all the monoglycerides, was used for calibration.

A synthetic mixture containing known amounts of ethyl esters (ee), of the monoglyceride standard (mono), of diglycerides (di), of RSO (tri), and of glycerol (gly) was prepared and analyzed by our HPSEC method. The correction factors were calculated by using glycerol as an internal standard by the following equation:

$$K_i = (x_i \times A_{gly})/(A_i \times x_{gly}); K_{gly} = 1 \quad [1]$$

where K_i, x_i, and A_i stand for the correction factor, the concentration, and the peak area of the component, respectively.

A typical chromatogram is presented in Figure 1, and the different correction factors and the retention times of each component is given in Table 1.

The relative percentage of each component is given by the following equation:

$$x_i = (K_i \times A_i) \times 100/(A_{gly} + K_{ee}A_{ee} + K_{di}A_{di} + K_{mono}A_{mono} + K_{tri}A_{tri}) \quad [2]$$

The rate of conversion of RSO to ethyl esters has been defined as:

$$R = (A_{ee}K_{ee}) \times 100/(A_{ee}K_{ee} + A_{tri}K_{tri} + A_{di}K_{di} + A_{mono}K_{mono}) \quad [3]$$

RESULTS AND DISCUSSION

Stoichiometry of rapeseed oil ethanolysis. The transesterification reaction consists of three consecutive and equilibrated reactions involving intermediate formation of mono- and diglycerides. The stoichiometry requires 3 mol of alcohol for 1 mol of triglyceride. An overall reaction is given in Scheme 1. Knowing the fatty acid (faac) composition of the RSO (Table 2), we calculated its average MW from Equation 4 and also the MW of the ethyl esters. From Equations 5 and 6, we deduced the MW of the mono- and diglycerides (Table 1).

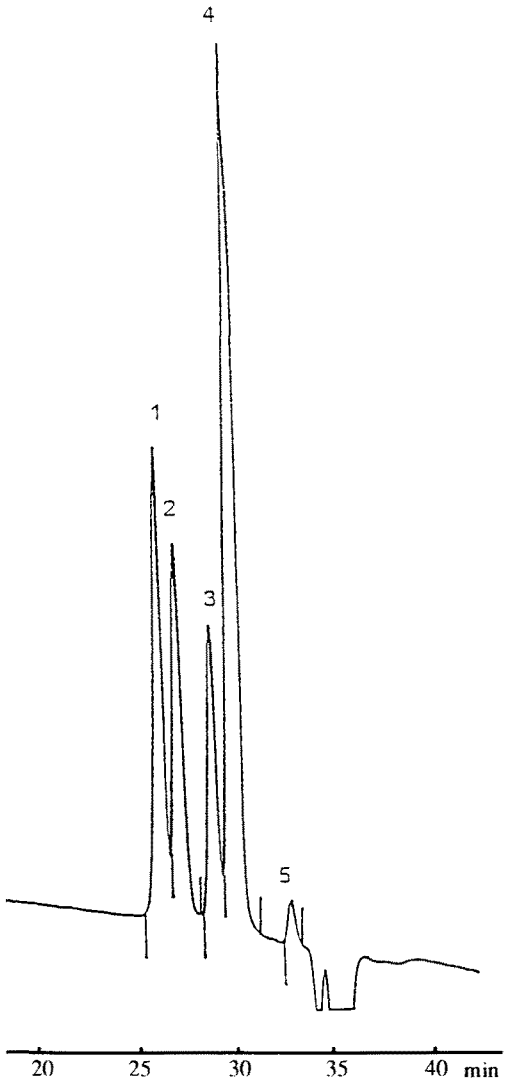


FIG. 1. Typical chromatogram of an ethanolysis reaction medium using the high-performance size-exclusion chromatography method: 1, triglycerides; 2, diglycerides; 3, monoglycerides; 4, ethyl esters; and 5, glycerol.

$$MW_{tri} = 3 \times MW_{faac} + MW_{gly} - 3 \times MW_{H_2O} \quad [4]$$

$$MW_{di} = MW_{tri} + MW_{EtOH} - MW_{ee} \quad [5]$$

$$MW_{mono} = MW_{gly} + MW_{ee} - MW_{EtOH} \quad [6]$$

TABLE 1
Molecular Weights, Correction Factors, and Retention Times for Each Lipid Class

Lipid class[a]	TG	DG	MG	EE	GL
Molecular weight	881	618	355	309	92
Correction factor	0.90	0.89	0.96	1.17	1.00
Retention time (min)	26.2	27.3	29.2	30.5	33.5

[a]TG, triglycerides; DG, diglycerides; MG, monoglycerides; EE, ethyl esters; GL, glycerol.

TABLE 2
Fatty Acid Composition of Rapeseed Oil

Fatty acid (wt%)									
16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	22:0	22:1
5.56	0.12	1.38	58.25	22.17	8.90	0.22	1.88	0.16	1.20

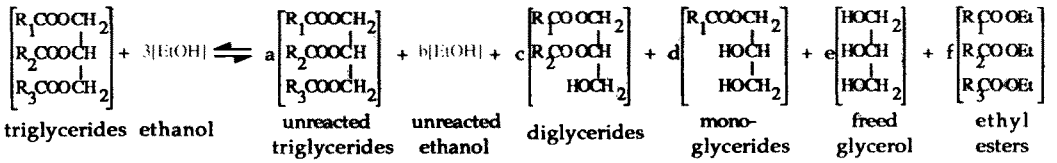
Stoichiometrically, 15.7 g of ethanol is required for 100 g of RSO, thus producing 105 g of ethyl esters and 10.4 g of glycerol. All our observations were compared to a reference reaction for which the conditions were: 100 g RSO, 30 g anhydrous ethanol, 1 g NaOEt, T = 80°C. We only changed one variable at a time to directly evaluate its influence on the course of the reaction.

Effect of time on the transesterification reaction. We examined the concentration changes of the ethanolysis reaction medium at a molar ratio of 6:1, catalyzed by 1% NaOEt by weight of RSO, at 80°C (Fig. 2). It appears that the reaction is very rapid because the ethyl ester conversion is near 90% after only 5 min, and stabilizes at around 94% after 30 min. The glycerol recovery is not directly representative of the ethyl ester conversion because there is no proportional relation between released glycerol and formed esters.

It seems that the concentrations of the di- and monoglycerides stabilize around 2 and 4%, respectively, as if there were an equilibrium. This equilibrium can be slightly displaced if the glycerine layer is withdrawn from the reaction medium after about 10 min of stirring and new alcoholic sodium ethoxide is added. The Henkel industrial process for methyl ester production is based on a two-stage reaction with separation of the glycerine after each stage (23).

Effect of temperature on ethyl ester conversion. The rate of ethyl ester conversion was studied for six temperatures ranging from 2.5 to 100°C (Fig. 3). An ethanol-to-RSO molar ratio of 6:1 and 1% of NaOEt by weight of RSO was used. It appears that temperature is not a sensitive parameter for the reaction. It seems to have its maximum effect during the first 30 min of stirring, otherwise there is only an increase of the conversion rate of about 10% between a reaction conducted at the ethanol's boiling point and a reaction at room temperature after one hour of agitation. Heating to temperatures above 80°C seems to have negative effects on the conversion.

A reaction conducted at a temperature as low as 2.5°C is still possible, but it is time-consuming. As the temperature increases, the solubility of ethanol in RSO increases and so does the speed of the reaction. As a matter of fact, at low temperatures, ethanol is not soluble in the oil; when the stirring is started, there is formation of an emulsion. The reaction takes place at the interface of the droplets of alcohol in the oil, and then, as soon as the first ethyl esters are formed, the alcohol solubilizes progressively because the esters are mutual solvents for the alcohol and the oil. The emulsion stage lasts a few seconds at 20°C and about 90 s at 2.5°C. We also noted that the separation of the glycerine was slower for decreasing



SCHEME 1

temperatures. For increasing temperatures, we noted a progressive coloration of the reaction medium to dark orange at 100°C. This may be caused by oxidation of the oil.

Influence of catalyst concentration on the ethyl ester yield. In most industrial chemical processes, the catalyst is expensive compared to the reagents. The catalyst also generates additional costs because it is necessary to remove it from the reaction medium at the end. We examined six catalyst concentrations, ranging from 0.15 to 1.5% by weight of RSO, at 80°C (Fig. 4). The ethanol/RSO molar ratio was 6:1. A catalyst concentration higher than 1.0% does not significantly increase the rate of conversion of ethyl esters, except for the speed of the reaction during the first 30 min. The minimum concentration of catalyst required is hard to evaluate because a compromise must be made between duration of the reaction and catalyst concentration. For example, 5, 10, and 48 min are needed to reach 80% conversion with 0.5, 0.35, and 0.25% catalyst, respectively. Sodium ethoxide is a light-yellow powder, and the more we used, the more the glycerine layer took on a darker color. Also, prolonged contact of this powder with air diminishes its efficiency because of interaction with moisture and carbon dioxide. As a result, the powder darkens and does not dissolve as well in the alcohol, and the ethanolic solution becomes turbid.

Effect of ethanol/RSO molar ratio on the ethyl ester conversion. The molar ratio of alcohol to vegetable oil has been stated by many investigators to be the most important variable affecting the transesterification reaction. Because this reaction is an equilibrium, according to Le Chatelier's principle, an excess of alcohol increases the ester conversion by shifting this equilibrium to the right. We examined five different molar ratios, ranging from the stoichiometric amount (3:1) to a 12:1 ratio (Fig. 5). The 6:1 ratio appears to give the best results, in agreement with the conclusion of many researchers. For ratios higher than 6:1, the excess of alcohol seems to favor conversion of di- to monoglycerides. There is, however, also a slight recombination of esters and glycerol to monoglycerides because their concentration kept increasing during the course of the reaction, in contrast to reactions conducted with molar ratios of 6:1 and lower. Krisnangkura and Simamaharnnop (24) also observed that when glycerol remained in solution it helped drive the equilibrium back to the left, lowering the yield of esters.

With a stoichiometric amount of alcohol, the conversion to esters is near 82% after 1 h of agitation. Under these conditions, the separation of the glycerine layer is fast, compared to reactions conducted with large excesses of ethanol, where separation takes time, is not complete, and increases costs for the alcohol recovery. Junek and Mittelbach (25) have developed a transesterification process with stoichiometric conditions and a catalyst concentration of 1.3 to 1.7 by weight of vegetable oil. According to these authors, most of the catalyst is drawn into the glycerine layer, in contrast with an excess of alcohol where the catalyst is evenly distributed in the ester layer and glycerine layer.

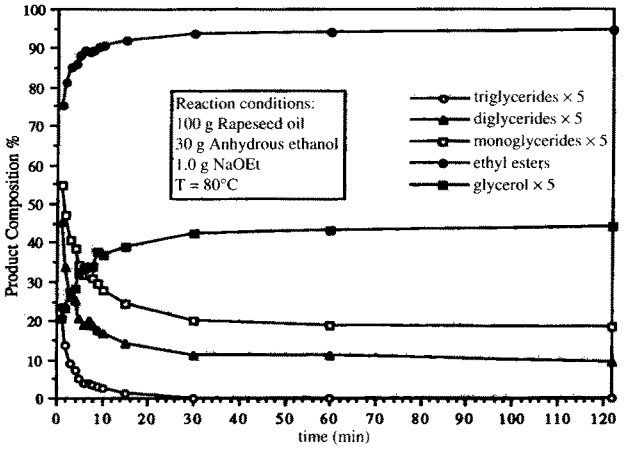


FIG. 2. Effect of time on the transesterification reaction using sodium ethoxide as catalyst. The tri-, di-, and monoglyceride and glycerol percentages have been multiplied by five.

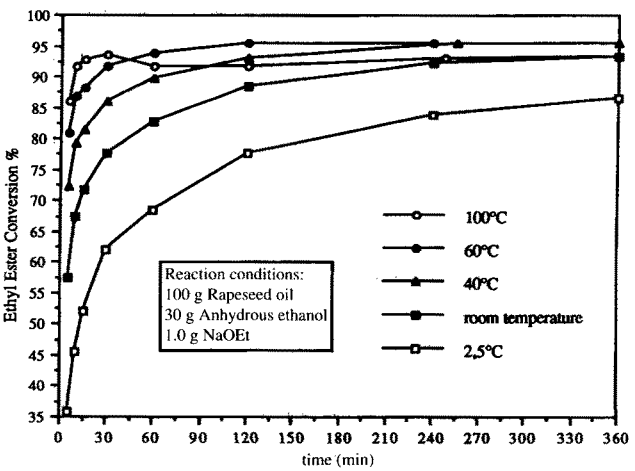


FIG. 3. Effect of temperature on the ethyl ester conversion.

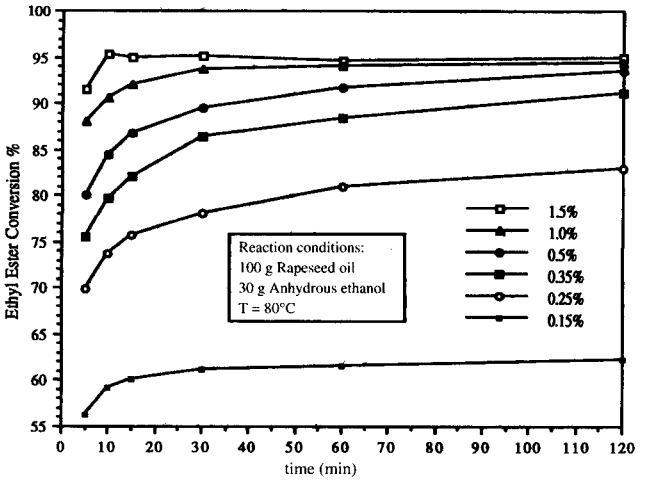


FIG. 4. Effect of catalyst concentration on ester conversion (wt% of NaOEt to rapeseed oil).

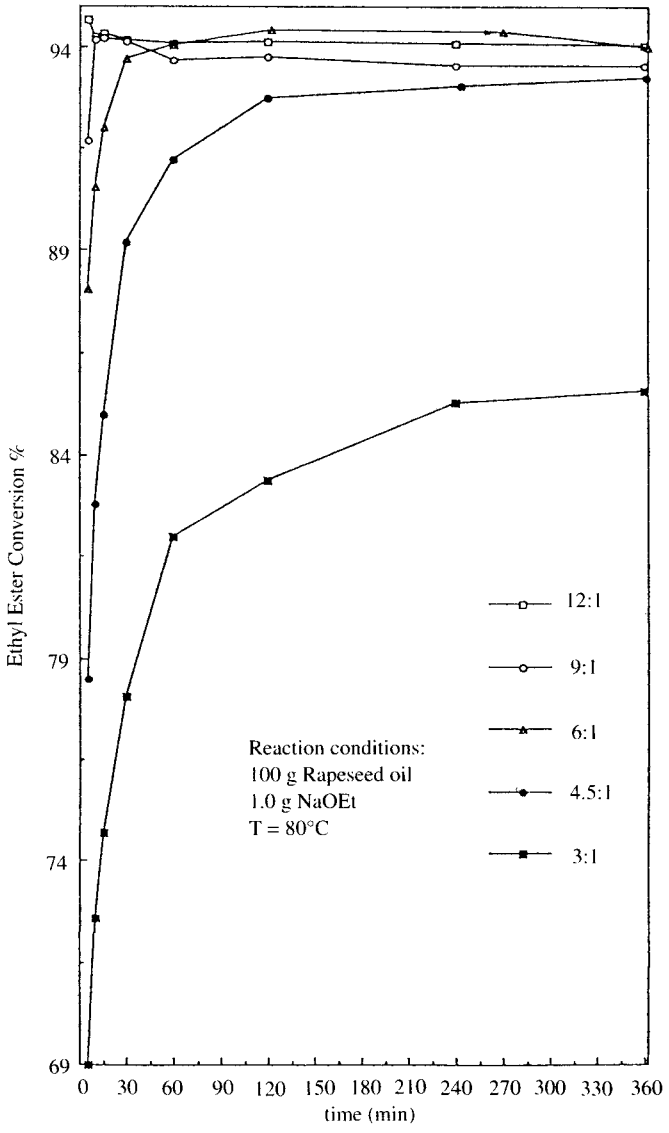


FIG. 5. Effect of ethanol/rapeseed oil molar ratio on the ethyl ester conversion.

Optimal conditions for ethanolysis. This study of the different variables affecting the yield of the ethanolysis of RSO has been conducted under laboratory-scale conditions. It is therefore hard to determine the choice of optimum process conditions. If an ethanol/RSO molar ratio of 6:1, 1% of NaOEt by weight of RSO, 80°C and 15 min of vigorous stirring gives best results, considering the observed high yield and short reaction time, this need not be the same for larger-scale operations. Indeed, economic compromises must be adapted among the four parameters examined.

This study was undertaken as a preliminary investigation of the ethanolysis reaction under anhydrous conditions. We have developed a new series of experiments with hydrated ethanol (95%). Water has a dramatic effect on the yield of the reaction because ethanolysis conducted under the same conditions given in the previous paragraph but with hydrated ethanol does not reach more than 30% conversion, in contrast with the usual 94–95%. The results of our study are now being interpreted, and optimum conditions are defined to minimize the saponification reaction.

REFERENCES

1. Kaufman, K.R., and M. Ziejewski, *Trans ASAE 27*:1626 (1984).
2. Pischinger, G.H., and A.M. Falcon, *Proceedings of the International Conference on Plant and Vegetable Oils as Fuels,* August 2–4, 1982, Fargo, pp. 198–208.
3. Geyer, S.M., M.J. Jacobus and S.S. Lestz, *Energy from Agriculture, Vegetable Oil as Diesel Fuel: Seminar III,* ARM-NC-28, October 19–20, 1983, pp. 157–162.
4. Bradshaw, G.B., and W.C. Meuly, U.S. Patent 2,360,844 (1941).
5. Feuge, R.O., and A.T. Gros, *J. Am. Oil Chem. Soc. 26*:97 (1949).
6. Graille, J., P. Lozano, D. Pioch, P. Geneste, A. Finiels and C. Moreau, *Rev. Fr. Corps Gras 32*:311 (1985).
7. Freedman, B., E.H. Pryde and T.L. Mounts, *J. Am. Oil Chem. Soc. 61*:1638 (1984).
8. Freedman, B., R.O. Butterfield and E.H. Pryde, *Ibid. 63*:1375 (1986).
9. Filip, V., J. Zajic and J. Smidrkal, *Rev. Fr. Corps. Gras 39*:91 (1992).
10. Reis, J.C., *Oléagineux 14*:249 (1959).
11. Gonçalves, D., *Arq. Biol. Technol. 27*:353 (1984).
12. Akoh, C.C., and B.G. Swanson, *J. Food Process. Preserv. 12*:139 (1988).
13. Clark, S.J., L. Wagner, M.D. Schrock and P.G. Piennaar, *J. Am. Oil Chem. Soc. 61*:1632 (1984).
14. Freedman, B., E.H. Pryde and W.F. Kwolek, *Ibid. 61*:1215 (1984).
15. Naudet, M., J. Pasero and S. Biasini, *Rev. Fr. Corps Gras 12*:525 (1965).
16. Mariani, C., P. Bondioli, S. Venturini and E. Fedeli, *Riv. Ital. Sostanze Grasse 68*:549 (1991).
17. Plank, C., and E. Lorbeer, *J. High Resolut. Chromatogr. 15*:609 (1992).
18. Prevot, A., and J.L. Coustille, *Rev. Fr. Corps Gras. 29*:17 (1982).
19. Goh, E.M., and R.E. Timms, *J. Am. Oil Chem. Soc. 62*:730 (1985).
20. Rosset, R., in *Techniques de l'Ingénieur—Analyse et Caractéri-*

sation, Vol. 2, Number P 1 465, edited by Techniques de l'Ingénieur, Paris, 1990, pp. 1–11.
21. Hopia, A.I., V.I. Piironen, P.E. Koivistoinen and L.E.T. Hyvönen, *J. Am. Oil Chem. Soc. 69*:772 (1992).
22. Christopoulou, C.N., and E.G. Perkins, *Ibid. 63*:679 (1986).
23. Assmann, G., G. Blasey, B. Gutsche, L. Jeromin, J. Rigal, R. Armengaud and B. Cormary, World Patent WO 91 05,034 (1991).
24. Krisnangkura, K., and R. Simamaharnnop, *J. Am. Oil Chem. Soc. 69*:166 (1992).
25. Junek, H., and M. Mittelbach, French Patent FR 2,603,296 (1987).

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