Synthesis and Properties of Erucic Acid Triacylglycerols

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The chemical synthesis of high-erucic acid triacylglycerols by a direct esterification approach was investigated in this study. A two-stage process was adopted in which reactants (without any catalyst) were heated at 160 ± 5°C for 4 h, followed by heating at 250 ± 10°C for 8 h. Purification of the esterified product was achieved by alkali refining, followed by alumina column chromatography. A greater than 85% yield of pure triacylglycerols was obtained, which contained ≈90% erucic acid, by using a 5% molar excess of erucic acid in the reaction. Oils containing erucic acid ranging from 45 to 91% were prepared by chemically interesterifying native high-erucic acid rapeseed (HEAR) oil and the synthetic high-erucic acid triacylglycerols. The melting point, cloud point, pour point, titer and viscosity of all oils exhibited positive correlations with the erucic acid content, whereas saponification value, iodine value and refractive index showed negative correlations. Randomization of native HEAR oil resulted in an increase in the melting point, cloud point and pour point.

KEY WORDS: Erucic acid, esterification, HEAR oil, interesterification, triacylglycerols, trierucin.

Vegetable oils rich in erucic acid have considerable industrial potential. These oils are used in steel casting, textile spinning and as engine oil supplements for their excellent lubricating properties (1,2). High-erucic acid oils are derived mainly from rapeseed containing from 40–60% erucic acid (3). To meet the rising demand, scientists are attempting to increase the level of erucic acid in these oils by possible genetic manipulation of the plant (4). Interest shown by industry in the physical and chemical properties of high-erucic acid oils has led to chemical synthesis of these oils.

Various methods have been tried to synthesize triacylglycerols with or without catalyst at high temperatures under vacuum. Triacylglycerols have been prepared by direct esterification of fatty acids with molar equivalents of glycerol in the presence of an acid or alkaline catalyst (5,6). Sulfuric acid, phosphoric acid, *p*-toluenesulfonic acid, zinc chloride, sodium hydroxide and other catalysts have been used. Interesterification of methyl esters of fatty acids and glycerol has also been attempted to obtain triacylglycerols. The use of most catalyst has resulted in dark-colored products and also posed problems during subsequent refining of the crude product. Triacylglycerols have also been synthesized without catalyst under severe conditions (6,7).

Triacylglycerols with high erucic acid content have been synthesized by means of direct esterification and interesterification approaches (8). Concentrated sulfuric acid was used as a catalyst for direct esterification, and the reaction yielded a dark reaction product with the undesirable *trans* isomer (10–12%) of erucic acid (brassidic acid). An oil product with up to 90% erucic acid was obtained with *p*-toluenesulfonic acid as catalyst but yield was not reported. In a second approach, crambe oil and methyl erucate were interesterified with NaOH as catalyst, and a product with ≈38% trierucin content was obtained (8).

The objective of this study was to produce, without discoloration, triacylglycerols with high levels of erucic acid. These products were then interesterified with high-erucic acid rapeseed (HEAR) oil to obtain products with various levels of erucic acid. The physical and chemical properties of these oils were determined.

MATERIALS AND METHODS

Chemicals. HEAR (*Brassica napus* var. Hero) oil was provided by CSP Foods Inc., Altona, Manitoba, Canada. Erucic acid (>90% pure) was obtained from Sigma Chemical Company, St. Louis, MO. Sodium-potassium alloy (22:78) was purchased from Aldrich Chemical Company, Inc., Milwaukee, WI. Glycerol and thin-layer chromatography (TLC) plates (aluminum-backed silica gel 60 F_{254} pre-coated plate 20 × 20 cm, layer thickness 0.2 mm) were purchased from BDH Inc., Edmonton, Alberta, Canada. Alumina (80–200 mesh) was purchased from Fisher Scientific, Edmonton, Alberta, Canada. All other chemicals and reagents utilized were of reagent grade.

Fatty acid analysis. The fatty acid compositions were determined with a Hewlett-Packard model 5750 gas chromatograph equipped with a flame-ionization detector (Palo Alto, CA). A stainless-steel packed column 1.8 m long, containing GP 3% SP-2310/2% SP-2300 on 100/120 Chromosorb WAW (Supelco Canada Ltd., Oakville, Ontario, Canada) was used. The column was held at 150°C for 1 min, raised to 240°C at 8°C/min and held at the same temperature for 4 min. Helium was the carrier gas at a flow rate of 40 mL/min. Methyl esters of fatty acids were prepared by the acid methanolysis method of Hitchcock and Hammond (9).

Trierucin synthesis. Preliminary experiments led to a final procedure for trierucin synthesis as developed and presented in Figure 1. Trierucin oil was synthesized directly by chemical esterification of erucic acid (>90% pure) onto glycerol. Glycerol and erucic acid (1:3 molar ratio) were placed in a 500-mL double-neck flask, and the mixture was heated stepwise under a vacuum of 25 ± 10 mm Hg with continuous stirring.

The reaction was conducted in two stages. Initially, a temperature of 160 ± 5°C was maintained for about 4 h. Subsequently, the temperature was raised to 250 ± 10°C and maintained for 8–10 h. The reaction was stopped by cooling the oil to room temperature before releasing the vacuum. The crude product was analyzed by TLC for the presence of triacylglycerols and other partial acylglycerols (10). The amounts of free fatty acids remaining in the reaction were determined by an AOCS official method (11). The crude product of esterification was alkali-refined and further purified through column chromatography with alumina as adsorbent, and the purity was confirmed by TLC analysis.

Preparation of oil samples. A portion of HEAR oil and the synthesized trierucin oil product were mixed in varying weight ratios. The binary mixtures were then randomized through chemical interesterification by continuously stirring the mixture of dried oils with 0.2% (w/w) sodium-potassium alloy for 2 h at 80°C under nitrogen

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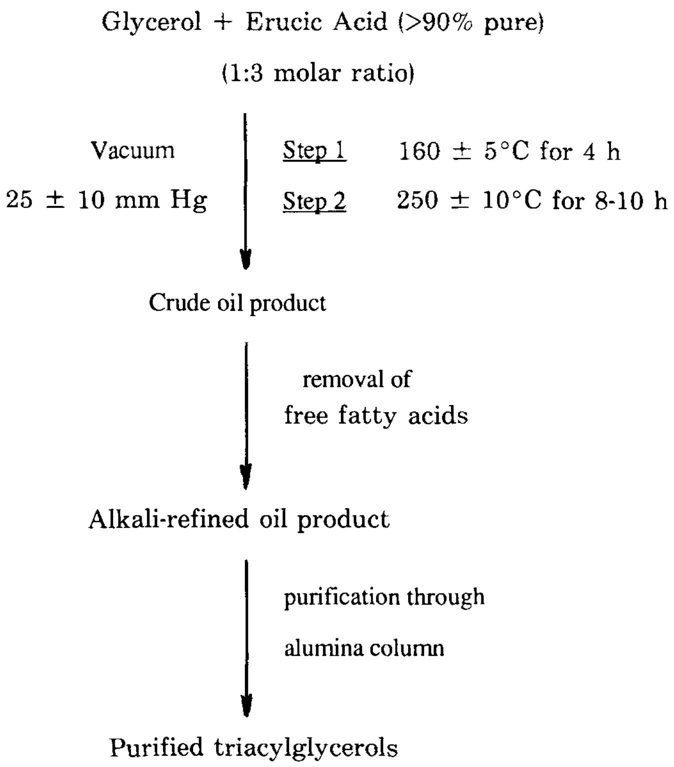

FIG. 1. Flow diagram for the synthesis of trierucin oil product.

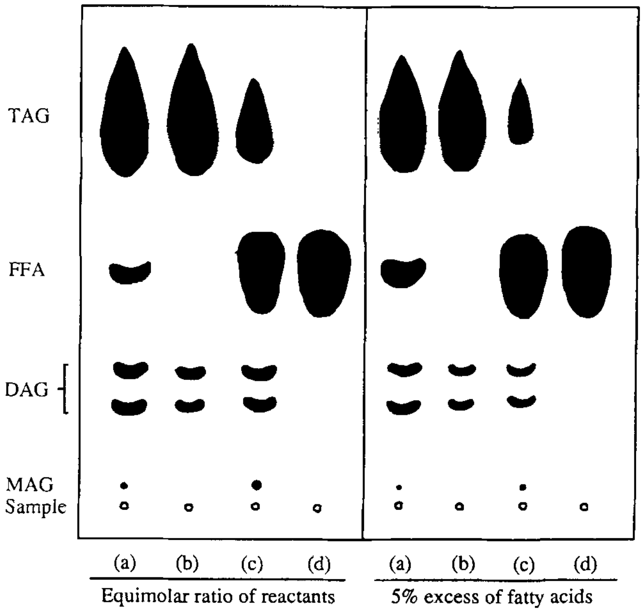

FIG. 2. Thin-layer chromatograms of trierucin oil product after reaction with equimolar and 5% excess erucic acid. (a) Crude trierucin oil product, (b) alkali-refined oil product, (c) fatty material recovered from soap, (d) erucic acid substrate. TAG, triacylglycerols; FFA, free fatty acids; DAG, diacylglycerols; MAG, monoacylglycerols.

in a water-jacketed Wheaton Flask. The HEAR oil was also randomized by the above method. The fatty acid composition of purified oil samples was determined by gas-liquid chromatography analysis as described above. The completion of the chemical randomization of the mixtures of HEAR oil and synthetic triurucin oil was confirmed by fatty acid positional analysis (10).

Physical/chemical characteristics. Melting point, titer point and refractive index of the synthetic oils were determined by AOCS official methods (12–14). Cloud point and pour point were determined according to ASTM methods (15,16). The viscosity measurements of the oil samples were obtained in a Haake rotational viscometer (Gebruder Haake, Berlin, Germany) with an NV sensor system and MK500 measuring head. All samples were measured at temperatures of 20 to 100°C at 10°C intervals, except the oil samples with 83 and 91% erucic acid, which were measured from 30 to 100°C at 10°C intervals. The iodine and saponification values of the oil samples were determined by AOCS official methods (17,18).

RESULTS AND DISCUSSION

Synthesis of triacylglycerols (trierucin). A direct esterification technique was adopted to synthesize trierucin from glycerol and erucic acid under vacuum. Molar equivalents of glycerol and fatty acid were heated with continuous stirring under 25 ± 10 mm Hg vacuum. Preliminary first-stage experiments at 180°C under 25 ± 10 mm Hg vacuum resulted in the loss of glycerol as condensate, and some charring of the contents was noted. A lower first-stage initial temperature of 160°C for 4 h eliminated these problems and produced a light-colored product, consisting mainly of unreacted fatty acid, mono- and diacylglycerol. Second-stage heating at 200–220°C was maintained for 8–10 h, during which the partial acylglycerol species were converted to triacylglycerol. The final product contained 10–14% free fatty acids. Alkali refining of the crude product yielded a refined fraction that accounted for about 80% of the theoretical yield. A higher second-stage temperature (250°C) resulted in a crude product with only *ca.* 5% free fatty acid content and 90–95% yield of alkali-refined product. The higher finishing temperature (250°C) was consistent with that recommended by Sonntag (7) for noncatalyzed synthesis of triacylglycerols. Purification of the alkali-refined product by column chromatography on alumina removed mono- and diacylglycerols, leaving an 85–90% yield of purified triacylglycerols. Figure 1 shows the scheme for preparation of purified trierucin oil product.

TLC analysis of the crude product demonstrated the presence of small amounts of mono- and larger amounts of diacylglycerols (Fig. 2a). TLC analysis of alkali-refined product (Fig. 2b) demonstrated effective removal of free fatty acids after saponification and low residual levels of monoacylglycerols because they tend to be removed along with saponified fatty acids.

The use of 5% molar excess of fatty acids resulted in decreased amounts of monoacylglycerols in the final product (Fig. 2), which helped in the alkali-refining process by faster and easier separation of soap from unsaponified oil, and thus less loss of neutral oil, resulting in improved yield of pure triacylglycerols. A 10% molar excess of fatty acids did not show any advantage over 5% excess (results not shown).

Fatty acid composition of synthetic erucic acid oils. The fatty acid compositions of native HEAR oil and different

TABLE 1

Fatty Acid Composition[a] of Native High-Erucic Acid Rapeseed (HEAR) Oil, Randomized HEAR Oil and Synthetic Erucic Acid Oils

	Oil product (% erucic acid)[b]						
	Native	Random	Synthetic oil				
Fatty acid[c]	45% HEAR	45% HEAR	55% Erucic	65% Erucic	75% Erucic	83% Erucic	91% Erucic
C16:0	2.8	2.8	2.2	1.6	1.0	0.5	0.0
C18:0	1.1	1.1	0.9	0.6	0.4	0.2	0.0
C18:1	17.5	17.4	13.7	10.3	6.4	3.2	0.2
C18:2	13.2	13.2	10.4	7.7	4.7	2.3	0.1
C18:3	8.6	8.6	6.9	4.9	3.0	1.4	0.0
C20:0	0.8	0.8	0.7	0.6	0.5	0.4	0.2
C20:1	8.6	8.5	7.2	5.8	4.3	3.2	1.9
C22:0	0.5	0.5	0.6	0.7	0.8	0.9	1.0
C22:1	45.2	45.5	55.1	64.7	74.9	83.3	91.7
C24:0	0.3	0.3	0.4	0.5	0.6	0.8	0.8
C24:1	0.9	1.0	1.2	1.4	1.6	1.8	2.0
Unidentified	0.5	0.4	0.8	1.1	1.7	2.1	2.0

[a] Components expressed as weight percent.
[b] Mean of three determinations.
[c] Fatty acids represented as number of carbon atoms:number of double bonds.

synthetic erucic acid oils are shown in Table 1. The HEAR oil used for this experiment contained, beside erucic acid, considerable amounts of oleic acid, linoleic acid, linolenic acid and gadoleic acid. The fatty acid composition of HEAR oil before and after randomization was the same, indicating no loss in fatty acids during chemical interesterification. Fatty acid analysis of randomized and purified oil mixtures of HEAR oil and synthetic erucic oil showed erucic acid levels of 45.5, 55.1, 64.7, 74.9, 83.3 and 91.7% by weight. Oleic, linoleic, linolenic and gadoleic acids decreased gradually in the oil samples as the erucic acid content increased. These fatty acids contributed less than 3% of the total in the 91% erucic acid oil, the major impurities being selacholeic (tetracosenoic) acid, gadoleic acid, behenic acid, lignoceric acid and unidentified compounds.

Properties of erucic acid oils. Melting point, cloud point, pour point and titer point of native HEAR, randomized HEAR and synthetic erucic oils are presented in Table 2. The melting point of randomized HEAR oil (16.8°C) was considerably higher than the native HEAR oil (5.8°C), possibly due to higher trierucin levels in the randomized HEAR oil. In native HEAR oil, only trace amounts of erucic acid were present at the *sn*-2 position, resulting in little or no trierucin. The melting points of oil samples in this study were dependent on erucic acid content and the positional fatty acid arrangement in the triacylglycerol molecule. The melting point was higher for the samples containing high contents of erucic acid because of presence of both long-chain and less-unsaturated (C20:1, C22:1, C24:1) triacylglycerols. Oil samples with erucic acid contents of 45, 55 or 65% showed a broader melting range (3-4°C) from the start of softening to completely clear liquid, whereas samples with higher erucic content exhibited relatively sharp values. The sharp melting of very high erucic acid oils is attributed to the presence of higher amounts of a single species of triacylglycerols (trierucin). Synthetic erucic oils had specific heats in the range 0.44-0.46 Cal/g°C (at 20 ± 3°C above the melting point), while for both native HEAR oil and randomized HEAR oil it was 0.43 Cal/g°C.

Cloud and pour point. Cloud and pour points followed patterns similar to that shown by the melting point. Pour points were, in general, slightly higher than the cloud points, and both were lower than the melting points. The cloud and pour points for randomized HEAR oil were −3 and −2°C, respectively, considerably higher than corresponding values (−12 and −14°C) for native HEAR oil,

TABLE 2

Melting, Cloud, Pour and Titer Points and Iodine and Saponification Values of Native High-Erucic Acid Rapeseed (HEAR) Oil, Randomized HEAR Oil and Synthetic Erucic Acid Oils[a]

Oil product	Melting point (°C)	Cloud point (°C)	Pour point (°C)	Titer point (°C)	SV[b]	IV[c]
45% Native HEAR	5.8 ± 0.2	−12 ± 1	−14 ± 1	16.0 ± 0.4	99 ± 0	176 ± 1
45% Random HEAR	16.8 ± 0.3	−3 ± 1	−2 ± 1	16.0 ± 0.4	99 ± 1	176 ± 0
55% Synthetic erucic	18.7 ± 0.3	0 ± 1	2 ± 0	19.6 ± 0.2	93 ± 0	173 ± 1
65% Synthetic erucic	21.8 ± 0.2	3 ± 1	3 ± 1	21.6 ± 0.1	87 ± 0	169 ± 1
75% Synthetic erucic	25.2 ± 0.2	7 ± 0	10 ± 1	25.6 ± 0.1	81 ± 0	164 ± 1
83% Synthetic erucic	26.8 ± 0.1	13 ± 1	15 ± 1	27.2 ± 0.1	76 ± 0	161 ± 1
91% Synthetic erucic	29.0 ± 0.1	16 ± 1	19 ± 1	29.6 ± 0.1	70 ± 1	158 ± 1

[a] Values are average of minimum three determinations.
[b] SV, saponification value.
[c] IV, iodine value.

due to the presence of high-melting triacylglycerols that crystallized at a higher temperature. During the experiment, a slight increase in the temperature of the oil sample was observed just before complete solidification of oil due to the heat released by crystallization, which warmed the oil sample (19).

Although oils with >75% erucic acid showed cloud and pour points ranging from 7 to 19°C, these oils started crystallizing at room temperature (23°C) if given enough time. For example, oil with 91% erucic acid started crystallizing in 3-4 h and became completely solid. Oils with 75 and 83% erucic acid required 8-12 h for onset of crystallization and remained partially liquid, particularly the oil with 75% erucic acid content. The time period for an oil sample to start solidifying at room temperature also is dependent on sample volume. Heat dissipation from the oil before and during crystallization may delay crystallization in larger volumes of oil.

Titer value. Titer point was higher for oil samples containing higher amounts of erucic acid. Titer values were similar to the melting points of randomized triacylglycerol samples.

Viscosity (dynamic). The viscosities of the oil samples decreased with increase in temperature. Figure 3 contains data from the randomized HEAR oil and from the synthetic 75 and 91% erucic acid oil samples. All other oils had data points intermediate to these. The decrease in viscosity was sharp when the temperature was increased from 20 to 60°C, beyond which an increase in temperature caused the viscosity to slowly but steadily decrease. A similar effect of temperature on viscosity of winter rapeseed oil has been previously observed (20,21). The viscosities of the oil samples ranged from 85 to 103 centipoise (cP) at 20°C. The difference in viscosities between different oils was greatly reduced with increase in temperature. There was almost no difference in viscosity at 90 and 100°C. Our results at 100°C agree well with reported values of 10-11 cP for high-erucic acid oils (22,23).

The randomization of HEAR oil did not have any effect on the viscosity of the oil over the temperature range (20—100°C) measured. The relatively higher viscosity values of high-erucic acid oils are ascribed to the higher content of long-chain triacylglycerols (24). The HEAR oil contained relatively more unsaturated fatty acids, which may have also contributed to lower viscosity (24).

Iodine and saponification values. Iodine and saponification values of native HEAR, randomized HEAR and synthetic erucic oils are presented in Table 2. These values were in close agreement to the theoretical values based on fatty acid composition. As expected, iodine value decreased with increase in erucic acid content because the synthetic oils contained lower amounts of polyunsaturated fatty acids.

The saponification value for the HEAR oil was in close agreement with reported values of 170-180 (22). An increase in erucic acid in the synthetic oils resulted in lower saponification values, hence 91% erucic oil had the lowest saponification value while 45% had the highest.

High-erucic acid oils, having a low degree of unsaturation and longer-chain fatty acids, should be resistant to oxidation, stable during high-temperature processing conditions and resistant to thermal stress. The increased viscosity of high-erucic acid oils at elevated temperatures will also add to the lubrication capability of these oils.

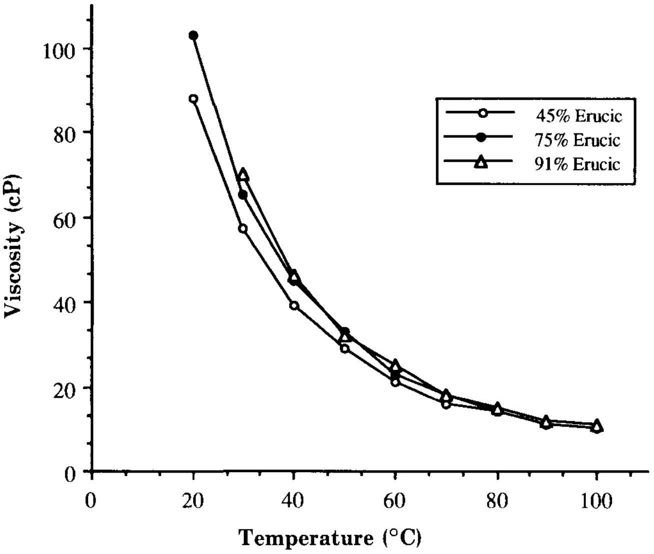

FIG. 3. Effect of temperature on viscosity of randomized high-erucic acid rapeseed oil (45% erucic) and synthetic erucic acid oils (75 and 91% erucic), cP, centipoise.

Oils with high-erucic acid may not be suitable for general lubrication purposes at room temperature or in cold weather conditions because oils with 75% or more erucic acid began crystallizing at room temperature. However, they might be successfully used where high temperatures are continuously maintained, as in the steel-casting industry. Also, at low temperatures, these oils may cause problems during transportation, handling and pumping operations.

In conclusion, we have characterized some of the properties of high-erucic acid triacylglycerol oils that can be indicators of characteristics of the high erucic acid oils that may be produced as a result of genetic modification of oilseeds. Such information would be beneficial to the industries utilizing these future oils and may help to guide the plant geneticists as to what level of erucic acid would be desirable.

REFERENCES

1. Latta, S., *INFORM 1*:434 (1990).
2. Nieschlag, H.J., and I.A. Wolff, *J. Am. Oil Chem. Soc. 48*:723 (1971).
3. Downey, R.K., in *High and Low Erucic Acid Rapeseed Oils*, edited by J.K.G. Kramer, F.D. Sauer and W.J. Pigden, Academic Press, Toronto, 1983, pp. 1-20.
4. Annual Report 1990/91, Plant Biotechnology Institute, Saskatoon, National Research Council Canada.
5. Hartman, L., *J. Am. Oil Chem. Soc. 43*:536 (1966).
6. Hartman, L., and D. Reimann, *Fat Sci. Technol. 91*:324 (1989).
7. Sonntag, N.O.V., *J. Am. Oil Chem. Soc. 56*:751A (1979).
8. Carlson, K.D., and M.O. Bagby, *Synthesis of Trierucin and Production of Very High Erucic Acid Oils*, poster session paper presented at American Oil Chemists' Society's Annual Meeting, May 3-7, Cincinnati, 1989.
9. Hitchcock, C., and E.W. Hammond, in *Developments in Food Analysis Techniques-2*, edited by R.D. King, Applied Science Publishers Ltd., London, 1978, pp. 185-224.

10. Tautorus, C.L., *The Effect of Fatty Acid Composition on the Autoxidative Stability of Native and Synthesized Triacylglycerols,* Ph.D. Thesis, University of Saskatchewan, Saskatoon, Canada, 1990.
11. *The Official Methods and Recommended Practices of the American Oil Chemists' Society,* Vol. 1, 3rd edn., edited by David Firestone, American Oil Chemists' Society, Champaign, 1989, Method Ca 5-40.
12. *Ibid.*, Vol. 1, Method Cc 1-25.
13. *Ibid.*, Vol. 1, Method Cc 12-59.
14. *Ibid.*, Vol. 1, Method Cc 7-25.
15. *Annual Book of ASTM Standards,* Section 5, American Standards and Testing Materials, Philadelphia, 1990, Method D-2500.
16. *Ibid.*, Method D-97.
17. *The Official Methods and Recommended Practices of the American Oil Chemists' Society,* Vol. 1, 3rd edn., edited by David Firestone, American Oil Chemists' Society, Champaign, 1989, Method Cd 1b-87.
18. *Ibid.*, Vol. 1, Method Cd 3-25.
19. Weiss, T.J., in *Food Oils and Their Uses*, 2nd edn., edited by T.J. Weiss, (ed), AVI Publishing Company Inc., Westport, 1983, pp. 1-33.
20. Peterson, C.L., D.L. Auld and R.A. Korus, *J. Am. Oil Chem. Soc. 60*:1579 (1983).
21. Noureddini, H., B.C. Teoh and L.D. Clements, *Ibid. 69*:1189 (1992).
22. Appelqvist, L.A., and R. Ohlson, in *Rapeseed: Cultivation, Composition, Processing and Utilization,* edited by L.A. Appelqvist, Elsevier Science Publishing Co., New York, 1972, pp. 257, 381.
23. Wagner, G.L., and C.L. Peterson, in *Vegetable Oils Fuels,* American Society of Agricultural Engineers Publication 4-82, 1982, pp. 329–336.
24. Formo, M.W., in *Bailey's Industrial Oil and Fat Products*, Vol. 1, edited by D. Swern, John Wiley and Sons, New York, 1979, pp. 177–232.

[Received March 12, 1993; accepted July 18, 1993]