Composition of Fatty Acids and Structure of Triglycerides in Medium and Low Erucic Acid Rapeseed

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ABSTRACT

Total triglycerides in medium (MEAR) and low (LEAR) erucic acid cultivars of rapeseed were fractionated by argentation chromatography into twelve and ten fractions, respectively. Gas liquid chromatography of the fatty acids in the triglyceride fractions and their 2-monoglycerides was used to evaluate the structural characteristics of the individual fractions. Fractionation occurred on the basis of degree of unsaturation, molecular weight and positional characteristics. The most mobile fractions contained 34-50% of saturated fatty acids while the less mobile had 59-65% of polyunsaturated fatty acids. In the medium erucic acid oil, long chain fatty acids (C20-C22) were found in all fractions, but four fractions of low erucic acid oil were essentially free of long chain acids. Two of these fractions in the latter oil, which represented 44% of the total triglycerides, were glycerol trioleate and dioleoyllinoleoylglycerol. The majority of the 2-positions were occupied by unsaturated C18 fatty acids, generally in the order of linoleic $\geqslant$ linolenic $>$ oleic acids. The saturated and long chain fatty acids occurred predominantly in the 1- and 3-positions. The various fractions of medium and low erucic acid oils were similar in structural composition except that eicosenoic and erucic acids substituted for oleic acid in some external positions. Erucic acid did not appear to substitute directly for oleic acid in the 2-position.

INTRODUCTION

The characteristics and distribution of fatty acids in rapeseed triglycerides, which constitute 95-98% of the commercial oil, have been the subject of several investigations (1-6). Oil obtained from the high erucic acid (HEAR) cultivars are reported to contain 61% of triglycerides with 62 carbon atoms in the fatty acid chains (mono-C18:1, mono-C18:2 or mono-C18:3 di-C22:1 glycerides), 17% of triglycerides with a carbon number of 60, and 6% with carbon numbers of 58 and 56 (1,2). About 80% of the rapeseed oil in low erucic acid (LEAR) cultivars consist of triglycerides with carbon numbers of 54 (tri-C18 glycerides), and 14% have 52 carbon atoms in the fatty acid chains. The rapeseed genotypes with medium erucic acid (MEAR) levels have been shown to contain less triglycerides with carbon numbers of 62 but more with values of 58 and 60 than HEAR cultivars.

Early studies using pancreatic lipase hydrolysis to determine the fatty acid structure of unfractionated HEAR oils demonstrated that the saturated acids and long chain mono- and diunsaturated acids (C20-C24) are esterified mainly in the external positions of the triglycerides (2,5). Unsaturated C18 fatty acids were initially built up in the 2-position of the triglycerides. Based on argentation chromatography of LEAR triglycerides, Sergiel (7) showed that linoleic and linolenic acids were preferentially esterified in the 2-position as compared to oleic and palmitic acids in most fractions. The above studies were designed to evaluate specifically the fatty acid characteristics of commercial (expeller and hexane-soluble) oils but did not consider the variable proportions of triglycerides which constitute the bound triglyceride fraction (8).

The objectives of the present study were to determine the fatty acid composition and structure of the individual fractions in the total (free and bound) triglycerides of MEAR and LEAR oils. Triglycerides were fractionated by argentation chromatography for fatty acid analysis and selective lipase hydrolysis to identify the 2-position fatty acids.

MATERIALS AND METHODS

Rapeseed used for analysis was obtained from paired plots of Sinus, a MEAR cultivar from Sweden, and Janpol, a Polish LEAR cultivar, grown at six locations in Poland. Analyses were conducted on bulked samples of each cultivar, the values reported being means of three determinations in all cases. The total lipids were extracted by repeated 1-hr extractions with chloroform/methanol (2:1, v/v), the solvent was removed in a rotary evaporatory under vacuum and nitrogen at 50-55 C, and the lipids were stored at -20 C under nitrogen.

The total triglycerides were separated from other lipid components by column chromatography on silica gel, suitable for chromatographic use (J.T. Baker Chemical Co., Phillipsburg, NJ). The silica gel was dried to constant weight at 200 C and hydrated with 5% (w/w) water. One gram of extracted lipid was dissolved in 15 ml chloroform and applied to the column of silica gel. The triglycerides were eluted with 200 ml of benzene at a flow rate of 1.5-2.0 ml/min. The elution of triglycerides was monitored by one dimensional thin layer chromatography (TLC) using benzene/diethyl ether/ethyl alcohol/acetic acid (50:40:2:0.2, v/v/v/v) (8).

The triglycerides were fractionated on 20 x 20 cm plates coated with a layer (0.75 cm thick) of Silica Gel G (Merck, Darmstadt, W. Germany) blended with a 20% $AgNO_3$ solution in water. The TLC plates were dried at room temperature for 12 hr before activation for 1 hr at 130 C (MEAR oil) and 125 C (LEAR oil) to improve the polarity of the gels and differentation of the triglycerides. Exactly 0.14 ml of 10% triglyceride solution was applied to each TLC plate and the chromatogram developed with benzene/diethyl ether (90:10 v/v). The developed plates were sprayed with an alcoholic solution of 2,7-dichlorofluorescein and the spots identified under UV-light. The individual fractions of triglycerides were scraped into 50 ml conical flasks containing 1 ml of internal standard (1% w/v margaric acid dispersed in diethyl ether) and 25 ml of diethyl ether. The contents of the flasks and two additional washings with 20 ml of diethyl ether were filtered through a fritted disc funnel and the filtrate evaporated to dryness in a rotary evaporator under nitrogen.

After weighing, the dried residues were dissolved in 1.5 ml of chloroform/methanol/sulfuric acid (100:100:1 v/v/v), transferred into 2 ml pharmaceutical vials and sealed hermetically (9). The methylations were carried out by heating the vials in a water bath at 90 C for 4 hr. After cooling, the vials were opened, the sulfuric acid precipitated with powdered zinc, and the contents of the tubes evaporated to dryness in a water bath at 60 C under nitrogen. The residues were dissolved in 1 ml hexane and 1.5 ml saturated NaCl solution, and the hexane phase transferred to a clean vial, followed by evaporation and solubilization

TABLE I

Yield and Fatty Acid Composition in Weight Percent of MEAR Triglycerides and Triglyceride Fractions

Fraction;	Total	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Recovered
						Triglyceride weight %[a]								
Yield:	100	4.5	11.0	6.7	12.3	11.7	7.4	10.7	3.2	13.2	8.7	6.5	4.4	100.3
						Fatty acid composition in weight % methyl ester								
C16:0	3.8	22.3	8.8	2.0	2.0	10.0	1.4	1.3	10.2	7.2	1.4	1.0	1.8	5.3
C16:1	0.1	—	0.8	0.2	0.9	0.2	—	0.1	—	0.5	1.3	0.7	0.6	0.5
C18:0	0.6	11.4	4.0	2.8	3.0	0.9	2.0	0.7	3.6	0.5	0.3	0.2	0.1	2.1
C18:1	39.2	33.7	46.8	54.1	73.4	20.3	33.4	63.0	26.4	20.3	50.8	33.7	25.7	42.2
C18:2	20.5	5.8	4.3	5.3	7.0	27.9	26.5	28.5	31.3	22.7	12.2	31.4	27.2	18.5
C18:3	9.2	—	—	—	—	—	—	—	8.3	17.2	20.9	21.5	31.5	7.1
C20:1	11.6	7.8	11.6	18.2	6.8	11.0	20.6	2.7	6.5	11.8	8.1	4.8	4.7	9.8
C22:0	0.1	—	—	—	—	—	—	—	—	—	—	—	1.5	0.1
C22:1	14.9	18.9	23.7	17.3	6.8	29.7	16.2	3.6	13.7	19.8	4.9	6.6	6.9	14.7

[a]Triglyceride fractions from TLC plates.

TABLE II

Yield and Fatty Acid Composition in Weight Percent of LEAR Triglycerides and Triglyceride Fractions

Fraction:	Total	I	II	III	IV	V	VI	VII	VIII	IX	X	Recovered
						Triglyceride weight %[a]						
Yield:	100	6.4	10.0	26.5	6.3	17.5	2.8	10.0	9.2	6.5	5.2	100.4
						Fatty acid composition in weight % methyl ester						
C16:0	5.2	31.4	20.9	1.1	23.4	1.1	18.1	7.7	1.6	4.1	4.3	8.0
C16:1	0.3	0.5	0.3	—	—	—	—	—	0.2	0.3	0.4	0.1
C18:0	1.9	16.2	9.7	—	6.6	—	6.3	3.1	—	1.6	2.3	3.1
C18:1	59.6	38.3	60.8	98.8	41.5	68.6	32.4	35.9	61.9	34.8	26.2	63.1
C18:2	20.4	8.3	3.8	—	28.2	30.0	37.9	42.9	10.2	32.9	25.0	17.7
C18:3	10.8	—	—	—	—	0.4	5.0	10.4	25.0	25.4	39.7	7.3
C20:0	0.4	2.7	2.2	—	0.1	—	—	—	—	—	—	0.4
C20:1	0.9	2.4	1.6	—	—	—	—	—	1.0	1.0	1.8	0.6
C22:1	0.6	—	0.8	—	—	—	0.3	—	—	—	—	0.1

[a]Triglyceride fractions from TLC plates.

in a few drops of hexane for GLC analysis. The methyl esters of the fatty acids in the triglyceride fractions were separated and quantitated by GLC on a Pye Unicam model 104 gas chromatograph with a hydrogen flame detector, in comparison with standard fatty acids. The glass column, 210 cm x 4 mm, was packed with 10% DEGS on 60/80 mesh Chromosorb W. A column temperature of 195 C was maintained with the argon carrier gas flow being 60 ml/min. The relative weight percent of each fatty acids was determined from the integrated peak areas.

Hydrolysis of the 1- and 3-position fatty acids was conducted on samples of each triglyceride fraction using pancreatic lipase in tightly sealed 10 ml flasks (7). Depending on the yield of each fraction, 1-5 mg of triglyceride, 1 ml of 1 M Tris buffer at pH 8.2, 0.1 ml of 20% $CaCl_2$ solution and 0.25 ml of 1% sodium taurocholate were added to the flasks. The flasks were placed in a water bath at 37 C and shaken for 5 min before adding 10 mg of pancreatic lipase (diluted in 1 ml of Tris buffer) and shaking for 5 additional min. The hydrolytic process was inhibited by adding 5 ml of 6 M HCl. The contents of the flask were transferred into a separatory funnel and the aqueous phase extracted three times with 15 ml of diethyl ether to remove the mixture of free fatty acids, mono-, di- and triglycerides. The collected eluant was dehydrated by draining through anhydrous sodium sulfate and evaporated to dryness under vacuum in a rotary evaporator under nitrogen.

Monoglycerides were separated from triglycerides and free fatty acids by TLC (7,10) on Silica Gel G plates which were activated for 1 hr at 110 C. The complete sample of hydrolyzed lipid material was taken up in a small quantity of diethyl ether and applied to the cooled TLC plate for development with hexane/diethyl ether/methanol/acetic acid (90:30:3:2, v/v/v/v). After drying, the developed plates were sprayed with 0.1% solution of dichlorofluorescein. Monoglycerides and other fractions were identified under UV-light in comparison with standards.

Monoglycerides were scraped from the plates, transferred into 50 ml flasks containing 1 ml of internal standard, and eluted three times with 20 ml of diethyl ether using a fritted disc funnel. The combined eluants were evaporated to dryness under nitrogen. The monoglycerides were then dissolved in 15 ml of chloroform/methanol/sulfuric acid (100:100:1, v/v/v) for hydrolysis and methylation. GLC analysis of the methylated fatty acids was conducted as for the above triglycerides.

RESULTS AND DISCUSSION

As reported previously (8), the average crude fat contents of the MEAR and LEAR samples were 42.4 and 42%, respectively, while the total lipids extracted with chloroform/methanol were 43.7 and 43%, respectively. The proportions of triglycerides in the lipid fractions were 91.9 ± 0.4% for the MEAR samples and 92.1 ± 0.6% for the LEAR samples, so that the yields of triglycerides from both samples were essentially equal.

Analysis of the total fatty acids in the MEAR and LEAR oils revealed that both types contained ca. 6% saturated, 64% monounsaturated and 30% polyunsaturated fatty acids (Tables I and II). The principal difference be-

TABLE III

Fatty Acid Composition in Weight Percent of 2-Monoglycerides from Lipase Hydrolysis of MEAR Triglycerides and Triglyceride Fractions

	Total	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Recovered
	Fatty acid composition in weight % methyl ester													
C16:0	0.2	2.6	2.7	1.7	1.5	1.5	1.5	1.6	3.6	1.3	1.2	1.2	2.0	1.7
C16:1	0.1	—	0.3	0.1	0.3	0.1	—	—	—	—	0.7	0.6	0.1	0.2
C18:0	0.2	2.6	1.8	0.9	0.9	0.5	0.5	0.6	3.9	0.6	0.2	0.2	0.4	0.9
C18:1	44.6	86.4	85.4	87.4	80.9	24.5	22.0	47.5	18.2	13.8	45.2	15.7	24.4	47.1
C18:2	37.1	4.4	6.2	7.5	16.4	72.3	76.0	49.9	52.8	35.8	8.1	49.6	38.6	34.9
C18:3	17.5	—	—	—	—	—	—	—	19.3	47.6	43.7	29.2	34.0	14.1
C20:1	0.2	3.0	1.9	1.4	—	0.7	0.1	—	1.6	1.0	0.9	2.1	—	0.9
C22:1	0.1	0.9	1.6	1.0	—	0.3	—	0.4	0.6	—	—	1.3	0.6	0.5

TABLE IV

Fatty Acid Composition in Weight Percent of 2-Monoglycerides from Lipase Hydrolysis of LEAR Triglycerides and Triglyceride Fractions

	Total	I	II	III	IV	V	VI	VII	VIII	IX	X	Recovered
	Fatty acid composition in weight % methyl ester											
C16:0	0.5	3.7	2.2	0.6	3.2	0.7	2.8	1.5	0.6	1.2	1.0	1.3
C16:1	0.1	0.4	0.5	—	—	—	—	—	tr	—	0.2	0.1
C18:0	0.2	1.5	1.1	—	1.2	—	1.6	0.9	—	0.8	0.5	0.5
C18:1	56.8	70.0	92.5	99.3	45.7	42.4	42.0	21.3	46.8	19.6	23.2	60.4
C18:2	29.1	23.9	3.1	—	50.0	56.4	46.1	56.9	9.9	46.9	34.8	27.6
C18:3	13.1	—	—	—	—	0.5	7.5	19.4	42.7	31.5	40.2	10.3
C20:0	0.1	0.2	0.4	—	—	—	—	—	—	—	—	0.1
C20:1	0.1	0.2	0.2	—	—	—	—	—	—	—	—	0.1
C22:1	—	—	—	—	—	—	—	—	—	—	—	—

tween the two oils was in the distribution of monounsaturated fatty acids, the MEAR triglycerides having 39.2% oleic, 11.6% eicosenoic and 14.9% erucic acids, while corresponding values in the LEAR oil were 59.6, 0.9 and 0.6%, respectively.

The MEAR triglycerides fractionated into twelve bands on the TLC plates while ten bands were resolved in the LEAR sample. The weight percentages of the MEAR fractions varied from 3.2 to 13.2% of the total triglycerides with no major fractions being evident (Table I). The LEAR oil contained high proportions of fractions III (26.5%) and V (17.5%) which showed intermediate to high mobility and, as discussed later, a narrow range in fatty acid composition (Table II).

Nonpolar triglycerides would be more mobile in the solvent system employed and, in general for both oils, the fractions appeared to be separated on the basis of degree of unsaturation, molecular weight and, possibly, positional effects (Tables I and II). Fraction I in the MEAR and I, II and IV in the LEAR oils contained over 30% of saturated acids in the triglycerides. The triglycerides with intermediate mobility, especially in the MEAR fractions, were high in monounsaturated fatty acids while the less mobile fractions showed increasing proportions of polyunsaturated fatty acids. Fraction XII in the MEAR and fraction X in the LEAR oils contained 58.7 and 64.7%, respectively, of polyunsaturated fatty acids. Fractions X in the MEAR and VIII in the LEAR oils were high in oleic acid but exhibited less mobility than would be predicted from the fatty acid distribution. Similarly, the minor fractions VIII (MEAR) and VI (LEAR) contained 14-24% of saturated acids but showed intermediate mobility. These deviations from expected mobilities were due, possibly, to positional effects since isomers of the unsaturated fatty acids occur in only low concentrations (11).

The MEAR triglycerides contained a significant proportion of 6-10 fatty acids in each fraction, but fewer fatty acids were found in several of the LEAR triglycerides (Tables I and II). In the latter oil, fraction III contained 98.8% of oleic acid while fraction V had 98.6% of oleic plus linoleic acids. It is significant that these two fractions represented 26.5 and 17.5%, respectively, of the total triglycerides in the LEAR oil. The variable composition of other fractions in both oils suggested that a number of triglycerides with similar mobility constituted each band on the argentation chromatograph.

Sergiel (7) fractionated a refined LEAR oil (Canbra) into seven bands by argentation chromatography, and these fractions demonstrated fatty acid compositions similar to those of II, III, IV, V, VII, VIII and possibly a combined IX and X fraction in the present study. In the present LEAR oil, fractions I and VI, which contained significant proportions of palmitic acid, were likely bound lipids that were not extracted readily with commercial hexane. As in the present study, Sergiel (7) found one fraction that contained 95.5% oleic acid and another with 96.7% of oleic plus linoleic acids, both of which represented 53.6% of the total triglycerides in the refined LEAR oil.

Early studies on unfractionated triglycerides of *Brassica* species using pancreatic lipase techniques and GLC analysis revealed that almost all saturated and long chain (C20-C24) fatty acids are esterified at the 1- and 3-positions, while unsaturated C18 acids are preferentially located at the 2-position (1,2,4,5). In LEAR oils (2) and triglyceride fractions (7) there was a preference for linoleic and linolenic acids at the 2-position. In the present study eicosenoic and erucic acids represented a small portion of the fatty acids in the 2-position of most MEAR fractions (Table III). Like the long chain acids, palmitic and stearic acids were esterified primarily at the 1- and 3-positions of both oils but somewhat higher proportions of available acids (Tables I and II) were found in the 2-position (Tables III and IV). The majority of the 2-positions were occupied by unsaturated C18 fatty acids, generally in the order of linoleic $\geqslant$ linolenic $>$ oleic acids in the two oils. In particular, linoleic acid showed preference over linolenic acid for the 2-position in fractions XI (MEAR) and IX (LEAR). Eicosenoic and erucic acids did not appear to substitute directly for

TABLE V

Positional Composition of the Major Triglycerides Based on 2-Position Analysis

MEAR fractions	Principal glycerides[a]	No. of double bonds	LEAR fractions	Principal glycerides[a]	No. of double bonds
I	POE	2	I	POO,PLP	2
II	EOO,POE	2-3	II	POO	2
III	EOO	3	III	OOO	3
IV	OOO,EOO	3	IV	OLP,POL	3
V	ELP,ELO,ELE	3-4	V	OLO,OOL	4
VI	ELO	4	VI	OLP,LOP	3
VII	OLO,OOL	4	VII	OLL,OLO,LLnP	4-5
VIII	ELO,ELP	3-4	VIII	OLnO,OOLn	5
IX	ELnO,ELL	5	IX	OLLn,OLnL,LOLn	6
X	OLnO,EOLn	5	X	OLnL,OLLn,LnOLn	6-7
XI	OLLn,ELnO	5-6			
XII	OLnL,ELLn,LOLn	6			

[a]P = palmitic plus stearic acids; O = oleic acid; L = linoleic acid; Ln = linolenic acid; E = eicosenoic plus erucic acids.

oleic acid in the 2-position, despite the high proportions of the latter acid in the 2-position of several triglyceride fractions.

Based on the fatty acid compositions and positional analyses, the triglycerides in the LEAR fractions appeared to differ from the MEAR fractions primarily in the substitutions of oleic for erucic acid in the external positions (Tables III and IV). In both oils, fractions I and II contained diunsaturated triglycerides in which oleic acid occupied the 2-position (Table V). In these triglycerides, the 1- and 3-positions would contain one of the saturated acids plus either erucic (MEAR) or oleic (LEAR) acids.

Glycerol trioleate was the major component of fraction III in the LEAR oil while dioleoylerucoylglycerol or glycerol trioleate were the principal components in fractions II, III and IV of the MEAR oil (Table V).

Major components of fractions V and VIII in the MEAR oil and IV and VI in the LEAR oil had linoleic acid in the 2-position with palmitic or stearic acids occupying one of the external positions (Table V). These glycerides were less mobile than other triunsaturated glycerides.

The large fractions V in the LEAR oil and VII in the MEAR oil were dioleoyllinoleoylglycerol with either linoleic or oleic acids in the central position of the tetraunsaturated glycerides (Table V). Similar glycerides appeared in fractions V, VI and VIII of the MEAR oil with eicosenoic and erucic acids replacing oleic acid in one external position. Dilinoleoyloleoylglycerol and dilinoleoylerucoylglycerol were probably glycerides in fractions VII (LEAR) and IX (MEAR), respectively.

Fractions X, XI and XII (MEAR) and VIII, IX and X (LEAR) contained polyunsaturated triglycerides with 5-7 unsaturated bonds due to the high composition of linoleic and linolenic acids (Table V). Linolenic acid was the predominant fatty acid in the least mobile fraction of each oil.

While GLC analysis of the intact triglycerides was not attempted, the present results indicate that little C62 triglycerides occurred in the MEAR oil. The majority of triglycerides appear to contain 54-58 carbon atoms in the fatty acid chains.

Recoveries of various fatty acids in triglyceride fractions and their 2-monoglycerides showed an increase in levels of saturated and monounsaturated fatty acids and a loss of polyunsaturated fatty acids (Tables I-IV). It was apparent that antioxidants should be used during analysis to protect the polyunsaturates. In addition, the present results would have been improved if a two solvent system had been employed to separate the triglycerides into their component fractions.

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