Composition of Total Lipids in Rapeseed

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ABSTRACT

Total lipids in medium and low erucic acid cultivars of rapeseed (*Brassica napus* var. Sinus and Janpol, resp.) were fractionated into polar and nonpolar constituents. Triglycerides, diglycerides, monoglycerides, free fatty acids, sterol esters, sterols, phospholipids and glycolipids were quantitated and their fatty acid compositions determined. Triglycerides and phospholipids constituted 92 and 3.4%, resp., of the total lipid from each cultivar. Triglycerides were lower in saturated fatty acids but higher in monounsaturated acids and linolenic acid than other lipid fractions. Phospholipids and glycolipids were higher in linoleic acid content than other lipid classes. Generally, the reduction in long chain, monoenoic, fatty acids was associated with a corresponding increase in oleic acid in most low erucic acid fractions.

INTRODUCTION

Rapeseed ranks fifth in total world production of edible vegetable oils, but cultivation is limited to regions where soybean and subtropical oilseed crops are poorly adapted. Rapeseed is one of the cruciferous crops which normally contains a high proportion of erucic acid in the seed oils. Within the past decade, much nutritional and pathological data has been presented to show the undesirability of consuming oils and hydrogenated fats which contain high proportions of the long chain fatty acids, especially the 22-carbon, monoenoic, erucic acid (1). Plant breeders have been successful in developing low erucic acid cultivars which are more acceptable for the edible oil market. In Canada there has been a progressive decrease in the long chain fatty acid content of commercial rapeseed oil from over 35% to less than 3% (2). A similar conversion is now underway in several European countries (1,2,3).

The fatty acid compositions of refined rapeseed oils and lecithin obtained during the processing of the oil have been extensively investigated (3,4,5,6). Generally, the yield and composition of triglyceride and phospholipid components depended to a certain extent on the processing parameters (4). Little published information is available on the proportions of these components in the total lipids of mature rapeseed or the characteristics of other lipid constitutents such as monoglycerides, diglycerides, free fatty acids, sterol esters and glycolipids. The fatty acid compositions of these lipid components are of considerable importance in oil extraction, oil refining and meal quality.

The objectives of the present study were to obtain quantitative data on the yields and fatty acid compositions of nonpolar and polar lipid components in rapeseed oil. Lipid fractionations were conducted on the total lipids in medium (MEAR) and low (LEAR) erucic acid cultivars of winter rapeseed (*Brassica napus* L.) to show the effects of erucic acid reduction on the distribution of fatty acids in each lipid component.

MATERIALS AND METHODS

The seeds used for analysis in the present study were obtained from paired plots of Sinus, a MEAR cultivar from Sweden, and Janpol, a Polish LEAR cultivar, grown at six locations in Poland. One or more analyses of each constituent were made on mature seed from each location, and the data reported in the present paper are the means of at least six determinations.

Seed was ground to 60-mesh before analysis and lipid extraction. Moisture content was determined by drying at 95 C in a vacuum oven for 5 hr. The crude fat content of the seed was determined by petroleum ether extraction in a soxhlet-type apparatus for 16 hr according to the AOAC procedure 7.048 (7).

The total lipids were extracted by repeated 1-hr extractions of ground seed with chloroform: methanol (2:1, v/v) (8). The solvent was evaporated in a rotary evaporator under vacuum and nitrogen at 50-55 C. The dried lipids were weighed and stored under nitrogen at -20 C. The preliminary separation of lipids into polar and nonpolar fractions was conducted by silicic acid adsorption chromatography (9). A 25-g sample of lipid was dissolved in 300 ml of chloroform and then shaken for 20 min with 100 g of silicic acid. The 100-mesh silicic acid (Mallinchrodt Chemical Works, St. Louis, Mo.) had been previously activated by heating at 120 C for 24 hr. The mixture was poured into a fritted disc funnel for elution of nonpolar lipids with 1500 ml of chloroform and polar lipids with 1000 ml of methanol. The solvents were evaporated at reduced pressure under nitrogen at 50 C and the lipid fractions stored under nitrogen at -20 C.

Polar lipids were separated by rechromatography on 4 x 30 cm columns of activated silicic acid (10). Polar lipids (400 mg) were added to the column in 3-5 ml of chloroform: methanol (2:1, v/v). Glycolipids were eluted with 1500 ml of acetone and phospholipids with 800 ml of methanol. The rate of polar lipid fractionation on the column was monitored to the 1500 ml change by two dimensional thin layer chromatography using chloroform-methanol-7N ammonium hydroxide-water (70:25:3.5:1.5, v/v/v/v) followed by chloroform-methanol-acetic acid-water (80:15:2:0.75, v/v/v/v). The solvents were evaporated under vacuum and nitrogen at 40 C and lipids weighed before storage under nitrogen at -20 C.

The nonpolar classes of lipid were isolated by thin layer chromatography (11) on Silica Gel G (Merck, Darmstadt, W. Germany) using 20 x 34 cm plates. One gram of the nonpolar fraction was dispersed in 10 ml chloroform and several 10 µl samples were applied to the plate. The chromatograms were developed with diethyl ether: benzene: ethyl alcohol: acetic acid (40:50:2:0.2 v/v/v/v). When the solvent front reached the height of 25 cm, the plates were dried and developed further in hexane:diethyl ether (94:6 v/v) to 32 cm height to separate sterol esters and hydrocarbons from the triglycerides. Standards of each particular class of neutral lipid were included in all chromatographic plates. The developed plates were sprayed with 50% sulfuric acid for identification of glycerides and fatty acids while Liebermann-Burchard reagent was used to locate the sterols and sterol esters. Replicate chromatographic plates were placed in an iodine-saturated chamber for spot identification and, after iodine evaporation, the spots were scraped from the plates and the lipids eluted with diethyl ether. The solvent was evaporated to dryness under nitrogen and dried residues were weighed. The diethyl ether used in the present study was purified especially for chromatographic use.

For additional quantitation of the nonpolar or neutral lipids, and the determination of their fatty acid compositions, the chromatograms were sprayed with 0.1% solution

TABLE I

Weight Percentage Composition of MEAR and LEAR Rapeseed Lipids[a]

Lipid fractions	MEAR cultivar	LEAR cultivar
Nonpolar lipids	95.8 ± 0.37	95.5 ± 0.41
Triglycerides	91.9 ± 0.41	92.1 ± 0.59
Diglycerides	1.5 ± 0.20	1.2 ± 0.15
Monoglycerides	trace	trace
Free fatty acids	0.8 ± 0.17	0.5 ± 0.15
Sterol esters	1.0 ± 0.10	1.1 ± 0.12
Sterols	0.6 ± 0.15	0.6 ± 0.15
Polar lipids	4.2 ± 0.37	4.5 ± 0.41
Phospholipids	3.2 ± 0.31	3.6 ± 0.29
Glycolipids	0.9 ± 0.10	0.9 ± 0.10

[a]Extracted with chloroform-methanol.

TABLE II

Percentages of Fatty Acids in the Principal Classes of Nonpolar and Polar Lipids in MEAR and LEAR Rapeseed

Lipid fractions and cultivars	Fatty acid composition in weight % methyl ester								
	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$	$C_{20:1}$	$C_{22:1}$
Nonpolar lipids									
Triglycerides									
MEAR	3.8	0.1	1.6	39.2	20.5	9.2	---	11.7	14.9
LEAR	5.2	0.3	1.9	59.8	20.4	10.8	0.4	0.9	0.6
Diglycerides									
MEAR	17.8	0.6	5.6	42.9	16.0	3.7	---	5.3	8.0
LEAR	18.9	1.5	6.0	46.4	17.6	5.4	0.9	3.2	---
Free fatty acids									
MEAR	18.1	0.8	6.9	37.8	14.3	4.1	---	8.0	10.0
LEAR	19.7	2.8	7.3	53.5	7.8	4.8	1.0	3.1	---
Sterol esters									
MEAR	17.0	2.1	7.7	31.7	20.4	5.2	---	6.9	8.9
LEAR	22.1	2.1	7.8	41.6	19.7	5.1	1.5	---	---
Polar lipids									
Phospholipids									
MEAR	12.1	1.1	0.8	43.9	33.1	5.9	---	1.9	1.2
LEAR	14.2	1.2	0.8	47.1	31.0	5.6	---	---	---
Glycolipids									
MEAR	13.2	1.5	2.3	35.7	26.2	6.9	---	5.7	8.3
LEAR	12.7	0.8	2.1	42.3	31.4	8.3	---	1.2	1.1

of 2,7-dichlorofluorescein in methanol. After comparison with standards on the same plates, the individual fractions of lipid 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 additional 25 ml washings were filtered through a fritted disc funnel and the filtrate evaporated to dryness in a rotary evaporator under nitrogen. 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. The methylations were carried out by heating the vials in a water bath at 90 C for 4 hr. Samples (10-50 mg) of the phospholipids and glycolipids were also dissolved in the above solvent for methylation of the fatty acids. The methyl esters of the fatty acids in the nonpolar and polar lipid fractions were separated and quantitated by GLC on a Pye Unicam model 104 gas chromatograph with a hydrogen flame detector. Glass columns 210 cm x 4 mm were packed with 10% DEGS on 60-80 mesh Chromosorb W. The column temperature was maintained at 195 C with argon carrier gas flow being 60 ml/min. Peaks were identified by comparison with standards. The relative weight percent of each fatty acid, as determined from peak area, was used to calculate the proportions of nonpolar lipid fractions.

RESULTS AND DISCUSSION

The average crude fat contents of the MEAR and LEAR samples were 42.4 and 42.0%, resp., while the total lipids extracted with chloroform-methanol were 43.7 and 43.0%, resp., for the two types of rapeseed. The weight percentage yields of nonpolar and polar lipid fractions were essentially the same in both cultivars (Table I). The ratio of nonpolar to polar lipids was ca. 22:1 in these samples. Triglycerides were the predominant nonpolar fraction, averaging 92% of total lipids while diglycerides and sterol esters each constituted over 1% of the total. Free fatty acids, sterols and monoglycerides were minor components of the nonpolar fraction. The polar lipids were primarily phospholipids (3.2-3.6%), but a significant amount of glycolipid (0.9%) was also found in each oil. McKillican (5) obtained similar results with hexane-extracted oils, although the yield of triglyceride was higher (94.3%) and polar lipid content lower (1.8%) than occurred in the present study. These differences could be attributed to the lipid extraction technique used in each study.

GLC analyses of the individual fatty acids in the triglycerides showed that the MEAR samples averaged about 40% oleic, 20% linoleic, 9% linolenic, 12% eicosanoic and 15% erucic acids (Table II). The LEAR triglycerides, however, contained 60% of oleic acid and only 2 % of 20- and 22-carbon fatty acids. These results substantiate those of other investigators (1,3,5,6). The poor digestibility and utilization of HEAR oil has been ascribed both to its low level of saturated fatty acids and its high content of erucic acid (1,12). The higher level of palmitic acid in the LEAR triglycerides, as well as the lower content of long chain fatty acids, can be considered as improvements in the nutritive value of the edible oil.

Palmitic acid levels in the diglyceride, free fatty acid and sterol ester fractions were 3-5 times greater than in the triglycerides, varying between 17.0 and 22.1% of the total fatty acids (Table II). Stearic acid levels were also substantially higher than in the triglycerides, the range being 5.6-7.6% in the various nonpolar fractions and cultivars. These nonpolar lipids also contained small quantities of lauric, myristic and palmitoleic acids. As compared to the triglycerides, the other nonpolar fractions were lower in linolenic and erucic acids in both MEAR and LEAR cultivars. In total, the triglycerides were much lower in saturated fatty acids but higher in monounsaturated acids and linolenic acid than the other lipid fractions.

The phospholipids and glycolipids were intermediate in palmitic acid content (12.1-14.2%) but showed high levels of linoleic acid (26.2-33.1%) (Table II). The phospholipids were particularly low in the long chain C20:1 and C22:1 fatty acids. While the complete fractionation of the principal classes of lipids in rapeseed has not been reported in the literature, several investigators have determined the fatty acid compositions of rapeseed oil gums (3,4,5). In these studies the levels of palmitic, linoleic and erucic acids in the phospholipids were reported to be somewhat higher than was obtained in the present study.

Among all lipid classes, the MEAR and LEAR samples appeared to differ primarily in the contents of the monounsaturated C18:1, C20:1 and C22:1 fatty acids (Table II). Relative to the MEAR triglyceride composition, the LEAR triglycerides were 20% higher in C18:1 and 25% lower in long chain fatty acids. The levels of long chain fatty acids in the other MEAR lipid classes, especially the phospholipids, were generally lower than were found in the MEAR triglycerides. However, most of the LEAR lipid classes also showed increased C18:1 levels that corresponded to the lower contents of long chain fatty acids. In the diglycerides and glycolipids, the LEAR fractions were higher than the MEAR fractions in C18:2 and C18:3 as well as C18:1. There is considerable evidence that the biosynthetic pathway for the long chain fatty acids involves chain elongation by single and two stage acetate addition to C18 and C16 fatty acids (3), while the polyunsaturated fatty acids are derived from C18:1 by one or two desaturation steps.

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