Composition and Development of Turnip Rapeseed (*Brassica campestris*) Oil Triacylglycerols at Different Stages of Maturation

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Triacylglycerols (TAGs) were extracted with supercritical CO_2 or diethyl ether from low erucic acid turnip rapeseeds harvested at various stages of maturity. The acyl carbon number (ACN) species of TAGs were quantified with supercritical fluid chromatography (SFC), and the fatty acids were analyzed as methyl esters with gas chromatography–mass spectrometry. The major acids of mature seeds were *cis*-9-octadecenoic (55%), *cis,cis*-9,12-octadecadienoic (22%), *all-cis*-9,12,15-octadecatrienoic (13%), *n*-hexadecanoic (3%), *cis*-11-octadecenoic (2.5%), *n*-octadecanoic (1.5%), eicosenoic (1%), *n*-eicosanoic (0.5%), *cis*-13-docosenoic (0.5%), and *cis*-15-tetracosenoic (0.5%) acids covering 99% of the TAG fatty acids. Proportions of the molecular weight species within the most abundant ACN fractions (ACN 54, 79%; ACN 52, 10%; ACN 56, 3%) were determined by ammonia chemical ionization mass spectrometry. The dominating double-bond species in ACN 54 varied between three and seven. Proportions of the 19 fatty acids and the 18 MW species analyzed remained fairly constant during the last 3 weeks of the growing season.

INTRODUCTION

Fatty acid compositions of the seeds of various *Brassica* varieties have been widely studied during the past two decades and reviewed by Ackman (1983). Erucic acid (*cis*-13-docosenoic acid) and *cis*-11-eicosenoic acid contents are known to increase and oleic acid (*cis*-9-octadecenoic acid) content is known to decrease in the high erucic acid variety oilseed rape (*Brassica napus*) during development and maturation (Norton and Harris, 1983). This reflects the whole triacylglycerol pool in *Cruciferous* plants, where the major species have three to five double bonds per molecule and the position *sn*-2 is typically occupied by the unsaturated C_{18} acids (Brockerhoff and Yurkovski, 1966; Brockerhoff, 1971; Litchfield, 1971; Appelqvist, 1972; Zadernowski and Sosulski, 1979; Norton and Harris, 1983). In the low erucic acid varieties the level of oleic acid is increased and "the excess" will be distributed mainly into the positions *sn*-1 and *sn*-3 (Ackman, 1983).

The aim of this study was to analyze the proportions of the fatty acids, various acyl carbon number species, and molecular weight species of triacylglycerols during the ripening of low erucic acid turnip rapeseeds.

MATERIALS AND METHODS

Samples and Sample Extraction. Turnip rape (*Brassica campestris*), a double-zero variety Kova, was grown on a commercial cultivation basis in Paimio in southwest Finland. The samples (100 g of seeds) were collected manually at different stages of growth, i.e., every 3–4 days during 3 weeks prior to maturation (August 1989) making a total of seven samples. The seeds were dried to a moisture level of 5–6% and crushed in a spice mill; the oil was extracted by both Soxhlet extraction and supercritical fluid extraction (SFE) (Suprex SFE/50, Suprex Corp., Pittsburgh, PA) methods.

When the Soxhlet method was used, 2 g of milled seeds was extracted with 200 mL of diethyl ether for 4 h. In SFE the volume of the extraction cartridge was 1 mL, and an exactly known amount (about 400 mg) of crushed seeds was weighed into it. Two-hour extractions were carried out with supercritical CO_2 at 60 °C and 45 MPa. The rear end of the extraction cartridge was equipped with a pressure restrictor, 10 cm long fused silica capillary tubing (i.d. 25 μm), which was introduced as a liner into an autosampler bottle through the septum. The depressurized CO_2 escaped via an injection needle which penetrated the septum. The pressure drop resulted in the oil precipitating in the bottle. Due to the expanding CO_2 no extra cooling device was needed and the collection bottle could be kept inside the oven during the whole extraction procedure.

Aliquots of all rapeseed samples from various stages of maturity were extracted once with Soxhlet and in triplicate with SFE methods. To compare the different isolation methods, one pooled rapeseed sample was extracted in triplicate with both Soxhlet and SFE.

All extracts were diluted with chloroform to a concentration of 6 mg of oil in 1 mL of $CHCl_3$ for triacylglycerol analyses by SFC.

Supercritical Fluid Chromatography of Triacylglycerols. The TAGs were analyzed with supercritical fluid chromatography (SFC) (Suprex SFC/200A, Suprex). Samples were injected with a Valco 4-port injector (Valco Instruments Co. Inc., Houston, TX) equipped with a 1.0-μL sample loop. The column (100 mm, i.d. 1.0 mm) was a packed Nucleosil C_{18} column (Keystone Scientific Inc., State College, PA) with 5-μm particle size. CO_2 with helium backpressure in the tank was used as the mobile phase. The flame ionization detector (FID) was connected to a Shimadzu C-R3A Chromatopac integrator (Shimadzu Corp., Kyoto, Japan).

The temperature of the FID was 385 °C and that of the oven 150 °C. The pressure was programmed from 10 to 45 MPa at a rate of 1 MPa/min. An integral-type pressure restrictor connected to the end of the column was used (Guthrie and Schwartz, 1986).

Each extract was analyzed twice with SFC, and the proportions of the seven major peaks were calculated. Each peak was a mixture of TAGs of one acyl carbon number (ACN).

Retention indices of the SFC peaks were determined by co-injecting a sample with a standard mixture of saturated, even-number, monoacid TAGs. The retention index of each standard TAG was defined as ACN multiplied by 100.

Gas Chromatography of Fatty Acids. The TAG extracts were purified by silicic acid column chromatography (Carroll and Serdarevitch, 1967). The methyl esters of the TAG fatty acids (FA) were prepared by NaOH saponification followed by BF_3/CH_3OH treatment according to the method of Metcalfe et al. (1966) and further developed according to the procedure of

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Table I. Extraction Yields, Standard Deviations (SD), and Coefficients of Variation (CV) in Soxhlet and SFE Extractions of Turnip Rapeseeds

extraction method	yield, %	SD	CV, %
Soxhlet	38.9		
	39.3	1.1	2.8
	37.3		
SFE	33.8		
	32.7	1.0	3.0
	31.8		

van Wijngaarden (1967). The dried hexane extracts were stored concentrated at −18 °C under nitrogen until analyzed.

Analysis of the fatty acid methyl esters was carried out by using a Varian 3300 gas chromatograph (Limerick, Ireland) equipped with a fused silica capillary column (NB-351, 25 m, i.d. 0.32 mm, d_f 0.20 μm; Nordion, Helsinki, Finland), a flame ionization detector, and a Shimadzu C-R3A integrator. The split exit of the injector (225 °C) was kept closed for 15 s after injection. The temperature program was 2 min at 70 °C, 3 °C/min to 230 °C, and 20 min isothermal. The detector temperature was 240 °C.

Retention indices (I_r) of fatty acid methyl esters were determined by co-injecting *n*-alkanes, from nonadecane to tetratriacontane, with the sample. Known amounts of *n*-alkanes (C_{22}, C_{23}, C_{25}, and C_{30}) were used as internal standards to quantify the ratios of the fatty acid methyl esters. Retention indices of commercial reference compounds, their mass spectra, and library mass spectra were used to verify the structures of the fatty acid methyl esters.

Mass Spectrometry. The fatty acid methyl esters were analyzed by a VG 7070E double-focusing mass spectrometer and a VG11-250 data handling system (Wythenshave, U.K.). The same column and temperature program as in the GC analysis were used. The temperature of the ion source (EI mode) was 280 °C and the ionization energy 70 eV.

Identification and quantitative analysis of the molecular weight species (degree of unsaturation) of the TAGs were performed by desorption chemical ionization (DCI), using ammonia as the reagent gas. The method is a modification of that of Murata and Takahashi (1977). The positive ion distribution was recorded after 6-kV acceleration in full scan mode at the resolution of 1000 (10% valley). The speeds of scans and interscan times were set to 2 s/decade and 0.5 s, respectively. The temperature of the ion source was kept constant at 180 °C. The pressure of the vacuum chamber measured above the diffusion pump in CI conditions was kept at 1.4×10^{-4} Pa. This was 10% higher than the value detected for proper plasma creation. The electron energy to create the CI plasma was 100 eV. The current through the coil-shaped platinum wire of the DCI probe was programmed to increase from 200 mA at the speed of 3.2 mA/s (2 °C/s).

The mass fragmentograms of the quasimolecular ions m/z $(M + 18)^+$ were plotted from the spectra. The results were corrected according to the varying intensities of $(M + 18)^+$ due to the size and saturation levels of the TAGs. The standard compounds (Sigma) for the molecular size corrections were 1,2,3-tri(tridecanoyl)glycerol, 1,2,3-tri(heptadecanoyl)glycerol, and 1,2,3-tri(nonadecanoyl)glycerol, which were added to an authentic turnip rapeseed oil sample and analyzed at the same time with the seed TAGs. The effect of the number of double bonds on the formation of $(M + 18)^+$ ions was verified by using 1,2,3-tri(octadecanoyl)-glycerol, 1,2-di(octadecanoyl)-3-(*cis*-9-octadecenoyl)-*rac*-glycerol, 1,3-di(*cis*-9-octadecenoyl)-2-octadecanoyl-*rac*-glycerol, 1,2,3-tri(*cis*-9-octadecenoyl)-*rac*-glycerol, and 1,2,3-tri(*cis*,*cis*-9,12-octadecadienoyl)glycerol as reference compounds. The overlapping ^{13}C isotope species were subtracted from the $(M + 18)^+$ ions.

RESULTS AND DISCUSSION

Isolation and Fractionation of the TAGs. The extraction yields of the Soxhlet and SFE methods are listed in Table I. The standard deviations and coefficients of variation show that both procedures were acceptably reproducible.

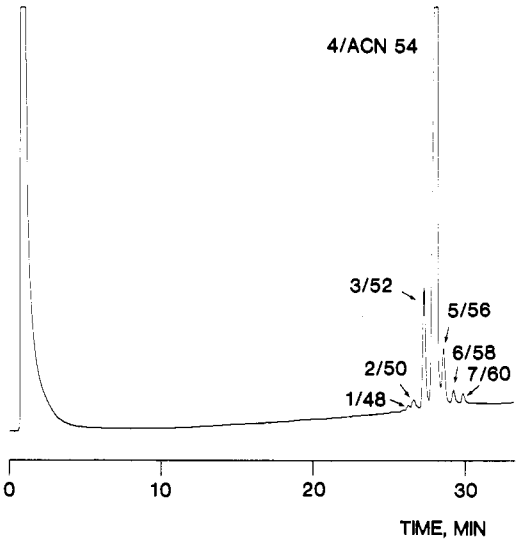

Figure 1. SFC chromatogram (C_{18} column, 100 × 1 mm) of triacylglycerols of turnip rapeseed oil harvested on August 30 and extracted by supercritical CO_2 (isothermal at 150 °C, linear pressure gradient from 10 to 45 MPa, 1 MPa/min). Each peak is numbered at elution order/acyl carbon number.

Table II. Retention Indices (I_r) and Distribution of the Seven TAG Fragments of Turnip Rapeseed Oil (August 30), Standard Deviations (SD), and Coefficients of Variation (CV)

extraction method	peak	I_r	%	SD	CV, %
Soxhlet	1	4800	0.3	0.03	10.0
	2	4950	1.1	0.1	9.1
	3	5130	9.7	0.2	2.1
	4	5330	78.1	0.5	0.6
	5	5490	5.3	0.04	0.8
	6	5700	2.0	0.1	5.0
	7	6000	3.4	0.6	17.6
SFE	1	4800	0.3	0.02	6.7
	2	4950	1.1	0.02	1.8
	3	5130	9.5	0.3	3.2
	4	5330	79.3	0.5	0.6
	5	5490	5.4	0.1	1.9
	6	5700	2.0	0.1	5.0
	7	6000	2.5	0.6	24.0

It is common knowledge that the yield of total lipids in Soxhlet extraction with diethyl ether is almost quantitative, and in our experiment it exceeded the yield of SFE by 15%. The reasons for this can be many. The particle size and shape of the material to be extracted have significant effects on the efficiency of the SFE (Snyder et al., 1984). The larger the particles or the smaller the surface area, the lower the extraction yield. The thinner the particle, the better the CO_2 diffusion and, as a result, the better the yield. Collection of the extract is always a problem in SFE. At this stage of the work some loss may have occurred. All of the oil was possibly not precipitated in the bottle but some was flushed out with gaseous CO_2. Also, the amount of CO_2 used in extraction affects the yield. The more CO_2, the better the yield (Snyder et al., 1984).

Diethyl ether is a more polar solvent than CO_2 at the conditions applied, and the fractions collected by SFC contained smaller amounts of polar lipids than the ether extracts.

The extraction methods were also compared by using SFC analysis. Figure 1 shows a chromatogram of a CO_2 extract of mature seeds harvested on August 30, 1989. Table II summarizes the proportions, standard deviations, and retention indices of the seven chromatogram peaks

Table III. Distribution (mol %) of Fatty Acids in Turnip Rapeseed Oil at Varying Stages of Maturity

			date of collection						
no.	I_r	FA	Aug 7	Aug 10	Aug 14	Aug 17	Aug 21	Aug 24	Aug 30
1	2000	14:0	0.1	0.0	0.1	0.1	0.0	0.1	0.1
2	2100	15:0	0.1	0.1	0.1	0.1	0.1	0.1	0.1
3	2209	16:0	3.5	3.5	3.4	3.2	3.3	3.5	3.3
4	2234	16:1ω9	0.2	0.2	0.2	0.2	0.2	0.2	0.2
5	2313	17:0	0.1	0.0	0.0	0.1	0.0	0.0	0.1
6	2419	18:0	1.6	1.5	1.5	1.4	1.5	1.4	1.5
7	2439	18:1ω9	52.0	53.5	55.3	55.4	56.2	55.0	54.6
8	2445	18:1ω7	3.4	3.1	2.7	2.5	2.6	2.5	2.4
9	2485	18:2ω6	22.6	22.0	21.5	21.7	21.3	22.2	21.8
10	2554	18:3ω3	13.0	12.4	12.4	12.6	12.6	12.7	12.5
11	2630	20:0	0.5	0.4	0.4	0.4	0.4	0.4	0.4
12	2651	20:1[a]	1.0	1.1	1.0	0.9	0.9	0.9	1.1
13	2706	20:2ω6	0.0	0.1	0.1	0.1	0.1	0.0	0.1
14	2775	20:3ω3	0.2	0.2	0.2	0.2	0.1	0.2	0.2
15	2849	22:0	0.2	0.2	0.2	0.2	0.2	0.2	0.2
16	2868	22:1ω9	0.3	0.6	0.2	0.1	0.2	0.2	0.5
17	3039	22:5ω3[b]	0.2	0.1	0.1	0.1	0.1	0.1	0.1
18	3059	24:0	0.3	0.2	0.2	0.2	0.2	0.2	0.2
19	3090	24:1ω9[b]	0.0	0.0	0.0	0.0	0.0	0.2	0.5

[a] Possibly ω9 isomer. [b] Tentative identification.

measured, i.e., the ACN species of the TAGs. The percentages of the ACN groups were identical with both isolation methods, and standard deviations and coefficients of variation remained a reasonable level. The resolution between the ACN species was sufficient but less than that of the GC analysis of Monseigny et al. (1979) due to the packed, low-resolution SFC columns used in our work.

The retention indices of the fractions presented in Table II deviate from the retention characteristics of the saturated monoacid TAGs used as reference compounds. This is due to the high number of unsaturated fatty acids (at least up to seven double bonds in the TAGs according to the fatty acid analysis) in rapeseed oil. Triacylglycerols of the low erucic acid variety Candle of *B. campestris* are known to contain about 3% fatty acids with 16 carbon atoms, about 95% with 18 carbon atoms, and about 2% with 20 carbon atoms (Sebedio and Ackman, 1981). These numbers are in good agreement with the SFC results on the variety Kova (4%, 93%, and 2%, respectively), shown in Table III. Figure 1 also shows that the acyl carbon numbers (ACN) of the three largest peaks were 54, 52, and 56. The carbon numbers of the four smaller peaks were 48, 50, 58, and 60, which was also confirmed by the mass spectral results.

Figures 2 and 3 show the variations in the proportions of the seven ACN–TAG groups during the last month of the growth period. Figures 2a and 3a represent Soxhlet extractions and Figures 2b and 3b SFE. The percentages of the three dominating fractions remained quite constant during August, and no statistically significant changes were recognized. The irregularities in the ACN species 58 and 60 (Figure 3) might be connected to the deviation of erucic acid (22:1ω9) shown in Table III. However, no detailed study was carried out because of the minor practical importance of these ACN species in the variety Kova. As a whole the variations between the seven ACN clusters were extremely small. SFC did not give any direct information concerning the possible changes in the unsaturation levels of the fatty acids and TAGs within the ACN fractions.

Fatty Acids. The 19 most abundant TAG fatty acids identified and quantified during the season are listed in Table III. Structure elucidation of some of the minor FAs was tentative. Concentrations of any other FAs detected were below 0.1% level, except one unknown which showed

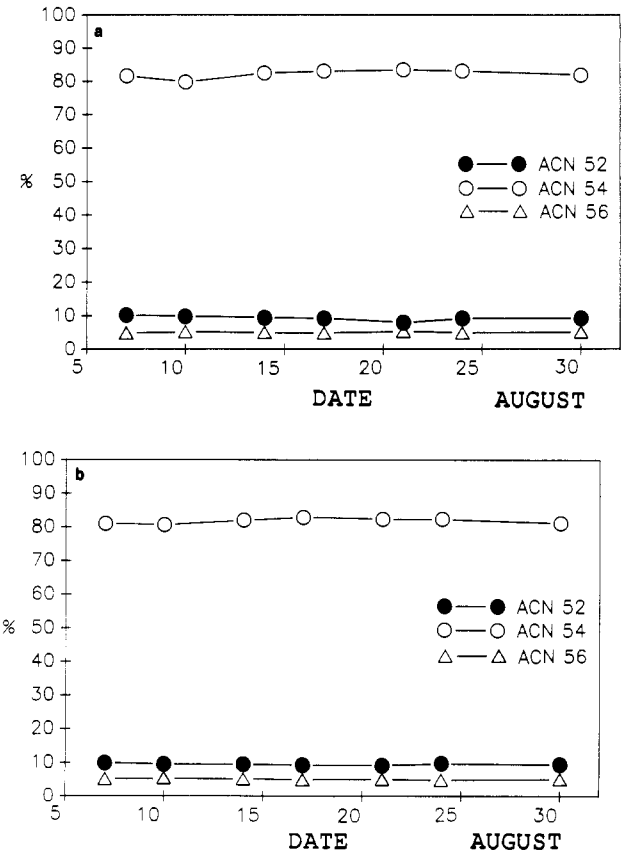

Figure 2. Development of the three major ACN fractions (54, 52, and 56) of turnip rapeseed oil separated by SFC: (a) Soxhlet extraction with diethyl ether; (b) supercritical fluid extraction with CO_2.

I_r value of 1934 and 0.1–0.3% abundance. The most abundant fatty acid in every sample was *cis*-9-octadecenoic acid (oleic, 18:1ω9), which comprised 52–56% of the total FA pool, followed by *cis,cis*-9,12-octadecadienoic (linoleic, 18:2ω6, 21–23%) and *all-cis*-9,12,15-octadecatrienoic acid (linolenic, 18:3ω3, 12–13%). The other fatty acids exceeding 0.5% proportion were hexadecanoic (palmitic, 16:0), *cis*-11-octadecenoic (18:1ω7), octadecanoic (stearic, 18:0), eicosenoic (probably ω9 species), eicosanoic (20:0), *cis*-13-docosenoic acid (erucic acid, 22:1ω9), and *cis*-15-tetracosenoic acid (24:1ω9). There were no clear exceptions when compared to the earlier corresponding studies summarized by Ackman (1983).

The cultivar Kova is known to be a low erucic acid variety, and this is also seen in Table III. The variation in the total proportion of 22:1ω9 acid was analogous with SFC fraction 6 (ACN 58) in Figure 3 as expected. Both showed slight maxima at August 10 and 30, and the proportions were statistically highly significantly higher than the corresponding values of other days. In the high erucic acid variety rapeseed oils the content of 22:1ω9 acid is known to increase during maturing of the seeds, which affects the composition as well as distribution of the fatty acids in the TAGs (Norton and Harris, 1983).

The fatty acid pattern in Table III supported the identification of the ACN fractions shown in Figure 1 and Table II. The most abundant combinations had acyl carbon numbers 54 followed by 52 and 56 regardless of the nonrandom distribution of fatty acids in the TAGs.

Molecular Weight Distribution of Triacylglycerols. Intensities of the $(M+18)^+$ ions of TAGs in ammonia CI-MS analysis are affected by the ion source conditions (Murata and Takahashi, 1977; Schulte et al., 1981; Kuksis

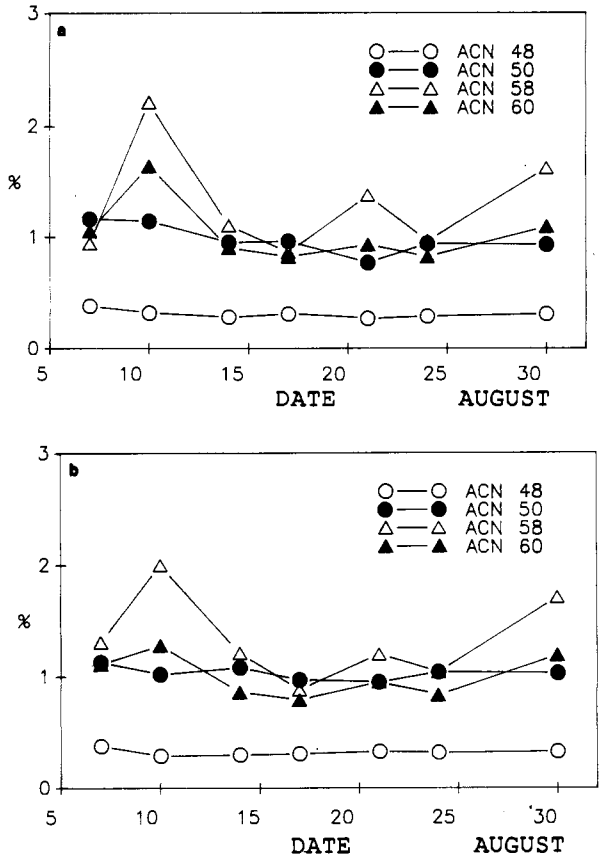

Figure 3. Development of the four minor ACN fractions (48, 50, 58, and 60) of turnip rapeseed oil separated by SFC: (a) Soxhlet extraction with diethyl ether; (b) supercritical fluid extraction with CO_2.

Table IV. Mass Spectrometry Correction Factors of TAGs Varying in Chain Length and Degree of Unsaturation

no. of double bonds	acyl carbon no. 52	54	56
0	0.550	0.480	0.410
1	0.509	0.444	0.379
2	0.440	0.384	0.328
3	0.366	0.319	0.273
4	0.294	0.257	0.219
5	0.223	0.194	0.166
6	0.151	0.132	0.113
7	0.080	0.070	0.059

et al., 1983). This is why a highly constant temperature gradient and pressure in the source are required for quantitative analysis. The regression equation used to correct the effect of varying molecular weight is

$$y = -0.1043x + 2.3600 \ (r = -0.994)$$

where x is ACN in the saturated monoacid TAGs and y the relative response of $(M + 18)^+$ ions, with the intensity of m/z 698.6, the $(M + 18)^+$ ion of TAG 3 × 13:0 being 1.0. The corresponding regression equation applied to correct the effect of varying unsaturation levels of TAGs is

$$y = -0.1301x + 1.0562 \ (r = -0.988)$$

where x is the number of double bonds and y the relative response of $(M + 18)^+$, with the intensity of m/z 908.7, $(M + 18)^+$ of 3 × 18:0 being 1.0. Schulte et al. (1981) claimed that the intensity of the QM^+ ion was almost independent of the number of double bonds in the MS under the conditions they used.

A correction factor table (Table IV) was calculated on the basis of both correction equations. The positions of double bonds within the fatty acids and the position isomers of TAGs are known to affect the MS fragmentation pattern of TAGs (Kuksis et al., 1985). However, the accuracy achieved by using Table IV is better than no corrections at all. The less abundant TAG species were omitted from Table IV because of lack of proper reference compounds.

Table V. Corrected Distribution of Various MW Species of Turnip Rapeseed Oil Analyzed by Ammonia CI Mass Spectrometry

acyl carbon no./ double-bond no.	distribution, %, for date Aug 7	Aug 10	Aug 14	Aug 17	Aug 21	Aug 24	Aug 30
52/0	2.0	1.8	1.8	1.9	2.1	1.9	2.0
52/1	1.1	1.2	1.0	1.2	1.3	1.3	1.3
52/2	2.3	2.6	2.5	2.4	2.4	2.5	2.4
52/3	2.3	2.3	2.2	2.1	2.1	2.2	2.2
52/4	2.0	2.2	2.0	1.9	1.9	2.0	2.0
54/0	0.1	0.1	0.1	0.1	0.1	0.1	0.1
54/1	0.3	0.2	0.2	0.0	0.1	0.1	0.1
54/2	2.0	2.0	2.0	1.7	1.7	1.6	1.7
54/3	18.9	19.4	20.1	18.9	19.3	18.6	17.8
54/4	18.4	18.3	18.7	18.3	17.6	18.5	17.5
54/5	19.1	20.1	20.7	21.3	21.0	21.0	21.1
54/6	12.7	14.8	14.6	15.3	15.0	15.5	15.0
54/7	14.8	11.5	11.0	12.2	12.8	12.4	13.4
56/1	0.2	0.1	0.0	0.0	0.0	0.1	0.0
56/2	1.0	0.5	0.5	0.4	0.3	0.3	0.4
56/3	1.1	1.1	1.1	0.8	0.9	0.7	1.0
56/4	0.9	1.0	0.9	0.8	0.8	0.7	1.0
56/5	0.6	0.8	0.6	0.7	0.7	0.5	0.9

The $(M + 18)^+$ ions were recorded of the TAG species isolated from the oilseeds harvested during the last 3 weeks of the growing period of the plant. The proportions of the various molecular weight species were corrected according to the varying chain lengths, number of double bonds, and overlapping ^{13}C isotope fragments, and the results are presented in Table V.

As clearly shown (Table V), in a low erucic acid turnip rape variety such as Kova, the chemical composition of the TAG fraction remains extremely constant during the final weeks of the growing season. This trend was even predictable, because the highest changes in the fatty acid balance in the developing *Brassica* seeds are known to be caused by the drastic increase in the total content of erucic acid (Norton and Harris, 1983), but the highly constant composition was a surprise. The results are fairly congruent with the SFC measurements shown in Figures 2 and 3.

Only the three major ACN species were analyzed by mass spectrometry because they comprised more than 95% of the total TAG pool. Five of the MW species were in abundance, i.e., those having ACN 54 and three to seven double bonds. They evidently consisted mainly of the 18:0, 18:1ω9 (+18:1ω7), 18:2ω6, and 18:3ω3 species, the more close structural analysis of which is in progress by tandem mass spectrometry (MS-MS).

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