Lipid Class Separation by HPLC Combined with GC FA Analysis: Comparison of Seed Lipid Compositions from Different *Brassica napus* L. Varieties

Christopher Beermann*, Angelika Green, Michael Möbius, Joachim J. Schmitt, and Günther Boehm

Numico Research Germany, D-61381 Friedrichsdorf, Germany

ABSTRACT: A reliable HPLC method was established to evaluate the lipid composition of useful plants modified by breeding techniques. This study focused on the polar lipid distribution and polar lipid FA compositions of four rapeseed varieties. Structure and quantity of the distinct lipid classes were compared by HPLC using ELSD followed by a GC FA analysis. A baseline separation of 14 lipid classes could be achieved within one step by using an eluent gradient of hexane, *tert*-methylbutyl ether, isopropanol, acetonitrile, chloroform, triethylamine, acetic acid, and water supplemented with ammonium sulfate with a polyvinyl alcohol column. After automatic fractionation, the FA compositions of the distinct lipid classes were characterized by a subsequent complementary GC FA analysis through direct acetylchloride methylation. The rape varieties analyzed showed diversity in polar lipid content and distribution, dominated by PC, PE, PI, monoglycosyldiacylglycerols, and phytosterols. Extensive variations were detected in FA within the lipid classes of rape varieties with predominantly oleic acid, linoleic acid, and α-linolenic acid observed followed by palmitic acid and gondoic acid. Oleic acid was mainly connected to PC and linoleic acid to PE, whereas α-linolenic acid and γ-linolenic acid were predominantly linked to PI in all varieties.

Paper no. J10381 in *JAOCS 80*, 747–753 (August 2003).

KEY WORDS: Fatty acids, light scattering detection, lipid class separation, quantification, rapeseed lipids.

The effort to refine vegetable sources for dietary, cosmetic, or technical applications often requires compositional modification of plant constituents. Many of those modifications refer to the FA composition of vegetable oil sources. One of the most common sources for traditional and modified vegetable oils is the rape plant (*Brassica* sp.). In Central Europe both *Brassica napus* L. and *B. rapa* L. are grown extensively and have even become two of the most important oilseeds worldwide. Several studies have been conducted to alter the pattern of protein and fat metabolism of seed oil from *B. napus* L. by traditional breeding techniques as well as by genetic modifications such as antisense expression (1,2) and gene transformation (3–6). The originally dark yellow oil of the unmodified rapeseed is dominated by erucic acid glycerides (22:1n-9), followed by oleic acid (18:1n-9) and linoleic acid (18:2n-6) (7). However, erucic acid and seed glucosinolates can cause adverse health effects and negatively affect the taste of this vegetable oil for dietary use. Therefore, both plant components have been eliminated by conventional plant breeding techniques to create dietary rapeseed oil, which is low in erucic acid (0-Rape) as well as seed glucosinolates (00-Rape). The substances have been replaced by oleic acid and linoleic acid (7). These new oil varieties are now mainly used as edible vegetable oils and as raw material for margarine formulations. In addition to the nutritive value of the oil as a caloric source, other possible applications of lipids and FA are attractive. Those alternative applications are basically related to the molecular structure of the polar lipids, because polar lipids and their physiological properties are important for emulsification applications and for dietary supplements (8). Several lipid class separations by HPLC have been done for vegetable oils and animal fats using silica (9), cyanopropyl (10), diol (11), or polyvinyl alcohol chemically bonded stationary phases (12) to characterize the composition of the corresponding raw materials in detail. Previous studies have demonstrated that the properties of an ELSD are useful for a quantitative HPLC detection of lipid classes within different polarity classes (13). This detection method has been described as highly sensitive for neutral lipids and phospholipids, with an acceptable reproducibility to enable quantification (14,15). A clear benefit of this technique is the possibility of detecting lipophilic substances independent of the absorption properties of eluents or samples and without any probe labeling of detected molecules (16). The signal intensity of an ELSD seems to be dependent on the development of droplets of eluents by the nebulizer/evaporator, as well as on the detector temperature. Thus, the nebulizer/evaporator parameters and detector temperature have to be optimized for an adequate detection and quantification of lipids of different polarities derived from plants within a wide concentration range.

The current study establishes an improved HPLC method for the baseline separation and quantification of both simple and complex lipids in one continuous analysis process. After automatic HPLC fractionation, a direct-sample methylation with acetylchloride is provided for an immediate FAME analysis by GC within one operation sequence to characterize

*To whom correspondence should be addressed.
E-mail: milupa.research@T-online.de

the distinct FA pattern of individual lipid classes. A complex triple eluent gradient using a polyvinyl alcohol stationary phase is utilized for the separation of a wide range of lipid classes varying in polarity between sterol esters and lysophosphatidylcholine.

By focusing on the lipid class distribution and FA profiles, the current study compares four *B. napus* L. variations with different genetic backgrounds (winter and summer rape, low sinapine rape, and high oleic acid rape). The combination of an improved HPLC method for separation and quantification of complex plant lipids with direct methylation and GC FAME analysis to determine their FA patterns provides a rapid technique to evaluate plant breeding or molecular-genetic technical modifications of the total lipid composition of seeds.

MATERIALS AND METHODS

Rapeseed varieties. Winter oilseed rape *Low sinapin-NPZ*; winter oilseed rape oleic acid *HO-NPZ*; yellow seed spring oilseed rape-*DSV*, and yellow seed summer rape *RAMSCH-DSV* were obtained from the Norddeutsche Pflanzenzucht, Hans-Georg Lembke KG (NPZ, Hohenlieth, Germany), and the Deutsche Saatgutveredlung (DSV, Lippstadt, Germany).

Total lipid extraction and solid phase extraction (SPE) of polar lipids. Seeds (10 g) were ground with an M 20 labor mill M (IKA Labortechik, Staufen, Germany) and the total lipids were extracted with chloroform/methanol/water (10:10:9, by vol) according to Bligh and Dyer (17). Because rapeseed lipids are dominated by MAG, DAG, and TAG as well as FFA, the glyco- and phospholipids were purified with SPE on aminopropyl cartridges by sequential elution. The total lipid extract was dissolved in chloroform to a maximal concentration of 40 mg/mL. The neutral lipids and polar lipids were separated using an aminopropyl-silica column (ISOLUTE SPE; ITC, Bad Homburg, Germany) with 3 mL lipids/500 mg column material. The fractionation process was as follows: (i) The column was conditioned with 4 mL *n*-hexane, (ii) a 2-mL sample aliquot was applied onto the column, (iii) neutral lipids were eluted with 10 mL chloroform/isopropanol (2:1, vol/vol), (iv) FFA were eluted with 6 mL 2% acetic acid in diethyl ether, and (v) polar lipids were eluted with 6 mL methanol/chloroform/water (10:5:4, by vol). The separated fractions were dried under a stream of nitrogen. The recovery rate for total phospholipids was ≥96%.

Lipid class separation and fractionation by HPLC. The rapeseed lipids were separated by HPLC and fractionated *via* automatic fractionation. An aliquot of the lipid extract was dissolved in chloroform/methanol (2:1, vol/vol) to a concentration of 0.5–2.5 mg/mL.

Lipid class separation was completed with an HPLC Alliance 2690 separation module from Waters (Waters GmbH, Eschborn, Germany) coupled with a PL-ELS 1000 ELSD system (Polymer Laboratories, Darmstadt, Germany). Detection was established at 100°C for the nebulizer and at 100°C for the evaporator. For lipid class separation, a polyvinyl alcohol chemically bonded stationary-phase PVA Sil column (5 μm, 250 × 4 mm; YMC Europe, Schermbeck, Germany) was used. The eluent system followed that of Christie (9): eluent A: *n*-hexane/*tert*-methylbutyl ether (98:2, vol/vol); eluent B: isopropanol/acetonitrile/chloroform/acetic acid (84:8:8:0.025, by vol); eluent C: isopropanol/water/triethylamine (50:50:0.2, vol/vol) plus 1 mM ammonium sulfate (Sigma Chemical, St. Louis, MO) modified according to Guan *et al.* (18).

All solvents used were of HPLC grade (Merck Eurolab, Darmstadt, Germany). The solvent gradient system was as follows: 0–1 min A/B/C (%) 100:0:0; 1–5 min A/B/C (%) 80:20:0; 5–20 min A/B/C (%) 42:52:6; 20–27 min A/B/C (%) 32:52:16; 27–30 min A/B/C 30:54:16; 30–33 min A/B/C (%) 30:70:0; 33–40 min A/B/C (%) 100:0:0. The flow rate was 1 mL/min.

Distinct lipid classes were collected by peak signal recognition with an automatic fraction sampler from Waters. Fractions were collected in 5-mL lockable glass tubes.

Quantification of lipid classes by HPLC. Stock solutions of 1 mg/mL of lipid standards were prepared in chloroform/methanol (1:1, vol/vol); response factors were determined by measuring several dilutions of standards (0.025–1.5 mg/mL) and 5 α-cholestane (5 μg/mL) as an internal standard to quantify the separated lipid components. The following lipid standards were used: steryl oleate (SE), TAG/tripentadecanoin (TG), stigmasterol/sitosterol (Sterol), sulfoquinovosyldiacylglycerol (SQDG), dehydroxycerebroside (DHCer), cerebroside (Cer), digalactosyldiacylglycerol (DGDG), PE-dipalmitoyl (PE), PA-diheptadecanoyl (PA), PI, PS, PC-diheptadecanoyl (PC), sphingomyelin (SPH), and lysophosphatidylcholine (LPC) (Sigma Chemical).

Injections (20 μL) contained 0.5–30 μg lipid. The calibration curves contained five points representing two replicate injections. The concentration equation was calculated with the Millenium^{32} Chromatography Documentation software package (Waters GmbH).

FAME analysis by capillary GC. The fractions separated by HPLC fractionation were flushed with nitrogen to remove the solvents. For the derivatization, samples were dissolved in 2 mL methanol/hexane (4:1, vol/vol) plus 0.5% pyrogallol and were methylated according to Lepage and Roy (19) with 200 μL acetylchloride at 100°C for 1 h; 5 mL 6% K_2CO_3 was added and the mixture was centrifuged for 10 min at 2200 × *g*. The upper hexane phase containing FAME was removed and dried with Na_2SO_4.

FAME were analyzed by capillary GC on a Varian Chrompack LS 32 (Varian Chrompack, Middelburg, The Netherlands) fitted with a cold split/splitless injector to prevent FA discrimination, as described previously by Kohn *et al.* (20). A chemically bonded 50% cyanopropyl-methylpolysiloxane capillary column DB23, 40 m Fisons (Varian Chrompack), was used for the separation of FA species. The chromatographic conditions were as follows: injector: programmable temperature vaporization (PTV): 65 to 270°C, split ratio 15:1; carrier gas flow: helium at a 40 cm/s flow. Signals were identified by an FID at 280°C. FA were identified

according to their retention times. The split was time-programmed for the solvent split injection mode, thus amplifying the signal-to-noise ratio: After sample injection, the split was opened for 90 s to omit the solvent signal. The split was closed again and the temperature program was started. The column oven temperatures were as follows: initial temperature: 60°C for 0.1 min; from 60 to 180°C at 40°C/min; 180°C for 2 min; from 180 to 210°C at 2°C/min; 210°C for 3 min; from 210 to 240°C at 3°C/min; 240°C for 10 min.

RESULTS AND DISCUSSION

A reliable HPLC method was established to evaluate the lipid composition of useful plants modified by breeding techniques. The aim of this study was to establish a sequential procedure whereby the lipid classes of plant seeds could be separated, quantified, and their FA pattern analyzed. An improved HPLC method was developed with a subsequent GC FA analysis to compare the polar lipid distribution and polar lipid FA composition of four rapeseed varieties with different genetic backgrounds. This method allowed a baseline separation of all investigated lipid classes with symmetrical peaks of 14 lipid standards covering a polarity range between steryl esters, TAG, and several glyco- and phospholipids in one operation sequence within 40 min (Fig. 1). The eluent system used in this application was established as described previously by Christie (9) and performed on a chemically bonded polyvinyl alcohol stationary phase. Gradient and retention times were changed to establish a precise lipid class separation. The separation gradient was developed over 33 min, with an additional 7 min to reactivate the column before the next analysis (deviation of retention time ±0.2 min). In addition, the water eluent was supplemented with 1 mM ammonium sulfate according to Guan *et al.* (18) to prevent column degeneration and improve reproducibility. The flow rate remained constant at 1 mL per minute. This gradient system is likewise suitable for the quantitative analysis of major lipid classes of plant materials. The detection of HPLC separation was established by the ELSD technique with the possibility of quantifying the distinct lipid classes. Previous studies have demonstrated that the properties of evaporative light scattering are useful for quantitative HPLC detection of different lipid classes with different polarities (13). Detection is highly sensitive for neutral lipids and phospholipids, with an acceptable reproducibility (signal-to-baseline distance ±5%) to enable quantification, regardless of the absorption properties of eluents and sample molecules (14–16). Despite the considerable increase in polarity of the eluent, the detector baseline remained stable, which is a typical feature of this type of detection (16). The calibration curves and equations for the lipids presented in Figure 2 indicated that the detector response was quadratic. Corresponding to the literature (14–16), the detector response intensities of the distinct lipid classes were markedly different. PA had an especially low response but Sterol, TG, and phospholipids had strong signals. The amount of saturation of FA did not affect detection by influencing the dispersion properties of lipids (data not shown). It has been discussed that for ELSD, the signal intensity depends on the development of droplets in the nebulizer/evaporator. The polarity of lipids, the presence of carbohydrates,

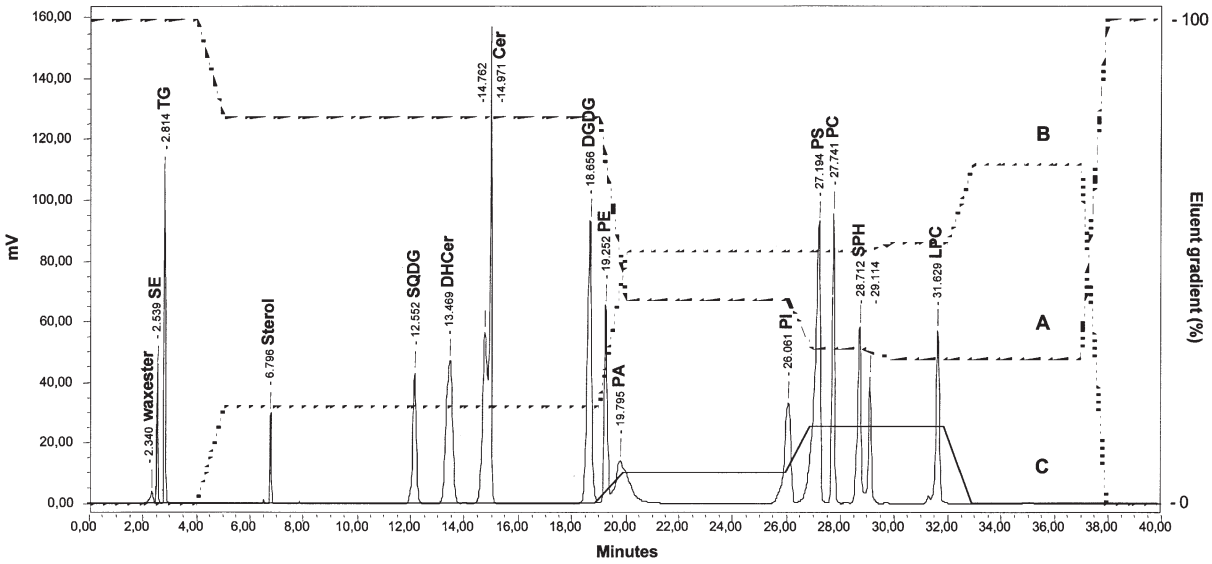

FIG. 1. HPLC separation of a lipid standard mixture: steryl oleate (SE), TAG/tripentadecanoin (TG), stigmasterol/sitosterol (Sterol), sulfoquinovosyldiacylglycerol (SQDG), dehydroxycerebroside (DHCer), cerebroside (Cer), digalactosyldiacylglycerol (DGDG), PE-dipalmitoyl (PE), PA-diheptadecanoyl (PA), PI, PS, PC-diheptadecanoyl (PC), sphingomyelin (SPH), and lysophosphatidylcholine (LPC). For separation, a polyvinyl alcohol chemically bonded stationary phase was used. Eluent gradient A: *n*-hexane/*tert*-methylbutyl ether (98:2, vol/vol); B: isopropanol/acetonitrile/chloroform/acetic acid (84:8:8:0.025, by vol); C: isopropanol/water/triethylamine (50:50:0.2, by vol), plus 5 mM ammonium sulfate. The samples were measured by ELSD.

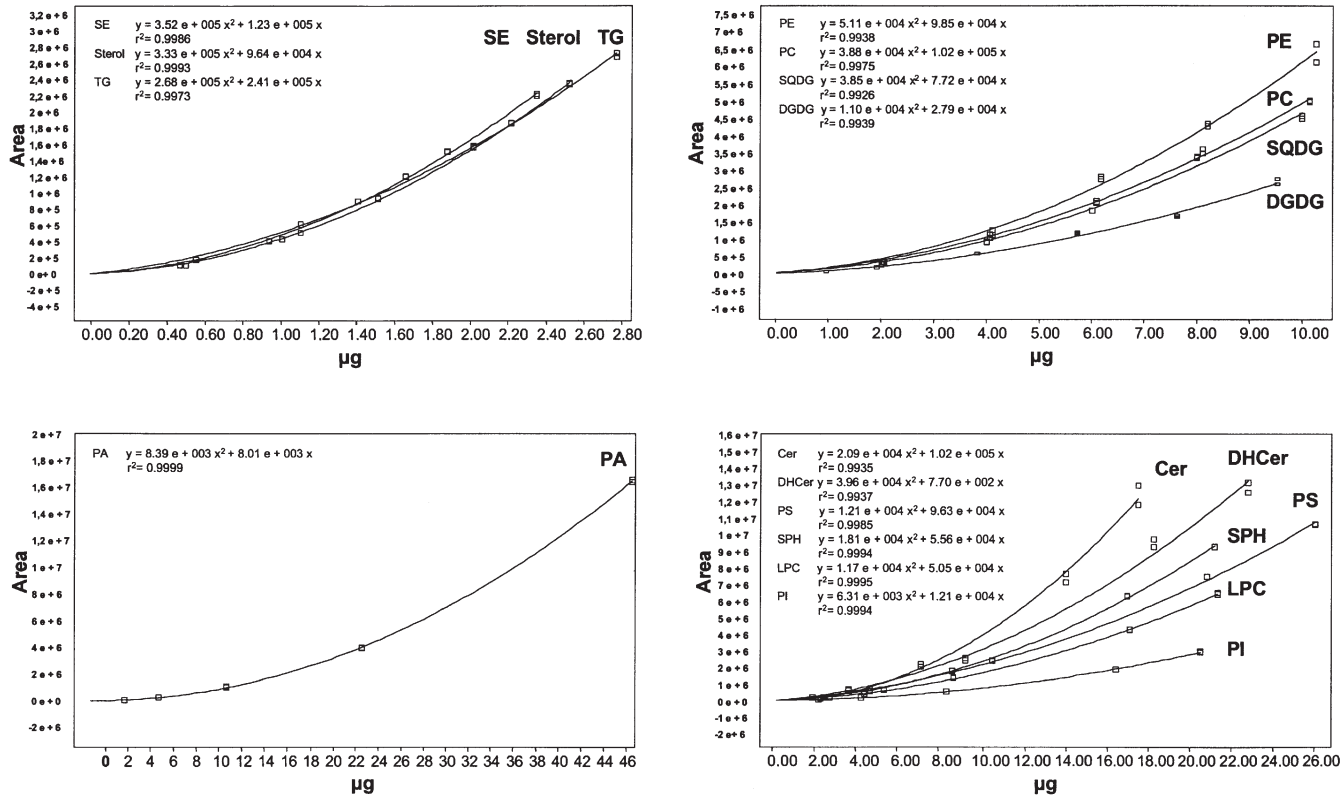

FIG. 2. Calibration curves and equations for the lipid standards. The different response factors were determined by measuring several dilutions of standards (0.025–1.5 mg/mL). See Figure 1 for abbreviations.

and the detector temperature may influence droplet development and size and therefore their recognition. For this work, the nebulizer/evaporator and detector temperatures were optimized to detect lipids derived from plants. The measurement of the standard mixture by different detector temperatures indicated that the signal intensity was maximal at 100°C (data not shown).

The total fat content of all seeds, determined gravimetrically, was between 32 and 40%, in accordance with the literature (21). The rapeseed oil of all four varieties was dominated by TG, which represented 98% of total lipid extract (Table 1). As a consequence, quantification of the polar lipid classes required purification of the total lipid extract with aminopropyl cartridges by SPE. After SPE, the main rapeseed lipid components PC, PE, PI, SQDG, and phytosterols were separated with symmetrical peaks as shown in Figure 3. Crude deoiled rape lecithin has been described as having the following phospholipid distribution: PC (25 wt%), PE (22 wt%), and PI (15 wt%) (7).

The rape varieties analyzed showed differences in their polar lipid contents (11.8–15.5 mg/g total fat) as well as in the distribution of distinct lipid classes such as PC, PE, PI, SQDG, and phytosterols (Table 2). PC was the predominant phospholipid in all rape varieties, in a range between 7.1 and 8.1 mg/g of total fat, followed by PE with 2.8–3.7 and PI with 0.1–4.1 mg/g of total fat (Table 1). Each variety characterized

TABLE 1
Lipid Compositions of *Brassica napus* L. Varieties at a Glance[a]

Lipid class	Winter oilseed rape *low sinapin-NPZ*	Winter oilseed rape *HO-NPZ*	Yellow seed spring oilseed rape-*DSV*	Yellow seed summer rape *RAMSCH-DSV*
		mg/g total fat		
TG	983.6	982.5	984.1	980.3
Sterol	0.1	0.6	0.3	0.2
Polar lipids	14.2	11.8	12.6	15.5
SQDG	0.3	0.4	0.5	0.6
PE	2.8	3.4	2.7	3.7
PI	4.1	0.6	2.3	3.1
PC	7.1	7.4	7.1	8.1

[a]TG, TAG/tripentadecanoin; Sterol, stigmasterol/sitosterol; SQDG, sulfoquinovosyldiacylglycerol; PE, PE-dipalmitoyl; PC, PC-diheptadecanoyl.

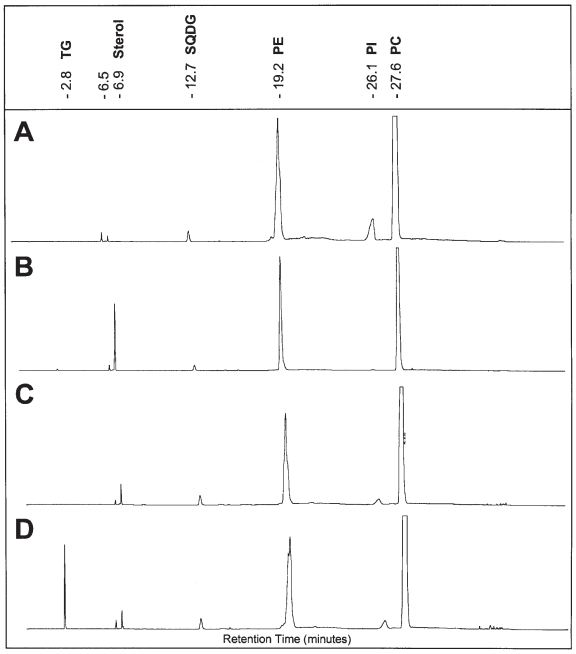

FIG. 3. HPLC separation and fractionation of the polar lipid extracts derived from different *Brassica napus* L. varieties after solid-phase extraction: (A) winter oilseed rape *low sinapin-NPZ*; (B) winter oilseed rape *HO-NPZ*; (C) yellow seed spring oilseed rape-*DSV*; (D) yellow seed summer rape *RAMSCH-DSV*. Before HPLC separation, the neutral lipids and polar lipids were separated by aminopropyl-silica solid-phase extraction. See Figure 1 for abbreviations.

possesses a special lipid class distribution. Both winter oilseed rape *low sinapin-NPZ* and yellow seed summer rape *RAMSCH-DSV* showed a high content of polar lipids. Winter oilseed rape *low sinapin-NPZ* showed the highest content of PI, and yellow seed summer rape *RAMSCH-DSV* had the highest contents of PC and SQDG. The winter oilseed rape *HO-NPZ* had the lowest PI content. Phytosterols were detected only in minor amounts (0.1–0.6 mg/g total fat) in all rape varieties.

The method described in this study is mild and nondestructive. The automatically sampled fractions could be methylated directly in the fractionation tubes for the combined FAME analysis following Lepage and Roy (19). The combination of these analytical methods enables the comparison of integrated FA profiles within the lipid classes of the rape varieties in a continuous process. Extensive variations of the total and integrated FA patterns were determined (Table 2). Generally, rapeseed oil is rich in oleic acid (18:1n-9) (56 wt%) and also contains linoleic acid (18:2n-6) (26 wt%) and α-linolenic acid (18:3n-3) (10 wt%) (7). In this study, the total FA compositions of the rape varieties were dominated by monounsaturated FA as previously reported (7). Oleic acid, linoleic acid, and α-linolenic acid were the most important components, followed by palmitic acid (16:0), *cis*-vaccenic acid (18:1n-7), and gondoic acid (20:1n-9). Stearic acid (18:0), which is a significant FA in several oils, such as soy oil and palm oil, is a minor component in rapeseed oil. Winter oilseed rape *HO-NPZ* had the highest quantity of monounsaturated

TABLE 2
Total FA Composition and Integrated FA Composition of Distinct Lipid Classes of *Brassica napus* L. Varieties[a]

	Winter oilseed rape *low sinapin-NPZ* (wt%)					Winter oilseed rape *HO-NPZ* (wt%)					Yellow seed spring oilseed rape-*DSV* (wt%)					Yellow seed summer rape *RAMSCH-DSV* (wt%)				
FA	Total	SQDG	PE	PI	PC	Total	SQDG	PE	PI	PC	Total	SQDG	PE	PI	PC	Total	SQDG	PE	PI	PC
14:0	0.1	2.8		1.0		0.04	1.0		1.2		0.1	1.4		1.0		0.1	2.0		1.8	
14:1n-5		6.6		4.8			3.9		7.1			4.8		5.7			6.7		4.1	
16:0	5.5	20.5	9.6	17.1	9.1	3.3	9.7	7.0	12.1	4.6	4.5	14.5	9.6	14.4	7.7	5.2	15.8	11.7	16.1	11.4
16:1n-7	0.3	0.7	0.5	0.5	0.6	0.3	0.5	0.6	0.9	0.6	0.3	0.6	0.5	0.8	0.6	0.3	0.6	0.4	0.8	0.6
17:0	0.1	0.9	0.1	0.3	0.1	0.1	0.4	0.1	0.3	0.04	0.04	0.5	0.1	0.2	0.04	0.1	0.7	0.1	0.4	0.1
17:1n7	0.1	0.4	0.2	0.4	0.1	0.1	0.3	0.3	0.9	0.2	0.1	0.3	0.3	0.9	0.2	0.2	0.4	0.6	2.2	0.5
18:0	1.5	16.2	1.1	5.6	0.7	2.8	8.6	1.7	5.7	0.8	1.7	9.6	1.2	4.6	0.9	1.8	10.7	1.3	4.2	1.0
18:1n-9	57.8	26.1	37.8	39.0	58.4	80.0	58.3	65.4	47.8	80.2	56.6	34.2	38.9	26.2	56.7	43.4	25.1	21.6	14.8	38.9
18:1n-7	4.1	3.9	3.3	2.8	2.6	3.3	4.4	2.8	2.4	2.0	4.3	7.6	3.3	2.6	2.5	4.1	8.6	3.7	2.5	3.1
18:2n-6	20.2	10.3	40.7	22.8	26.6	5.2	4.6	18.4	15.8	9.5	19.2	13.6	39.4	34.6	27.2	28.7	16.6	47.1	39.4	36.6
18:3n-6		0.9		0.3			0.3		0.5			0.2		0.4			0.5		0.4	
18:3n-3	8.2	3.7	5.6	3.0	3.1	1.7	1.7	2.2	3.0	1.1	8.6	4.4	5.1	6.5	3.4	13.2	5.0	12.1	11.5	7.1
18:4n-3													0.1					0.1		0.05
20.0	0.5	0.7	0.1	0.2		0.9	0.1	0.1			0.6	1.0	0.1	0.1		0.7	1.2	0.1	0.1	
20:1n-9	1.0	0.4	0.1		0.1	1.4	0.1	0.2	0.2	0.1	1.8	1.5	0.3	0.2	0.2	1.1	0.7	0.1	0.1	0.1
22:0	0.3	0.9	0.1	0.2		0.4	0.7	0.1	0.1		0.4	0.8	0.1	0.1		0.5	1.0	0.1	0.2	
22:1n-9	0.04	2.5		1.1	0.3	1.3	0.1	1.4		1.3	2.6	0.2	1.3				1.7	0.1	0.9	
23:0											0.2						0.2			
24:0	0.1	1.7		0.2		0.2	1.1	0.1	0.2		0.2	1.5	0.1	0.2		0.3	1.8	0.1	0.5	
24:1n-9	0.1	0.6		0.2	0.1	0.1	0.4				0.2	0.6	0.04			0.3	0.6	0.1		
SAFA	8.0	43.9	10.8	24.5	10.3	7.7	22.3	8.9	19.8	5.7	7.5	29.5	11.8	20.7	8.9	8.6	33.4	13.7	23.4	12.6
MUFA	63.5	41.2	42.7	49.3	61.9	85.5	71.2	70.5	60.8	83.6	64.6	52.3	43.5	37.7	60.6	49.4	44.3	26.9	25.2	43.5
PUFA	28.5	14.9	46.5	26.2	27.8	6.8	6.5	20.6	19.4	10.7	27.9	18.2	44.7	41.6	30.5	42.0	22.3	59.4	51.4	43.9

[a]The fractions of HPLC separations were measured by FAME GC analysis. All data are presented in weight percent (wt%). SAFA, saturated FA, MUFA, monounsaturated FA. See Table 1 for other abbreviations.

FA due to the high content of oleic acid. In contrast, the yellow seed summer rape *RAMSCH-DSV* possessed huge amounts of linoleic acid and α-linolenic acid but small amounts of oleic acid.

Likewise, there was considerable variation in the FA patterns of the lipid classes of the rape varieties (Table 2). In general, myristic acid (14:0) and myristoleic acid (14:1n-5) were linked to only SQDG and PI. In all rape varieties, oleic acid was mainly connected to PC, linoleic acid to PE, and α-linolenic acid predominantly to PE and PI, whereas γ-linolenic acid was specifically linked to PI and SQDG. In general, SQDG was relatively high in saturated FA, PC contained monounsaturated FA, and PE was high in PUFA. Interestingly, tricosanoic acid (23:0), which was exclusively linked to SQDG, was detectable in only yellow seed spring oilseed rape-*DSV* and yellow seed summer rape *RAMSCH-DSV*. Monoglycosyldiacylglycerol contains a high proportion of PUFA and is abundant in plant leaves, mainly in the chloroplasts. In this study, the sulfated monoglycosyldiacylglycerol SQDG was detected in seeds of all varieties. According to the definition of Gurr and Harwood (21), plants can be divided into two groups: 16:3 plants, which contain hexadecatrienoate, and (ii) 18:3 plants, which contain α-linolenic acid in glycosyldiacylglycerols. In this study α-linolenic acid was determined to be linked to SQDG in all the rapeseed oils.

In addition to environmental influences, the lipid class distribution as well as the FA patterns of the varieties analyzed may reflect their genetic background, e.g., expressions of acyl carrier proteins, phospholipid synthases, acyltransferases, desaturases, or elongases.

Because the properties of lecithins and oils are determined by the lipid class distribution and heterogeneity of integrated FA, detailed information on the composition is essential for efficient applications. Today, rapeseed oil is itself an oil with an already modified FA composition, since traditional rapeseed oil was rich in erucic acid. In the future, more complex modifications of crop constituents will be generated and will need to be characterized.

As a result, the combination of this improved HPLC lipid class separation into 14 classes with a direct GC FAME analysis offers a rapid qualitative characterization of lipid structures derived from plant matrices. Additionally, the quantification of the separated lipid classes to evaluate technical modifications in the field of cultivation or molecular genetics has been made possible.

ACKNOWLEDGMENTS

We would like to express our gratitude to Sabine Brandt for her basic work on this issue and to Anja Frings for her critical review. In addition, we are grateful to the breeding companies Norddeutsche Pflanzenzucht, Hans-Georg Lembke KG (NPZ), and the Deutsche Saatgutveredlung (DSV) for providing the different rapeseed varieties. The work for this publication was supported by the Federal Ministry of Education and Research of Germany under grant number 0312252M. The authors are responsible for the content of the publication.

REFERENCES

1. Knutzon, D.S., G.A. Thompson, S.E. Radke, W.B. Johnson, V.C. Knauf, and J.C. Kridl, Modification of *Brassica* Seed Oil by Antisense Expression of a Stearoyl-Acyl Carrier Protein Desaturase Gene, *Proc. Natl. Acad. Sci. USA 89*:2624–2628 (1992).
2. Kohno-Murase, J., M. Murase, H. Ichikawa, and J. Imamura, Effects of an Antisense Napin Gene on Seed Storage Compounds in Transgenic *Brassica napus* Seeds, *Plant Mol. Biol. 26*:1115–1124 (1994).
3. Verwoert, II, K.H. van der Linden, M.C. Walsh, H.J. Nijkamp, and A.R. Stuitje, Modification of *Brassica napus* Seed Oil by Expression of the *Escherichia coli fabH* Gene, Encoding 3-Ketoacyl-Acyl Carrier Protein Synthase III, *Plant Mol. Biol. 27*:875–886 (1995).
4. Zou, J., V. Katavic, E.M. Giblin, D.L. Barton, S.L. MacKenzie, W.A. Keller, X. Hu, and D.C. Taylor, Modification of Seed Oil Content and Acyl Composition in the Brassicaceae by Expression of Yeast *sn*-2 Acyltransferase Gene, *Plant Cell 9*:909–923 (1997).
5. Stoutjesdijk, P.A., C. Hurlestone, S.P. Singh, and A.G. Green, High-Oleic Acid Australian *Brassica napus* and *B. juncea* Varieties Produced by Co-suppression of Endogenous Δ12-Desaturases, *Biochem. Soc. Trans. 28*:938–940 (2000).
6. Pongdontri, P., and M. Hills, Characterization of a Novel Plant Acyl-CoA Synthetase That Is Expressed in Lipogenic Tissues of *Brassica napus* L., *Plant Mol. Biol. 47*:717–726 (2001).
7. Gunstone, F.D., and M.R. Pollard, Vegetable Oils with Fatty Acid Composition Changed by Plant Breeding or by Genetic Modification, in *Structured and Modified Lipids*, edited by F.D. Gunstone, Marcel Dekker, New York, 2001, pp. 155–184.
8. Koletzko, B., P.J. Aggett, J.G. Bindels, P. Bung, P. Ferré, A. Gil, M.J. Lentze, M. Roberfroid, and S. Strobel, Growth, Development and Differentiation: A Functional Food Science Approach, *Br. J. Nutr. 80*:S5–S45 (1998).
9. Christie, W.W., Separation of Lipid Classes from Plant Tissues by High-Performance Liquid Chromatography on Chemically Bonded Stationary Phases, *J. High. Resolut. Chromatogr. 18*:97–100 (1995).
10. Samet, J.M., M. Friedman, and D.C. Henke, High-Performance Liquid Chromatography Separation of Phospholipid Classes and Arachidonic Acid on Cyanopropyl Columns, *Anal. Biochem. 182:*32–36 (1989).
11. Patton, G.M., and S.J. Robins, Separation and Quantitation of Phospholipid Classes by HPLC, *Methods Mol. Biol. 110*:193–215 (1998).
12. Nordbäck, J., E. Lundberg, and W.W. Christie, Separation of Lipid Classes from Marine Particulate Material by HPLC on a Polyvinyl Alcohol-Bonded Stationary Phase Using Dual-Channel Evaporative Light Scattering Detection, *Marine Chem. 60*:165–175 (1998).
13. Homan, R., and M.K. Anderson, Rapid Separation and Quantification of Combined Neutral and Polar Lipid Classes by High-Performance Liquid Chromatography and Evaporative Light Scattering Mass Detection, *J. Chromatogr. B 708*:21–26 (1998).
14. Grizard, G., B. Sion, D. Bauchart, and D. Boucher, Separation and Quantification of Cholesterol and Major Phospholipid Classes in Human Semen by High-Performance Liquid Chromatography and Light Scattering Detection, *Ibid. 740*:101–107 (2000).
15. Silversand, C., and C. Haux, Improved High-Performance Liquid Chromatographic Method for the Separation and Quantification of Lipid Classes: Application to Fish Lipids, *Ibid. 703*:7–14 (1997).
16. Seppänen-Laasko, T., I. Laasko, H. Vanhanen, K. Kiviranta, T. Lehtimäki, and R. Hiltunen, Major Human Plasma Lipid Classes

Determined by Quantitative High-Performance Liquid Chromatography, Their Variation and Associations with Phospholipid Fatty Acids, *Ibid. 754*:437–445 (2001).
17. Bligh, E.G., and W.J. Dyer, A Rapid Method of Total Lipid Extraction and Purification, *Can. J. Biochem. Physiol. 37*:911–917 (1959).
18. Guan, Z., J. Grünler, S. Piao, and P.J. Sindelar, Separation and Quantification of Phospholipids and Their Ether Analogues by High-Performance Liquid Chromatography, *Anal. Biochem. 297:*137–143 (2001).
19. Lepage, G., and C.C. Roy, Improved Recovery of Fatty Acids Through Direct Transesterification Without Prior Extraction or Purification, *J. Lipid Res. 25*:1391–1396 (1984).
20. Kohn, G., P. van der Ploeg, M. Möbius, and G. Sawatzki, Influence of the Derivatization Procedure on the Results of the Gas Chromatographic Fatty Acid Analysis of Human Milk and Infant Formulae, *Z. Ernährungswiss. 35*:226–234 (1995).
21. Gurr, M.I., and J.L. Harwood, Lipids in Cellular Structures, in *Lipid Biochemistry*, edited by M.I. Gurr, Chapman & Hall, New York, 1991, pp. 246–263.

[Received July 10, 2002; accepted February 28, 2003]