Analysis of the Wax Ester Fraction of Olive Oil and Sunflower Oil by Gas Chromatography and Gas Chromatography–Mass Spectrometry

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ABSTRACT: The wax ester fractions of solvent-extracted sunflower oil and "extra virgin" olive oil were obtained by solid-phase extraction and subsequently subjected to gas-chromatographic and gas chromatographic–mass spectrometric analysis. The comprehensive qualitative analysis of these fractions, which was carried out by the interpretation of mass spectral data, revealed several types of wax esters. In olive oil, short-chain, even-numbered wax esters, saturated and unsaturated long-chain, even-numbered wax esters, benzyl esters, and the diterpenic esters phytyl and geranylgeranyl ester (the latter as a minor component) are present. With the exception of benzyl esters, all these esters occur in sunflower oil as well, but in considerably different amounts compared to those in olive oil. Whereas unsaturated wax esters are present in a negligible amount, diterpenic esters, mainly geranylgeranyl esters, represent the major part of the wax ester fraction.

Paper no. J9809 in *JAOCS 78*, 881–888 (September 2001)

KEY WORDS: Aromatic esters, gas chromatography, gas chromatography–mass spectrometry, geranylgeranyl esters, long-chain wax esters, phytyl esters, solid-phase extraction, vegetable oils.

Wax esters (WE) are part of the unsaponifiable matter in vegetable oils and are of interest to the oil-processing industry for various reasons. The WE content is used as a quality parameter for high-quality oils such as cold-pressed "extra virgin" olive oils (1). For instance, the European Union Regulation No. 183/93 requires the quantitation of WE in olive oils, because solvent-extracted olive oils contain a considerably higher amount of waxes than oils obtained by cold pressing (2). In addition, typical WE patterns or specific WE enable the recognition of adulteration of high-quality oils with cheaper oils, for example, the addition of sunflower oil to olive oil (3).

The isolation of WE in vegetable oils has been performed by on-line high-performance liquid chromatography–gas chromatography (HPLC–GC) (3–6), by column chromatography (7), by HPLC (8,9), and, recently, by solid-phase extraction (SPE) (10,11) with subsequent capillary GC analysis. None of these techniques requires saponification of the WE fraction. Therefore, WE can be determined without any alteration of their structure. These analytical methods allow isolation and quantitation of WE according to their number of carbon atoms and their degree of unsaturation, but a complete qualitative analysis as well as elucidation of isomeric constitution can only be carried out by mass spectrometric (MS) methods.

WE in olive and sunflower oils are of particular interest, as already mentioned, but there is a lack of data concerning their detailed structures and their isomeric composition. In the case of olive oil, a GC–MS analysis of WE in extracted olive oil published by Bianchi *et al.* (12) revealed the presence of esters with alcohols (AL) in the range of AL 1–AL 6, esters of oleic acid with long-chain aliphatic alcohols AL 22–AL 38, and benzyl alcohol esters of the long-chain saturated fatty acids (FA) FA 26 and FA 28.

The composition of sunflower WE has been determined only by the analysis of hydrolysis products. WE were hydrolyzed, and fatty acids and alcohols were analyzed separately, revealing FA 20:0 and FA 22:0 as the major fatty acids and AL 24:0 and AL 26:0 as the major alcohols (13–15).

The on-line liquid chromatography (LC)–GC chromatograms of the sunflower oil WE fraction published by Grob and co-workers (3,6) show a very significant peak with a retention time corresponding to the saturated WE 41. These results are inconsistent with the compositions of the fatty acids and alcohol obtained by hydrolysis, which both show even-numbered chain lengths. Trost (16) investigated the nonpolar material of sunflower oil by semipreparative LC and obtained semiquantitative data by LC, GC, GC–MS, and ^{13}C nuclear magnetic resonance (NMR) analysis of the fractions collected. One of these fractions contained compounds such as geranylgeranyl esters, which are esters of the diterpenic alcohol geranylgeraniol, and saturated and unsaturated fatty acids of different chain lengths.

Within the scope of a comprehensive investigation of minor and major compounds in vegetable oils produced in Europe, WE fractions were analyzed in particular, as sparse data are available for them. The lack of data on the WE fraction of olive oil and sunflower oil led us to carry out studies on the structure of WE compounds. They were separated from the triglyceride matrix by SPE and subsequently analyzed by capillary GC and GC–MS in electron impact (EI) mode. Along with saturated and unsaturated even-numbered, unbranched aliphatic esters, phytyl and geranylgeranyl esters with saturated and unsaturated fatty acid moieties, and, in smaller amounts, short-chain esters and, particularly in olive oil, benzyl alcohol esters were identified.

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MATERIALS AND METHODS

Samples and reagents. All solvents were obtained in *p.a. (pro analysi)* quality. Extra virgin olive oil of Greek origin (Kalamata) and sunflower oil of unknown origin were purchased in a local supermarket. Sunflower seeds (no high-oleic varieties) grown in the Netherlands (no. 90) and France (no. 94) were obtained from Saatbau Linz, Austria. Solvent extraction with *n*-hexane was performed by crushing 100 g of seeds, avoiding extensive warming of the ground material, and transferring the ground seeds into a 2-L Erlenmeyer flask, to which 1 L of *n*-hexane was added. After stirring for 30 min at 60°C, the dispersion was sucked through a glass filter (pore size G3). The residue was extracted once again and filtered. Combined extracts were evaporated with the use of a rotary evaporator at 50–60°C.

Solid-phase extraction. For sample preparation, the vegetable oil was subjected to SPE using an SPE-12G vacuum manifold column processor (J.T.Baker, Deventer, The Netherlands) and 3-mL disposable extraction columns packed with 500 mg of silica gel (J.T.Baker). To enable transfer of larger elution solvent volumes, these solid-phase columns were connected to 75-mL reservoirs.

Sample preparation was carried out according to the method published previously (10), with slight modifications. The SPE columns were conditioned with *n*-hexane, and then the sample (approximately 30 mg of vegetable oil dissolved in 500 μL of *n*-hexane) was transferred onto the column and drawn through the column at a flow rate of 2.5 mL/min. The WE fraction was eluted with 10 mL of *n*-hexane/diethyl ether (99:1, vol/vol). (In this procedure, the column must not be allowed to run dry.) The solvent was removed in a stream of nitrogen, and subsequently redissolved in 5 mL of *n*-hexane. This solution was subjected to GC–MS analysis.

GC analysis. An SFE 3000 series gas chromatograph (Carlo Erba, Milan, Italy) equipped with an on-column injector, a constant-pressure–constant flow module (CP–CF 516), and a flame-ionization detector (FID) using a ceramic flame jet was employed. A DB-1 fused-silica capillary column (12 m × 0.32 mm, film thickness 0.25 μm; J&W Scientific, Folsom, CA) was used. The sample (1 μL) was injected at 75°C; the oven was then heated to 230°C at a rate of 25°C/min and from 230 to 350°C at a rate of 10°C/min. The final temperature was held for 10 min. The detector temperature was held at 400°C. Hydrogen was used as a carrier gas at a constant flow rate of 1.8 mL/min. At higher temperatures the flow rates (controlled by a CP–CF module) could not always be adjusted precisely, which resulted in slightly different retention times (as can be seen in Figs. 1 and 4). This problem can be handled by carrying out GC analysis without adjusting the parameters during the analysis of a batch of samples.

GC–MS analysis. A GC 8000 series gas chromatograph (Fisons Instruments, Milan, Italy) was used which was equipped with a DB-1 fused-silica column (10 m × 0.32 mm, film thickness 0.25 μm; J&W Scientific) and an uncoated precolumn, deactivated with diphenyltetramethyldisilazane, 1.5 m × 0.53 mm i.d. (BGB Analytik, Rothenfluh, Switzerland). One microliter of the sample was injected onto the column at 75°C. GC conditions were similar to those described above for GC analysis. Helium was used as a carrier gas at a flow rate of 4.8 mL/min and a gas inlet pressure of 25 kPa. The GC was connected to a quadrupole mass spectrometer (MS 800; Fisons Instruments) in EI mode at an ionization energy of 70 eV. The mass range of the mass spectrometer was set to 50–800 amu, and the source and interface temperature were held at 200 and 300°C, respectively.

RESULTS AND DISCUSSION

GC and GC–MS analysis of WE fraction of olive oil. Prior to GC–MS analysis, a GC separation of the WE fraction of olive oil was performed, since separation efficiency and peak shape are significantly improved and peak assignment is simplified by this process. A GC–FID chromatogram of the WE fraction is given in Figure 1. The assignment of the components was performed by GC–MS analysis and is given in detail below.

The WE fraction of olive oil is composed of esters of different chain lengths, which are grouped as short-chain esters, saturated WE, unsaturated WE, benzyl esters, and diterpenic esters in the discussion below. The structures of WE are represented as R^1–COO–R^2, where R^1 is the acidic moiety and R^2 is the alcoholic moiety.

The major fatty acids in the short-chain esters are palmitic and oleic acid esterified with methanol and ethanol, respectively (see Fig. 1, peak nos. 1–4). These esters were eluted together with various hydrocarbons, as can be seen in Figure 1, and detected using molecular ions and selected diagnostic ions. The molecular ions of all these esters were found, but abundances were low. The diagnostic ion of methyl esters (ME) at *m/z* 74 is obtained by the McLafferty rearrangement. The unsaturated short-chain esters were identified by single-ion monitoring (SIM) of the ion $[R^1CO - 1]^+$ (for FA 18:1, *m/z* 264), which is characteristic of monoenoic esters and is discussed later in this section. The fraction analyzed contains methyl palmitate (FA 16:0 ME, peak no. 1), methyl stearate (FA 18:0 ME, peak no. 3), methyl oleate [FA 18:1(9*Z*) ME, peak no. 2], and ethyl oleate [FA 18:1(9*Z*) EE, peak no. 4]. The molecular ions and diagnostic ions for butyl and hexyl esters, which were found by Bianchi *et al.* (12), could not be extracted by SIM.

Saturated long-chain esters are present in lower concentrations than unsaturated ones and range from WE 38 to WE 46 (see Fig. 1, peak nos. 13, 18, 22, 25, and 27). These findings are not in agreement with those of Bianchi *et al.* (12), who detected the saturated WE 38, which is composed of FA 16 and AL 22 only (12). We took a closer look at the complete isomeric composition of the esters by carrying out SIM experiments with the molecular ion and the major diagnostic ion $[R^1CO_2H_2]^+$, which is formed by a double hydrogen rearrangement fragmentation at the ester group and described in numerous publications (17,18). The detailed isomeric composition is shown in Table 1, including the intensities of the ions $[M]^+$, $[R^1CO]^+$, and $[R^2 - 1]^+$. WE in the range from WE

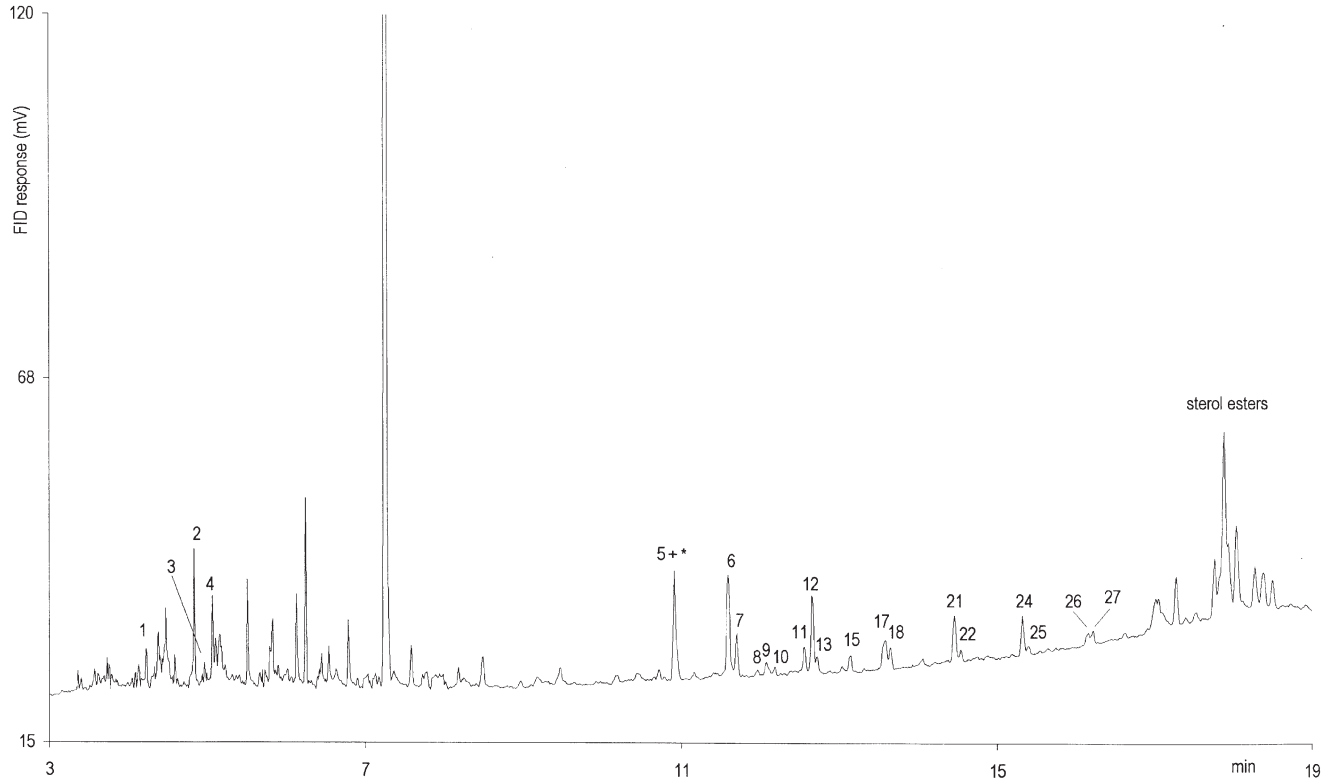

FIG. 1. Gas chromatography–flame-ionization detection (GC–FID) chromatogram of the wax ester fraction of olive oil. Peak assignments: 1, palmitic acid methyl ester; 2, oleic acid methyl ester; 3, stearic acid methyl ester; 4, oleic acid ethyl ester; 5, benzyl $C_{26:0}$; *, unidentified compound; 6, phytyl $C_{18:1}$; 7, phytyl $C_{18:0}$; 8, benzyl $C_{28:0}$; 9, geranylgeranyl $C_{18:1}$; 10, geranylgeranyl $C_{18:0}$; 11, phytyl $C_{20:1}$; 12, phytyl $C_{20:0}$; 13, wax ester 38:0; 14, geranylgeranyl $C_{20:1}$; 15, geranylgeranyl $C_{20:0}$; 16, phytyl $C_{22:0}$; 17, wax ester 40:1; 18, wax ester 40:0; 19, geranylgeranyl $C_{22:0}$; 20, phytyl $C_{24:0}$; 21, wax ester 42:1; 22, wax ester 42:0; 23, geranylgeranyl $C_{24:0}$; 24, wax ester 44:1; 25, wax ester 44:0; 26, wax ester 46:1; 27, wax ester 46:0.

38 to WE 42 are mainly formed from palmitic acid esterified to the corresponding alcohol. Beyond WE 42, WE are constituted of several fatty acids such as palmitic (FA 16:0), stearic (FA 18:0), arachidic (FA 20:0), and behenic acid (FA 22:0) and their corresponding alcohols.

Unsaturated WE are found in considerably higher quantities than saturated ones, ranging from WE 40 to WE 46 (see Fig. 1, peak nos. 17, 21, 24, and 26) and containing oleic acid as the major fatty acid component. These results are in agreement with those of Bianchi *et al.* (12), who found WE in the same range consisting of oleic acid as well, but in higher amounts. Abundances of molecular ions of saturated WE were rather low, and the base peak $[R^1CO - 1]^+$ and the double hydrogen rearrangement ion $[R^1CO_2H_2]^+$ were used for the determination of the isomeric composition, which is shown in Table 1. The ion $[R^1CO - 1]^+$, described in previous papers (10,17), is formed due to the loss of a fragment corresponding to the alcohol moiety.

Aromatic esters such as benzyl esters occur as minor constituents in the WE fraction. Benzyl octacosanoate and benzyl hexacosanoate (see Fig. 1, peak nos. 5 and 8) were found, the latter coeluting with a second compound that could not be identified. The spectra of these aromatic compounds are formed by the fission of the benzyl group $[C_7H_7]^+$ (*m/z* 91), and of the phenylmethoxy group $[C_7H_8O]^+$ (*m/z* 108). The molecular weight can be determined by SIM and the ions $[M - 91]^+$ and $[M - 109]^+$. Both compounds were identified by Bianchi *et al.* (12) as well.

Before the elution of saturated and unsaturated WE, a set of peaks was present that Artho *et al.* (6) presumed to be diterpenic esters, i.e., esters of long-chain fatty acids esterified with diterpenic alcohols such as phytol or geranylgeraniol. The diagnostic ion in the spectra of phytol ester is $[M - R^1CO_2H]^+$ (*m/z* 278), deriving from the phytyl group, which was described by Patterson *et al.* (19) and Cranwell *et al.* (20). Fragments within the phytyl chain of phytyl oleate are shown in Figure 2, and the spectrum of phytyl stearate is presented in Figure 3. Although $[M - 183]^+$ and $[M - 211]^+$ are very low in abundance, these ions enable the assignment of various phytyl esters, which are composed of saturated and unsaturated FA 18, FA 20, and FA 22 fatty acids (see Fig. 1, peak nos. 6, 7, 11, and 12). The positive charges of *m/z* 43 and *m/z* 113 are strongly stabilized, and therefore the ions $[M - 43]^+$ and $[M - 113]^+$ are not visible in the spectra. Table 2 shows the fatty acids that occur within phytyl esters.

After assuming the ion $[M - R^1CO_2H]^+$ is formed in the spectra of geranylgeranyl esters as well, a SIM experiment was performed with *m/z* 272, making three small peaks visible (see Fig. 1, peak nos. 9, 10, and 15). Owing to their low concentration in olive oil, there is a lack of any other ions, but since the retention times of geranylgeranyl esters in sunflower oil (which are discussed later) are identical, the assignment is very likely to be correct.

TABLE 1
Isomeric Composition of Saturated and Unsaturated Wax Esters in Olive Oil and Mass Spectral Data—Molecular Ions, Characteristic Fragments, and Base Peak Intensities (BPI%)

Peak no.	Wax ester CN:DB[a]	Components (acid–alcohol)	% of isomer[b]	$[M]^{+c}$ (BPI%)	$[R^1CO_2H_2]^+$ (BPI%)	$[R^1CO]^{+d}$ (BPI%)	$[R^2 - 1]^{+d}$ (BPI%)	$[R^1CO - 1]^+$ (BPI%)
13	WE 38:0	12:0–26:0	2.4	565 (0.90)	201 (0.10)	183 (0.44)	[364]	
		14:0–24:0	2.2		229 (0.19)	211 (0.24)	336 (0.04)	
		16:0–22:0	73.8		257 (14.73)	239 (1.36)	308 (0.05)	
		18:0–20:0	3.7		285 (0.16)	[267]	280 (0.65)	
		20:0–18:0	17.9		313 (3.23)	295 (0.68)	[252]	
18	WE 40:0	14:0–26:0	1.4	593 (1.09)	229 (0.20)	211 (0.25)	[364]	
		16:0–24:0	89.0		257 (26.30)	239 (1.97)	[336]	
		18:0–22:0	7.7		285 (1.97)	[267]	308 (0.49)	
		20:0–20:0	1.9		313 (0.60)	[295]	[280]	
17	WE 40:1	16:1–24:0	12.7	591 (0.08)	255 (0.69)			236 (1.39)
		18:1–22:0	87.3		283 (2.75)			264 (11.60)
22	WE 42:0	14:0–28:0	1.5	621 (0.97)	229 (0.12)	211 (0.28)	[392]	
		16:0–26:0	82.6		257 (20.83)	239 (1.38)	[364]	
		18:0–24:0	13.2		285 (3.09)	[267]	336 (0.45)	
		20:0–22:0	1.9		313 (0.42)	[295]	308 (0.08)	
		24:0–18:0	0.8		369 (0.18)	351 (0.05)	[252]	
21	WE 42:1	16:1–26:0	5.0	619 (0.13)	255 (0.39)			236 (1.02)
		18:1–24:0	95.0		283 (4.66)			264 (21.92)
25	WE 44:0	16:0–28:0	43.2	649 (9.75)	257 (60.78)	[239]	392 (0.60)	
		18:0–26:0	48.6		285 (68.61)	[267]	364 (0.51)	
		22:0–22:0	8.2		341 (6.26)	[323]	308 (5.31)	
24	WE 44:1	16:1–28:0	4.1	647 (0.17)	255 (0.41)			236 (1.37)
		18:1–26:0	94.3		283 (6.05)			264 (35.18
		20:1–24:0	1.6		311 (0.44)			292 (0.25)
27	WE 46:0	14:0–32:0	5.0	677 (1.06)	229 (0.06)	211 (0.96)	448 (0.32)	
		16:0–30:0	29.2		257 (7.82)	[239]	[420]	
		18:0–28:0	21.8		285 (5.50)	[267]	392 (0.35)	
		20:0–26:0	24.5		313 (4.05)	295 (2.52)	[364]	
		22:0–24:0	15.0		341 (3.18)	323 (0.67)	336 (0.18)	
		24:0–22:0	4.5		369 (0.99)	351 (0.21)	[308]	
26	WE 46:1	18:1–28:0	100.0	675 (0.23)	283 (8.74)			264 (36.19)

[a]CN, carbon number of wax ester; DB, number of double bonds; WE, wax ester.
[b]The percentage of a single isomer of a saturated wax ester was calculated based on intensities of the ions $[R^1CO_2H_2]^+$, $[R_1CO]^+$, and $[R^2 - 1]^+$. For unsaturated wax esters the ions $[R^1CO_2H_2]^+$, and $[R^1CO - 1]^+$ were used.
[c]The mass spectrometer calculates the digital mass peak based on isotopic masses (H = 1.0078, C = 12.000, O = 15.995); in this case, the mass of the molecular ion for compounds with more than 65 hydrogen atoms is rounded up (the molecular ion is not protonated).
[d]Fragments in square brackets are not visible in the spectrum; either the intensity of the peak is too low or, owing to mass discrimination, fragments with a molecular weight higher than 300 are not detected.

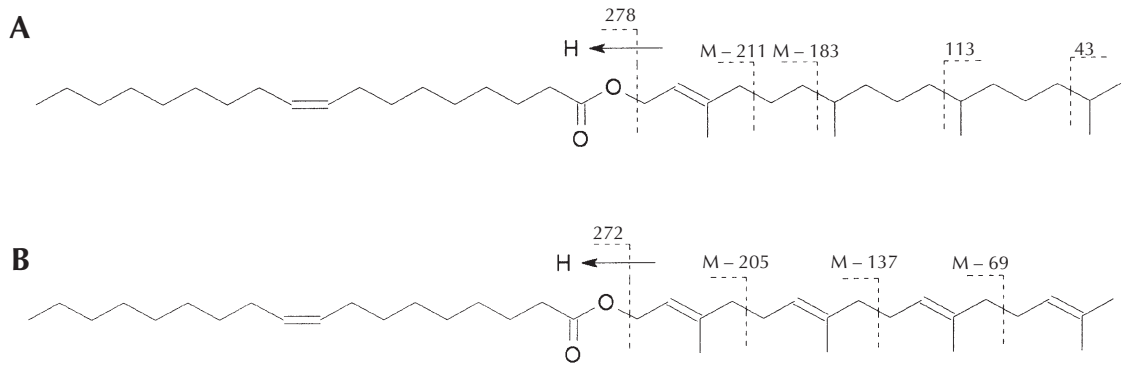

FIG. 2. Fragmentation pattern of the mass spectra of (A) phytyl oleate [phytyl – FA 18:1(9*Z*)] and (B) geranylgeranyl oleate [geranylgeranyl – FA 18:1(9*Z*)]. FA, fatty acid.

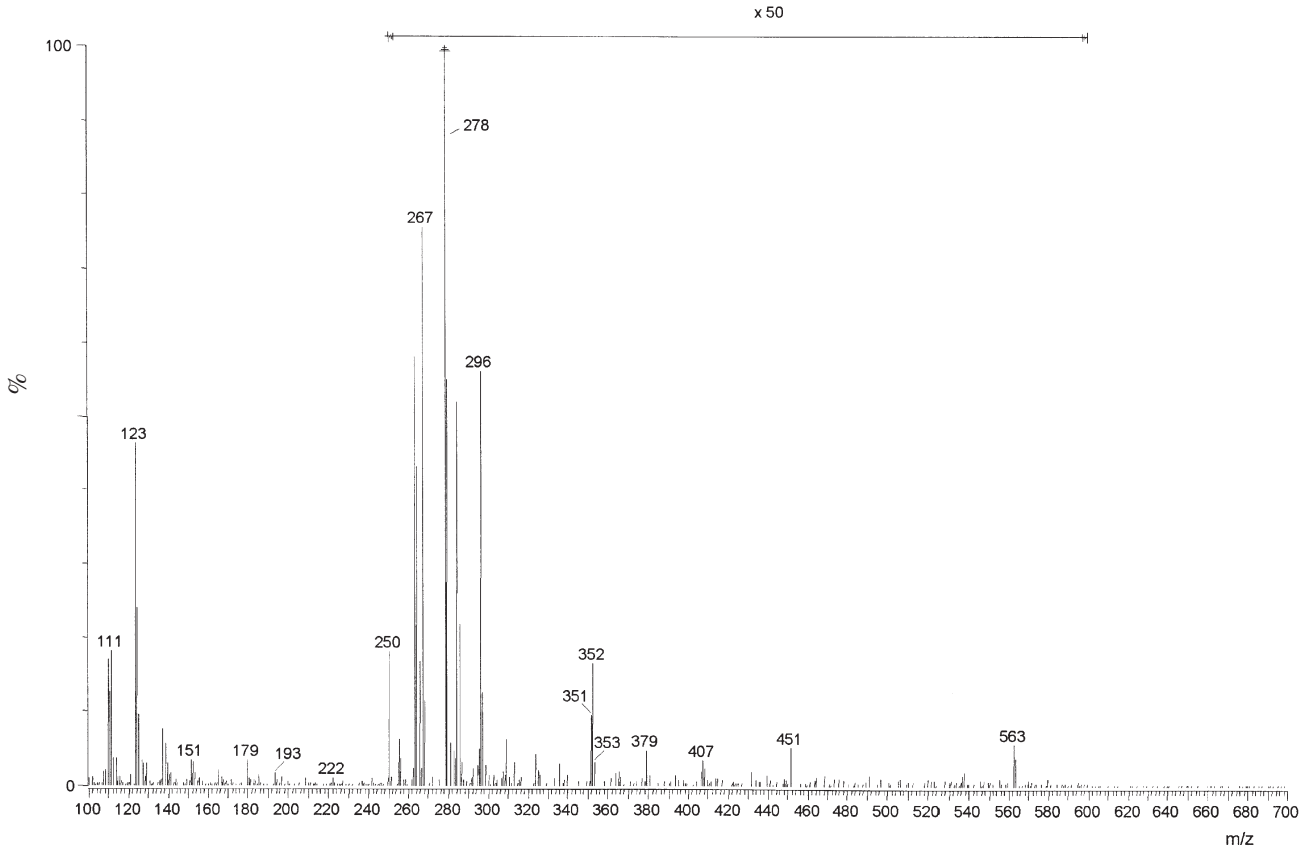

FIG. 3. Electron impact (EI) spectrum of phytyl stearate (phytyl–FA 18:0, peak no. 7 in Fig. 1). $[M]^+$, *m/z* 563; $[M - R^1CO_2H]^+$, *m/z* 278; $[M - 211]^+$, *m/z* 351; $[M - 183]^+$, *m/z* 379. See Figure 2 for other abbreviation.

GC and GC–MS analysis of WE fraction of sunflower oil. A SPE sunflower oil was subjected to solid-phase extraction and analyzed by GC prior to GC–MS analysis. A GC–FID chromatogram of the WE fraction of sunflower oil is presented in Figure 4. The peak assignment of each component was achieved on the basis of the GC–MS data and reveals the presence of a variety of esters, such as short-chain esters, saturated long-chain WE, diterpenic esters, and unsaturated long-chain WE as minor components. These groups of esters are discussed in greater detail below.

Short-chain esters occur to a very limited amount in the fraction analyzed. Methyl esters of palmitic and oleic acid (see Fig. 2, peak nos. 1 and 2) could be found only as small peaks by extracting the McLafferty rearrangement ion *m/z* 74. Traces of other methyl esters beyond FA 18 were found by means of a SIM experiment for the ion *m/z* 74, but no peaks could be seen in the total ion chromatogram (TIC).

Unsaturated WE are not present in sunflower oil in a noteworthy concentration. By means of SIM experiments for the ion $[R^1CO - 1]^+$, traces of unsaturated WE, mainly containing FA 18:1 as the major fatty acid, are detected but are not visible in the TIC.

TABLE 2
Determination of the Fatty Acid Pattern in Phytyl and Geranylgeranyl Esters in Olive Oil by Mass Spectrometry—Molecular Ions, Characteristic Ions, and Their Base Peak Intensities (BPI%)

Peak no.	Fatty acid	$[M]^{+\,a}$	$[M - R^1CO_2H]^+$ (BPI%)	$[M - 211]^+$ (BPI%)	$[M - 183]^+$ (BPI%)	$[M - 69]^+$ (BPI%)	$[M - 137]^+$ (BPI%)	$[M - 205]^+$ (BPI%)
Phytyl ester								
7	FA 18:0	563 (0.11)	278 (5.16)	351 (0.19)	379 (0.09)			
6	FA 18:1	561 (0.06)	278 (2.13)	349 (0.90)	377 (0.03)			
12	FA 20:0	591 (0.01)	278 (5.73)	379 (0.50)	407 (0.14)			
11	FA 20:1	589 (0.02)	278 (2.27)	377 (0.18)	405 (0.27)			
Geranylgeranyl ester								
10	FA 18:0	557 (0.01)	272 (0.43)			487 (0.09)	419 (0.18)	351 (0.01)
9	FA 18:1	555 (0.09)	272 (0.67)			485 (0.07)	417 (0.63)	349 (0.11)
15	FA 20:0	585 (0.05)	272 (0.46)			515 (0.09)	447 (0.08)	379 (0.12)

[a]See Table 1, footnote c.

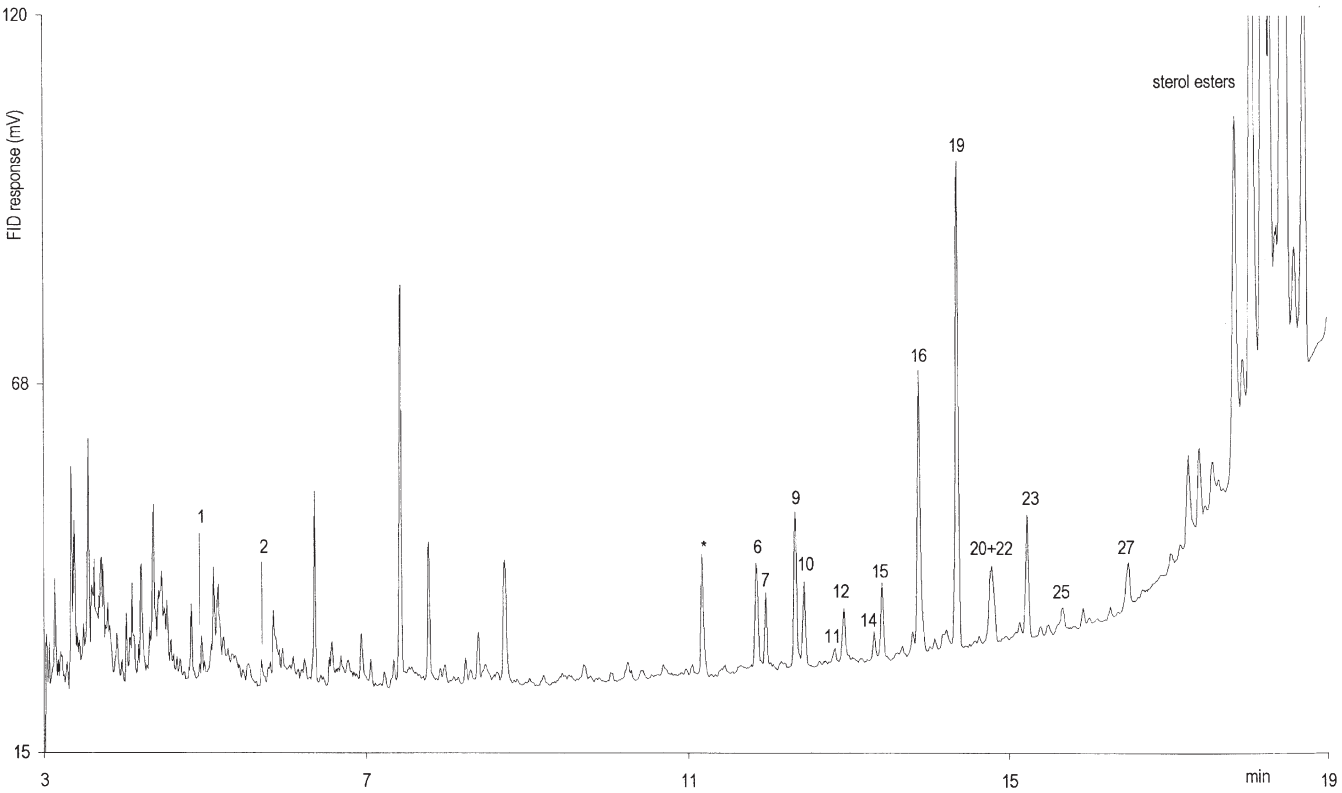

FIG. 4. GC–FID chromatogram of the wax ester fraction of sunflower oil. See Figure 1 for abbreviation and peak assignments.

However, saturated WE with higher molecular weights are found in considerable amounts, ranging from WE 42 to WE 46 (see Fig. 2, peak nos. 22, 25, and 27). The WE are mainly formed by arachidic (FA 20:0) and behenic (FA 22:0) acids. These results correspond to those obtained by several authors who examined the products of WE hydrolysis by GC and determined 55–80% of these fatty acids. Another major fatty acid is palmitic acid, particularly in WE 46:0. The full data on the isomeric composition of the saturated WE and their detection by means of molecular ions and fragment ions are listed in Table 3.

SIM experiments with two diagnostic ions $[M - R^1CO_2H]^+$ for saturated diterpenic esters (*m/z* 278) and unsaturated diterpenic esters (*m/z* 272), which were discussed in the olive oil section, revealed a considerable number of peaks in a homologous series. On closer inspection, weak molecular ions of

TABLE 3
Isomeric Composition of Saturated Wax Esters in Sunflower Oil and Mass Spectral Data—Molecular Ions, Characteristic Fragments, and Their Base Peak Intensities (BPI%)

Peak no.	Wax ester CN:DB[a]	Components (acid–alcohol)	% of isomer[b]	$[M]^{+c}$	$[R^1CO_2H_2]^+$ (BPI%)	$[R^1CO]^{+d}$ (BPI%)	$[R^2 - 1]^{+d}$ (BPI%)
22	WE 42:0	14:0–28:0	2.8	621 (2.14)	229 (0.66)	211 (0.44)	[392]
		16:0–26:0	10.3		257 (2.97)	239 (0.24)	364 (0.01)
		18:0–24:0	5.1		285 (1.62)	[267]	336 (0.02)
		20:0–22:0	63.8		313 (17.75)	295 (0.73)	308 (1.86)
		22:0–20.0	15.2		341 (3.33)	323 (0.15)	280 (1.37)
		24:0–18:0	2.8		369 (0.46)	351(0.43)	[252]
25	WE 44:0	16:0–28:0	10.9	649 (2.31)	257 (2.68)	239 (1.19)	[392]
		18:0–26:0	6.1		285 (1.07)	267 (1.09)	364 (0.02)
		20:0–24:0	55.4		313 (17.33)	295 (1.03)	336 (1.33)
		22:0–22:0	26.2		341 (8.00)	323 (0.18)	308 (1.13)
		24:0–20:0	1.4		369 (0.41)	351 (0.07)	[280]
27	WE 46:0	16:0–30:0	40.0	677 (2.50)	257 (12.06)	239 (1.34)	420 (0.36)
		18:0–28:0	6.9		285 (1.98)	[267]	392 (0.41)
		20:0–26:0	42.1		313 (13.39)	295 (0.13)	364 (0.97)
		22:0–24:0	11.0		341 (3.79)	[323]	[336]

[a–d]See Table 1.

TABLE 4
Determination of the Fatty Acid Pattern in Phytyl and Geranylgeranyl Esters in Sunflower Oil by Mass Spectrometry—Molecular Ions, Characteristic Ions, and Their Base Peak Intensities (BPI%)

Peak no.	Fatty acid	$[M]^{+a}$	$[M - R^1CO_2H]^+$ (BPI%)	$[M - 211]^+$ (BPI%)	$[M - 183]^+$ (BPI%)	$[M - 69]^+$ (BPI%)	$[M - 137]^+$ (BPI%)	$[M - 205]^+$ (BPI%)
Phytyl ester								
7	FA 18:0	563 (0.29)	278 (9.06)	351 (0.46)	379 (0.20)			
6	FA 18:1	561 (0.14)	278 (3.26)	349 (0.13)	377 (0.03)			
12	FA 20:0	591 (0.26)	278 (9.53)	379 (0.10)	407 (0.29)			
11	FA 20:1	589 (0.09)	278 (1.87)	377 (0.01)	405 (0.01)			
16	FA 22:0	619 (0.29)	278 (10.98)	407 (0.22)	435 (0.16)			
20	FA 24:0	647 (0.28)	278 (10.24)	435 (0.04)	463 (0.12)			
Geranylgeranyl ester								
10	FA 18:0	557 (0.04)	272 (1.49)			487 (0.16)	419 (0.05)	351 (0.09)
9	FA 18:1	555 (0.03)	272 (1.16)			485 (0.11)	417 (0.05)	349 (0.04)
15	FA 20:0	585 (0.04)	272 (1.57)			515 (0.20)	447 (0.05)	379 (0.09)
14	FA 20:1	583 (0.01)	272 (0.77)			513 (0.05)	445 (0.02)	377 (0.02)
19	FA 22:0	613 (0.05)	272 (1.97)			544 (0.19)	475 (0.05)	407 (0.08)
23	FA 24:0	641 (0.02)	272 (1.26)			572 (0.10)	503 (0.01)	435 (0.05)

[a]See Table 1, footnote *c*.

components corresponding to the exact masses of phytyl esters and geranylgeranyl esters could be found. Although the double-bond configuration of the latter compound could not be determined from EI spectra, consideration of biochemical pathways and the analyses of Trost (16) make the presence of these diterpenic esters very likely. In addition, the all-*trans* configuration of the double bonds within the geranylgeranyl chain causes the unsaturated diterpenic esters to elute after the saturated esters on the nonpolar stationary phase. Gunstone *et al.* (21) investigated the retention time behavior of monounsaturated *cis*- and *trans*-fatty acid methyl esters on a nonpolar stationary phase and showed that the *trans*-fatty acid methyl esters eluted after the *cis*-fatty acid methyl esters. For similar reasons, the same elution pattern can occur in the case of saturated phytyl esters and unsaturated geranylgeranyl esters.

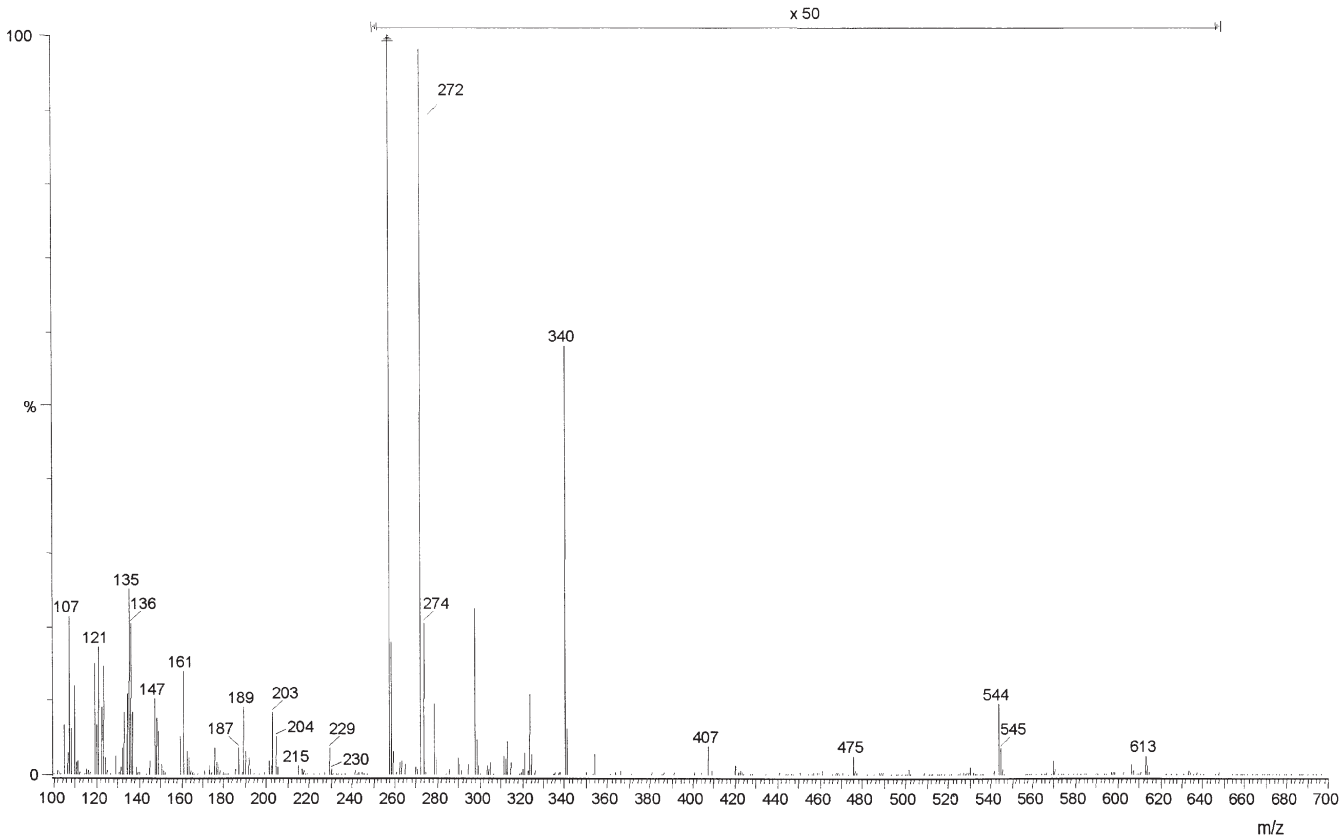

FIG. 5. EI spectrum of geranylgeranyl behenate (geranylgeranyl–FA 22:0, peak no. 19 in Fig. 2). $[M]^+$, *m/z* 613; $[M - R^1CO_2H]^+$, *m/z* 272; $[M - 205]^+$, *m/z* 407; $[M - 137]^+$, *m/z* 475; $[M - 69]^+$, *m/z* 544. See Figures 2 and 3 for abbreviations.

TABLE 5
Wax Ester Composition of the Three Different Sunflower Oils (in Percent of the Total Wax Ester Fraction)

	Sunflower no. 90	Sunflower no. 94	Sunflower oil (GC–MS)[a]
Phytyl–FA 18:1	1.7	5.9	6.6
Phytyl–FA 18:0	1.7	5.0	3.0
Phytyl–FA 20:0	1.5	4.8	1.8
Phytyl–FA 22:0	5.1	22.4	19.0
Phytyl–FA 24:0	—	2.6	2.0
Geranylgeranyl–FA 18:1	12.8	5.4	9.6
Geranylgeranyl–FA 18:0	6.1	3.1	4.7
Geranylgeranyl–FA 20:1	1.5	Trace	Trace
Geranylgeranyl–FA 20:0	5.2	3.3	3.9
Geranylgeranyl–FA 22:0	41.3	19.6	30.6
Geranylgeranyl–FA 24:0	9.2	3.4	7.3
Wax ester 42:0	6.2	6.9	6.1
Wax ester 44:0	3.8	12.0	1.9
Wax ester 46:0	4.1[b]	5.7	3.5

[a]GC–MS, gas chromatography–mass spectrometry.
[b]Sum of WE 46:0 and WE 46:1.

The full fatty acid composition of both phytyl and geranylgeranyl esters was determined. Molecular ions and fragment ions derived from fission within the phytyl chain of phytyl oleate are shown in Figure 3. Although all these ions are low in abundance, the fatty acid composition could be determined as mainly saturated and monounsaturated fatty acids ranging from FA 18 to FA 24 (Fig. 2, peak nos. 6, 7, 11, 12, 16, and 20) (Table 4).

Fissions within the unsaturated, branched geranylgeranyl chain occur due to allylic activation. The characteristic fragmentation of geranylgeranyl oleate is presented in Figure 2, and the EI spectrum of geranylgeranyl behenate is shown in Figure 5. As in the case phytyl esters, the fatty acid composition is mainly determined by saturated and monounsaturated FA 18 to FA 24 (Fig. 4, peak nos. 9, 10, 14, 15, 19, and 23); the largest portion within these diterpenic esters is generated by geranylgeranyl–FA 22:0. Analyses of the WE fraction of two additional sunflower oils (nos. 90 and 94) revealed that the concentration of diterpenic esters ranged from 75 to 90%. The distribution of diterpenic esters shows a wide variation. A low percentage of phytyl esters goes along with a high percentage of geranylgeranyl esters and vice versa. It is presumed that the generation of saturated and unsaturated diterpenic esters depends on the stage of maturation, but analysis of sunflower seeds in various stages of maturation is required to confirm this statement.

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[Received November 14, 2000; accepted June 1, 2001]