A Simple and Rapid Method for Concurrent Determination of Petroselinic and Oleic Acids in Oils

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ABSTRACT: A procedure to determine the petroselinic acid/oleic acid ratio in oils is described. The method is based on transesterification of the constituent fatty acids to methyl esters. An aliquot of the solution of the esters is then analyzed by capillary gas chromatography; another aliquot is used for epoxidation of the double bonds with 3-chloroperoxybenzoic acid and subsequent opening of the oxirane ring with hydrochloric acid to obtain the chlorohydrin derivatives. The hydroxy groups are then silanized, and the reaction mixture is analyzed by high-resolution gas chromatography–mass spectrometry. The procedure is precise, rapid, and reproducible, and several samples can be analyzed in one working day.
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KEY WORDS: High-resolution gas chromatography–mass spectrometry, petroselinic acid determination, petroselinic acid/oleic acid ratio.

Petroselinic acid (*cis*-Δ-6-octadecenoic acid) is a relatively rare fatty acid. Generally, it is one of the major fatty acids in some Umbelliferae seed oils, and it is present in parsley oil, extracted from dried seeds of *Petroselinum hortense* Hoffm. Moreover, this acid and/or its *trans* isomer can be found in some oleic-containing oils that are originally free of their presence; these acids can arise in these oils as a consequence of positional/geometric isomerization of oleic acid during some industrial treatments. In these situations, analytical checking is necessary to confirm the presence of these acids, which can serve as important markers of the processes used on the oil.

Oils rich in petroselinic acid can be used for some industrial processes (1), and the determination of the abundance of this acid is complicated by the presence of oleic acid (*cis*-Δ-9-octadecenoic acid). Petroselinic acid is always accompanied by some oleic acid, and a single chromatographic technique suitable for their separation and quantitation is not available. The analytical methods for quantitation of the two acids are based on a procedure first introduced in 1956 by von Rudloff (2). After gas-chromatographic analysis of fatty acid methyl esters, the same fraction is separated according to the degree of unsaturation by argentation thin-layer chromatography (TLC). The monoenoid ester band is recovered and analyzed by gas chromatography. The relative abundance of positional isomers in this fraction is then determined by an oxidation–gas chromatographic procedure (3,4); however, this method is not suitable for routine use.

In this paper, a simple and rapid procedure to determine the petroselinic/oleic acid ratio in oils is described. The method is based on transesterification of the fatty acids to methyl esters (5), epoxidation of the double bonds (6), and opening the oxirane ring to obtain the corresponding chlorohydrin derivatives (7). After silanization of the hydroxy groups, the mixture is analyzed using high-resolution gas chromatography (HRGC)–mass spectrometry (MS); obviously, simultaneous HRGC determination of fatty acid composition must be carried out. The procedure is precise and reproducible, and several samples can be analyzed in one working day.

MATERIALS AND METHODS

All reagents and solvents were of analytical grade (Aldrich, Milwaukee, WI). Standards of triacylglycerols were from Sigma (St. Louis, MO). Approximately 100 mg of each triacylglycerol standard was accurately weighed and dissolved in hexane to a final volume of 50 mL. Purity of the standard triacylglycerols was checked by the oxidation-HRGC procedure, and methyl petroselinate was found to be contaminated by 0.3% oleic acid methyl ester. This problem has been considered throughout this work, and proper corrections were introduced when necessary. Triacylglycerols from coriander seeds were extracted with a chloroform/methanol solution (2:1, vol/vol) and purified using TLC on silica by eluting them with hexane/diethyl ether (80:20, vol/vol).

Sample derivatization, HRGC of fatty acid methyl esters, and HRGC–MS of chlorohydrins-trimethylsilyl (TMS) derivatives. Triacylglycerols (10 mg) were methylated by sodium methoxide–catalyzed transesterification (5). A portion of the solution was used for HRGC analyses that were performed

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under the following conditions: gas-chromatograph apparatus Chrompack GC (Middelburg, The Netherlands), model 9001, equipped with a split-splitless injector (carrier gas flow rate: 1 mL He/min; split ratio 1:50); a flame-ionization detector (FID) (H_2 flow: 20 mL/min; N_2 make-up flow 19 mL/min; air flow: 150 mL/min) and a Mosaic software integration system; the capillary column was a Supelcowax 10 (length 30 m × 0.25 mm i.d., 0.25 µm film thickness) (Supelco, Bellefonte, PA). The system temperatures were injection port and FID, 270°C, and initial oven temperature, 160°C. The oven temperature was increased at a rate of 3°C/min to a final temperature of 240°C.

Another portion of the initial fatty acid methyl ester solution was used to purify these esters by TLC on silica with a mobile phase hexane/diethyl ether (80:20, vol/vol). The methyl ester band was scraped and extracted twice with hexane/diethyl ether. The organic fractions were pooled and, after solvent removal with a nitrogen stream, the test tube containing the residue was cooled in an ice/water bath (0°C). 3-Chloroperoxybenzoic acid (15 mg; 70%) was added. The mixture was then gently mixed with a polypropylenic stick. After 5 min, the mixture was removed from the cold bath and maintained at room temperature for a further 15 min. Diethyl ether (2 mL) and a 5% $Na_2S_2O_5$ water solution (2 mL) were then added to the mixture to reduce the unreacted peroxyacid. After separation of the aqueous phase, the organic layer was washed with a 5% $NaHCO_3$ aqueous solution (2 × 2 mL) to remove 3-chlorobenzoic acid.

The ether solution was then dried over anhydrous sodium sulfate. After solvent removal by evaporation in a nitrogen stream, the resulting epoxy compound was dissolved in a hydrochloric acid–saturated ether solution (0.5 mL) at room temperature. After standing a few minutes, the excess hydrochloric acid was removed by evaporation in a nitrogen stream (7). Hexamethyldisilazane (0.4 mL) and trimethylchlorosilane (0.2 mL) were added to the chlorohydrins dissolved in pyridine (0.6 mL) for hydroxy group silanization. The mixture was maintained at room temperature for 30 min prior to HRGC–MS analysis.

A Hewlett-Packard 5890 II series gas chromatograph, equipped with a split injector (split-ratio 60/1), was used throughout under the following conditions: HP-5MS fused silica column (1ength 25 m, 0.25 mm i.d., film thickness 0.25 µm, and phase ratio 250 (Hewlett-Packard, Palo Alto, CA); injection temperature, 280°C; oven temperature, 120°C for 2 min; then increased at a rate of 10°C/min to 280°C; and carrier gas flow-rate, 0.8 mL He/min. The system was interfaced with an HP 5971A mass spectrometer (Hewlett-Packard). The transfer line was held at 280°C, and the ion source at 180°C. Mass spectra were obtained at 70 eV, scanning over a mass range from *m/e* 50–450 for qualitative analysis and monitoring *m/e* 215.2 and 257.2 fragment ions in the single-ion monitoring mode for quantitative analysis. Data were acquired and processed by a 486 66 MHz PC, with HP ChemStation software (Hewlett-Packard).

RESULTS AND DISCUSSION

Epoxidation of unsaturated fatty acids is a well-known reaction (8), and it can be performed with a variety of oxidizing agents (mainly peroxyacids and hydroperoxides); the reaction time is quite long, generally more than 1 h. In this work, 3-chloroperoxybenzoic acid was added directly to the fatty acid methyl ester mixture and, when contact between the two reagents was maintained by careful mechanical stirring, the reaction was almost complete within a few minutes (6). The hydrochloric acid reaction that opened the oxirane ring was practically instantaneous and led to a mixture of positional isomeric compounds having TMS derivatives that are shown in Scheme 1, where **1** and **2**, from petroselinic acid, are methyl 7-trimethylsiloxy-6-chloride octadecanoate and methyl 6-trimethylsiloxy-7-chloride octadecanoate, respectively; **3** and **4**, from oleic acid, are methyl 10-trimethylsiloxy-9-chloride octadecanoate and methyl 9-trimethylsiloxy-10-chloride octadecanoate, respectively. The TMS derivatives of the chlorohydrins gave stable compounds, that could not be resolved under GC conditions described in a previous work (4).

A capillary column for HRGC separation resolved the two isomers obtained from petroselinic fatty acid methyl ester, while those obtained from oleic acid were not resolved. However, these findings do not influence the required result. The relative yield of the two possible isomers is constant within a defined set of experimental conditions, and reproducible results were obtained over several months.

The mass spectra recorded for the TMS derivatives of the chlorohydrins obtained from the fatty acids are shown in Figure 1. The mass spectrum of the TMS derivatives of the chlorohydrins obtained from oleic acid is the sum of the spectra of the two unresolved isomers. The *m/e* 257.2 ion from product 1 and the *m/e* 215.2 ion from product 3 in Scheme 1:

1 $CH_3OOC(CH_2)_4$–CHCl–CH(OTMS)–$(CH_2)_{10}CH_3$

163 ←|→ 257

2 $CH_3OOC(CH_2)_4$–CH(OTMS)–CHCl–$(CH_2)_{10}CH_3$

217 ←|→ 203

3 $CH_3OOC(CH_2)_7$–CHCl–CH(OTMS)–$(CH_2)_7CH_3$

205 ←|→ 215

4 $CH_3OOC(CH_2)_7$–CH(OTMS)–CHCl–$(CH_2)_7CH_3$

259 ←|→ 161

SCHEME 1

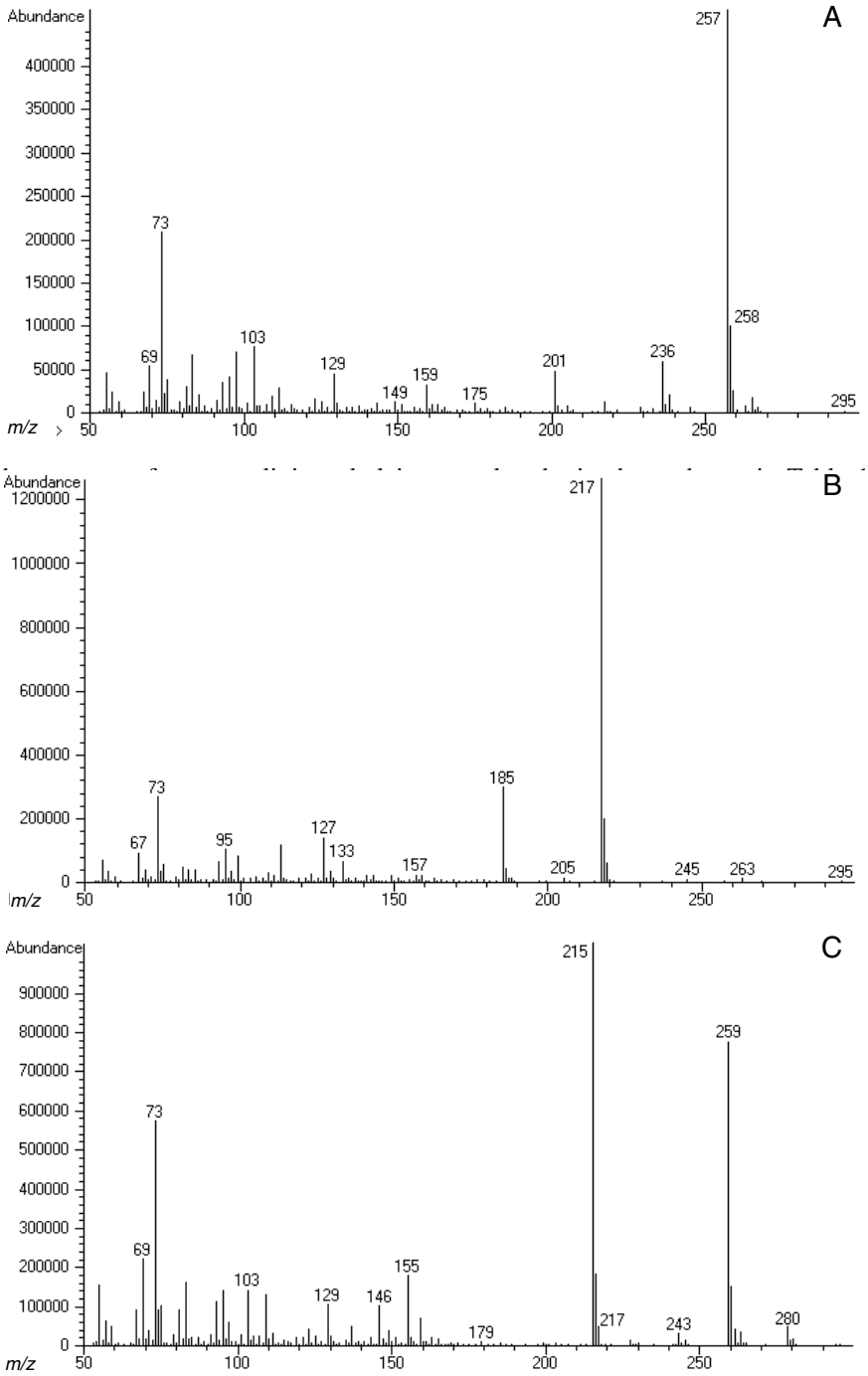

FIG. 1. Mass spectra of products shown in Scheme 1. (A) Product 1, (B) Product 2, and (C) Sum of products 3 and 4. Fragment ions of major interest are showed.

oleic acid ratio in a natural sample of coriander seed triacylglycerols, after HRGC analysis of fatty acid methyl esters from this oil was carried out under the conditions described. A total ion chromatogram, obtained for a natural sample of coriander seed oil, is reported in Figure 2. The peak with lower retention time is relative to methyl-7-trimethylsiloxy-6-chloride octadecanoate, the second peak represents the TMS derivatives of the other chlorohydrins. The

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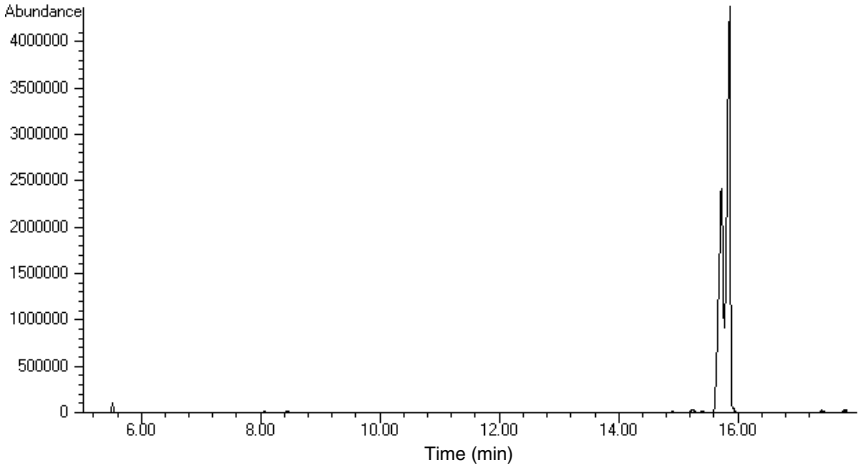

FIG. 2. Total ion chromatogram of trimethylsilyl derivatives of the chlorohydrins obtained from a natural sample of coriander fatty acid methyl esters.

TABLE 1
Percentage Relative Content of Petroselinic and Oleic Acids in a Natural Sample of Coriander Seed Oil

	Petroselinic (%)	Oleic (%)
Determination number 1	90.6	9.4
Determination number 2	91.0	9.0
Determination number 3	90.4	9.6
Determination number 4	90.8	9.2
Determination number 5	90.9	9.1
Average value	90.7	9.3
Coefficient of variation	0.24	2.24
Standard deviation	0.21	0.21

TABLE 2
Detection Limit for the Determination of Oleic Acid in a Petroselinic/Oleic Mixture (average of three determinations)

Oleic acid abundance		
Expected (%)	Found (%)	Coefficient of variation
0.30	0.46	46.0
1.29	1.25	3.4
2.25	2.01	3.7
3.20	2.99	9.4

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