Assessment of Olive Oil Adulteration by Reversed-Phase High-Performance Liquid Chromatography/Amperometric Detection of Tocopherols and Tocotrienols

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ABSTRACT: A method involving reversed-phase high-performance liquid chromatography with amperometric detection has been developed for the analysis of tocopherols and tocotrienols in vegetable oils. The sample preparation avoids saponification. Recoveries of α-tocotrienol and γ-tocotrienol in extra virgin olive oil were 97.0 and 102.0%, respectively. No tocotrienols were detected in olive, hazelnut, sunflower, and soybean oils, whether virgin or refined. However, relatively high levels of tocotrienols were found in palm and grapeseed oils. This method could detect small quantities (1–2%) of palm and grapeseed oils in olive oil or in any tocotrienol-free vegetable oil and might, therefore, help assess authenticity of vegetable oils.
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KEY WORDS: Adulteration, grapeseed oil, hazelnut oil, HPLC, olive oil, palm oil, soybean oil, sunflower oil, tocopherols, tocotrienols, vegetable oils.

Tocopherols and tocotrienols are derivatives of 2-methyl-6-chromanol with a side chain of three terpene units attached at C_2. The terpenoid side chain occurs in the saturated form (tocopherols) or in the unsaturated form (tocotrienols) with double bonds in positions 3′, 7′, and 11′. The difference between α, β, γ, and δ homologs of both tocopherols and tocotrienols lies in the number and position of methyl substituents on the chroman ring, as shown in Table 1. In addition, each tocotrienol can exist in four geometrical isomers (*cis–cis, cis–trans, trans–cis, trans–trans*). Tocopherols and tocotrienols are particularly important in vegetable oils for the following reasons : (i) they have a nutritional function for humans as vitamin E (1,2); (ii) tocopherols contribute, in association with polyphenols and other substances (3), to the prevention of oxidation of vegetable oils, and their quantity provides information about the oxidative state of the oil; and (iii) some authors (4–7) have suggested utilization of tocopherols as tracers for the identification and differentiation of vegetable oils. Unfortunately, the technique is applicable in only a few situations, and oils are often better identified by other distinctive parameters (fatty acids and sterols). However, the presence of tocotrienols may be potential markers for the presence of palm and grapeseed oils in olive oil (8,9).

TABLE 1
Position of Methyl Groups in Tocopherol and Tocotrienol Homolog Forms

Current name	R1	R2	R3
α-Tocopherol (α-T)	Me	Me	Me
β-Tocopherol (β-T)	Me	H	Me
γ-Tocopherol (γ-T)	H	Me	Me
δ-Tocopherol (δ-T)	H	H	Me
α-Tocotrienol (α-T3)	Me	Me	Me
β-Tocotrienol (β-T3)	Me	H	Me
γ-Tocotrienol (γ-T3)	H	Me	Me
δ-Tocotrienol (δ-T3)	H	H	Me

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Chemical–physical controls of olive oils, as established within the European Union (Commission Regulation, EEC, No. 2568/91 and further modifications), aim to guarantee their authenticity and quality. Some of the methods for authenticity assessment, even if effective, are particularly time-consuming and laborious. None of them involve tocopherols or tocotrienols. Moreover, no methods are available to detect specific types of adulteration, such as hazelnut oil or high-oleic sunflower oil, at a low addition percentage.

The present work was carried out to develop a reliable and sensitive method to measure tocopherols and tocotrienols in vegetable oils and to evaluate its applicability to the control of adulteration of olive oil.

Variable data for tocopherol and tocotrienol contents are reported in the literature as a result of the different analytical methods applied (8,10–12). Tocopherols and tocotrienols are highly sensitive to light and air, so procedures involving many manipulations can result in their partial degradation, and subsequently, in important quantification errors. Therefore, sample preparation is the critical point of the analysis. When direct analysis of the product is impossible, warm saponification at 70–100°C is the most frequently used pretreatment. However, even when working under protective conditions, recoveries are low, generally less than 80% (1,2). In the last ten years, high-performance liquid chromatography (HPLC) has been widely used. Reversed-phase HPLC (RP–HPLC) presents the advantage of short equilibrium and analysis times and high reproducibility of retention times.

The technique is limited by the coelution of β and γ isomers of both tocopherols and tocotrienols. Normal-phase HPLC (NP–HPLC), on the contrary, allows good separation of the eight molecules, but the analysis time is longer than that of RP–HPLC, and retention times are more variable (2). Spectrophotometric, fluorimetric, and amperometric detectors are generally used in this type of analysis. They differ in sensitivity, with the spectrophotometric detector being the least sensitive and the amperometric detector the most sensitive (1). Our objectives were to develop a method that avoids warm saponification, and has high recovery and a detection limit low enough to allow the detection of small amounts of foreign oils in olive oils.

EXPERIMENTAL PROCEDURES

Samples. The following samples were submitted for analysis: 19 extra virgin olive oils (9 from Italy, 5 from Spain, and 5 from Greece); 16 nonedible virgin olive oils, commercially called "virgin lampante olive oil" (5 from Italy, 6 from Spain, 3 from Greece, and 2 from Tunisia); 6 refined olive oils (blends of different origins); 14 fractionated and refined palm oils (blends of different origins); 1 mono-, 1 bi-, and 1 trifractionated palm oil (from the same initial sample); 3 virgin unrefined grapeseed oils (from France); 2 refined grapeseed oils (1 from Italy and 1 from Switzerland; commercial samples); 8 virgin hazelnut oils [2 from Italy and 1 from Turkey (both pressed in a pilot plant), 2 from Italy extracted with hexane in the laboratory, and 2 from Italy, and 1 from Switzerland (both commercial samples)]; 4 refined high-oleic sunflower oils (from Italy; commercial samples); and 3 refined soy oils (from Italy).

Solvent and chemicals. All analytical reagent-grade chemicals and HPLC solvents were purchased from Merck (Zurich, Switzerland).

Mobile phase. Two different methods were used to analyze tocopherols and tocotrienols: (i) NP–HPLC: hexane/2-propanol (99.7:0.3); and (ii) RP–HPLC: 0.05M sodium perchlorate aqueous solution/methanol (10:90). Both mobile phases were degassed under helium.

Standard solution. Two different standard solutions were used: (i) for NP–HPLC, 0.01 mg/mL solutions were made up in hexane for each α-, β-, γ-, and δ-tocopherol (abbreviated as α-, β-, γ-, and δ-T) (Merck) and each α-, β, γ-, and δ-tocotrienol (abbreviated as α-, β-, γ-, and δ-T3) (Merck). (ii) RP–HPLC, 0.25 μg/mL solutions were made up in methanol for each α-, β-, γ-, and δ-T and each α-, β-, γ-, and δ-T3.

Liquid chromatography (LC). NP–HPLC was performed with the following equipment: a Model LC-10AD pump (Shimadzu, Burkard Instruments, Zurich, Switzerland); a Model SIL-10A auto injector (Shimadzu) fitted with a 50-μL sample loop; a Model SPD-M10A diode array detector (Shimadzu) scanning between 280 and 350 nm; a Model RF-551 fluorescence detector (Shimadzu) with excitation set at 290 nm and emission set at 330 nm; a Model CBM-10A communication bus module (Shimadzu) and Class LC10 software package (Shimadzu) for data acquisition, analysis display, and system control.

The RP–HPLC was performed with the following equipment: a Model 114M pump (Beckman, Buchi, Switzerland); a Model LC-17A manual injector (BAS, Bucher, Switzerland) fitted with a 20-μL sample loop; a Model LC-4B amperometric detector (BAS) with potential set at 0.600 V; a Model R100A recorder (Perkin Elmer, Science Parc, Switzerland).

LC columns and chromatographic conditions. For NP–HPLC, a Lichrosorb Si 60 column (5-μm, 250-4 mm; Merck) was used at room temperature and flow rate 1.7 mL/min. For RP–HPLC, a Spherisorb ODS column (5-μm, 250-4.6 mm; Merck) was used at room temperature and flow rate 2.0 mL/min.

Sample preparation. For NP–HPLC/fluorimetric-photodiode array detector/direct analysis, the oil (1 g) was diluted to 100 mL with hexane and directly analyzed by HPLC. For RP–HPLC/amperometric detector/direct analysis, the 1 g of oil was diluted to 50 mL with tetrahydrofuran, and 15 mL of this solution was further diluted to 100 mL with methanol before the injection. For NP–HPLC/fluorimetric-photodiode array detector/cold saponification, 2 g oil was spiked with 2 mL of 0.01 mg/mL of α-T3 and γ-T3 standards and dissolved in a mixture of 3 mL water and 20 mL ethanol. Potassium hydroxide pellets (3 g) and sodium L(+)ascorbate (0.1 g) were added. The solution was shaken for 60 min at room temperature, in the absence of light, and extracted with hexane (2 × 30 mL). After the extraction, the organic phase was washed with water, taken to dryness, and diluted to 2 mL with hexane before the injection. A second saponification experiment was performed under the same conditions, with 2 × 30 mL diethyl ether for extraction instead of hexane.

Statistical calculations. Means, standard deviations, confidence limits, relative standard deviations, regressions and limits of detection were calculated, at a 95% confidence level, according to statistical procedures as reported by Miller and Miller (13). Two 50-μL vol of standard and sample solution were injected into the two different HPLC systems. Results were calculated according to the following procedure:

$$(H \times C' \times D)/(H' \times W) \qquad [1]$$

where H and H' = height of the tocopherol or tocotrienol peak in the sample and standard solutions, respectively; C' = concentration of tocopherol or tocotrienol in the standard solution (mg/mL); W = weight of sample taken (g); and D = dilution factor (10000 for NP–HPLC and 333.3 for RP–HPLC). All results were expressed in mg tocopherol or tocotrienol per 100 g oil.

Tocopherol identification. Separate isomeric forms of tocotrienols (Merck) were injected according to the previously described conditions. The identification of these compounds in the samples was performed by means of the retention time and the diode array spectrum.

Tocotrienol identification. Separate isomeric forms of tocotrienols, commercially available from Merck, were injected

according to the previously described conditions, and each showed a side peak. The diode array spectrum of each side peak was compared with a spectrum of each tocotrienol to establish the nature of these compounds. The results showed a perfect overlap of each tocotrienol with its side peak. It was assumed that each tocotrienol standard was a mixture of four geometrical isomers, which could be partly separated under the described HPLC conditions. For this reason, the calculations for each tocotrienol were made by considering the sum of the heights of the two peaks.

RESULTS AND DISCUSSION

Two different HPLC techniques were evaluated and compared. In the first method, tocopherol and tocotrienols were separated on an NP column and detected with a fluorimetric and a diode array detector. In the second procedure, tocopherols and tocotrienols were separated on an RP column and detected with an amperometric detector.

NP–HPLC/fluorimetric/diode array detection method evaluation. An NP–HPLC system was used because of its great ability to resolve all the compounds of interest and, also, because hexane could be used as solvent, which allowed the solubilization of oil and all analytes. To unambiguously identify the isomeric forms of tocopherols and tocotrienols, a fluorimetric and a photodiode array detector were connected in series after NP–HPLC. Different chromatographic conditions were examined to find the optimal separation conditions for each isomer (Table 2). In particular, separation between γ-T and β-T3 was considered as a discriminating criterion. Peak shape and analysis time also were considered. The best conditions were obtained with a Lichrosorb Si 60 column, a mobile phase of hexane/2-propanol (99.7:0.3), and a flow rate of 1.7 mL/min (Fig. 1). Even if the obtained separation between γ-T and β-T3 was not optimal, it was considered sufficient for the purposes of the present study.

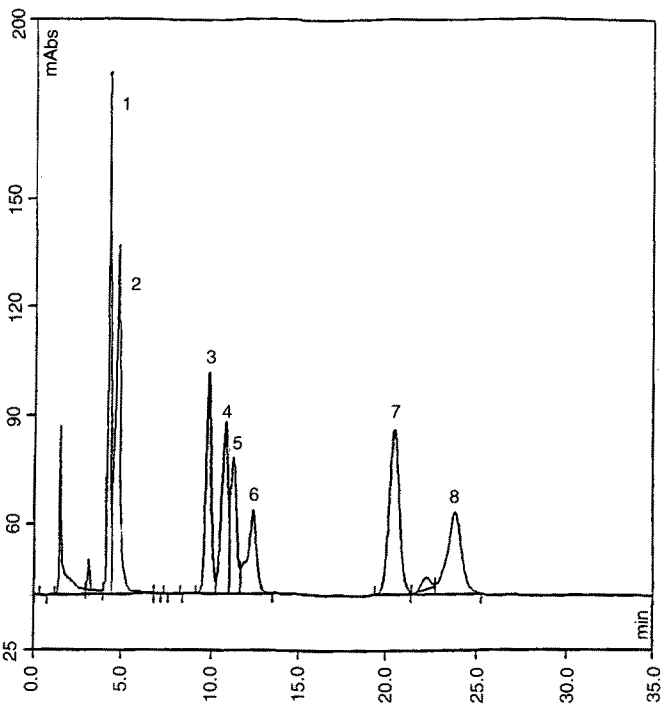

FIG. 1. Normal-phase high-performance liquid chromatography/fluorescence detection analysis of a mixture of tocopherol and tocotrienol standards (0.01 mg/mL for each compound). See conditions in the text. Peak identification: 1, α-tocopherol; 2, α-tocotrienol; 3, β-tocopherol; 4, γ-tocopherol; 5, β-tocotrienol; 6, γ-tocotrienol; 7, δ-tocopherol; and 8, δ-tocotrienol.

Linearity. Linearity was assessed for α-T and γ-T3 only. A linear detector response (correlation coefficient, r = 0.9996) was obtained over α-T and γ-T3 concentrations ranging from 1.0 to 20.0 μg/mL.

Detection limit (DL). Because the aim of the present work was to evaluate the applicability of tocotrienols as markers

TABLE 2
Influence of Chromatographic Conditions on the Separation of β-T3 and γ-T

Column[a]	Mobile phase	Flow (mL/min)	β-T3/γ-T separation
Spherisorb Si 60 (5 μm) 250-4.6	Hexane/*tert*-butyl-methylether (96:4)	2.0	–
Spherisorb Si 60 (5 μm) 250-4.6	Hexane/*tert*-Butyl-methylether (96:4)	1.5	–
Spherisorb Si 60 (5 μm) 250-4.6	Hexane/*tert*-butyl-methylether (98:2)	1.5	–
Spherisorb Si 60 (5 μm) 250-4.6	Hexane/*tert*-butyl-methylether/2-propanol (96.5:3.0:0.5)	1.5	–
Spherisorb Si 60 (5 μm) 250-4.6	Hexane/2-propanol (99.2:0.8)	1.5	–
Spherisorb Si 60 (5 μm) 250-4.6	Hexane/2-propanol (99.5:0.5)	1.5	–
Lichrospher Si 60 (5 μm) 250-4	Hexane/2-propanol (99.5:0.5)	1.5	–
Lichrospher Si 60 (5 μm) 250-4	Hexane/2-propanol (99.5:0.5)	1.0	–
Lichrospher Si 100 (5 μm) 250-4	Hexane/2-propanol (99.5:0.5)	1.5	–
Lichrosorb Si 60 (5 μm) 250-4	Hexane/2-propanol (99.5:0.5)	1.5	–
Lichrosorb Si 60 (5 μm) 250-4	Hexane/2-propanol (99.7:0.3)	2.0	+
Lichrosorb Si 60 (5 μm) 250-4	Hexane/2-propanol (99.7:0.3)	1.5	+
Lichrosorb Si 60 (5 μm) 250-4	Hexane/2-propanol (99.7:0.3)	1.7	+
Lichrosorb Si 60 (5 μm) 250-4	Hexane/2-propanol (99.8:0.2)	1.8	+

[a]Columns from Merck (Switzerland). See Table 1 for abbreviations.

for low levels (<5%) of olive oil adulteration, it was important to know their DL. The DL of γ-T3, the most important marker, was calculated with a statistical procedure (13) at 95% confidence. The DL was 1.9 mg of γ-T3 per 100 g of oil.

Recovery experiments. After optimization of the chromatographic conditions, the accuracy of the method was evaluated by recovery experiments of standard compounds. Known amounts (20 mg/100 g oil) of α-T and γ-T3 were added to a sample of fractionated palm oil and analyzed in triplicate by the previously described method. Recoveries were good, 98.6% for α-T and 99.0% for γ-T3, respectively.

Repeatability. The repeatability of the method was determined with a sample of fractionated palm oil with a high tocotrienol content. The sample was analyzed ten times within the same day and under the same analysis conditions. The repeatability relative standard deviations (RSDr) were 0.5% (α-T), 0.9% (α-T3), 6.8% (β-T3), 1.5% (γ-T3), and 5.3% (δ-T3). The values for β-T3 and δ-T3 were relatively high because of their low concentrations (1.5 and 6.8 mg/100 g, respectively).

Analysis of vegetable oils. The tocopherol and tocotrienol profiles of extra virgin olive oil and fractionated palm oil samples were determined with the optimized method (Table 3). Similar levels of α-T were found in olive and in palm oil. No tocotrienols were present in olive oil; however, all four tocotrienol homologs were detected in palm oil. γ-T3 was the major tocotrienol, with concentrations ranging from 18.7 to 35.6 mg/100 g. Therefore, γ-T3 may be a good tracer for the presence of palm oil in olive oil. Allowing for the lowest γ-T3 content determined in palm oil and for its measured DL (1.9 mg/100 g), it could be deduced that the method was not sensitive enough to detect the presence of less than 5% of palm oil in olive oil. Trials to lower this detection limit by cold saponification of the sample in the presence of sodium ascorbate to prevent oxidation were not successful.

TABLE 3
Tocopherol and Tocotrienol Content in Extra Virgin Olive Oils and Fractionated Palm Oils (mg/100 g)[a]

Oil	α-T	β-T	γ-T	δ-T	α-T3	β-T3	γ-T3	δ-T3
Oliver oil								
Italy 1	21.2	—	—	—	—	—	—	—
Italy 2	15.1	—	—	—	—	—	—	—
Italy 3	16.3	—	—	—	—	—	—	—
Italy 4	16.1	—	—	—	—	—	—	—
Palm oil								
Palm 1	15.0	—	—	—	5.5	1.4	20.0	5.7
Palm 2	21.8	—	—	—	11.3	1.5	25.7	6.8
Palm 3	20.7	—	—	—	11.4	1.8	28.9	8.5
Palm 4	22.7	—	—	—	11.7	1.3	28.7	8.1
Palm 5	15.3	—	—	—	8.4	1.0	18.7	4.8
Palm 6	22.8	—	—	—	7.8	1.6	35.6	6.7

[a]Normal-phase high-performance liquid chromatography/fluorimetric detection method. Abbreviations as in Table 1.

RP–HPLC/amperometric detection method evaluation. A further attempt to lower the DL was to use RP–HPLC with amperometric detection, which is generally reported as a sensitive technique (2). The RP–HPLC method does not separate β-T3 and γ-T3. However, this was not considered a problem to determine olive oil authenticity.

Amperometric potential evaluation. A sample of fractionated palm oil was analyzed at four different amperometric potentials—0.500, 0.600, 0.700, and 0.800 V—to establish the optimal working potential. The potential of 0.600 V was chosen because it offered the best compromise for the various detector responses of the different isomeric forms of tocopherols and tocotrienols.

Linearity and calibration curve for γ-T3. The linearity was assessed for γ-T3 only. A linear detector response (correlation coefficient, r = 0.9999) was obtained over γ-T3 concentrations ranging from 0.025 to 0.500 μg/mL.

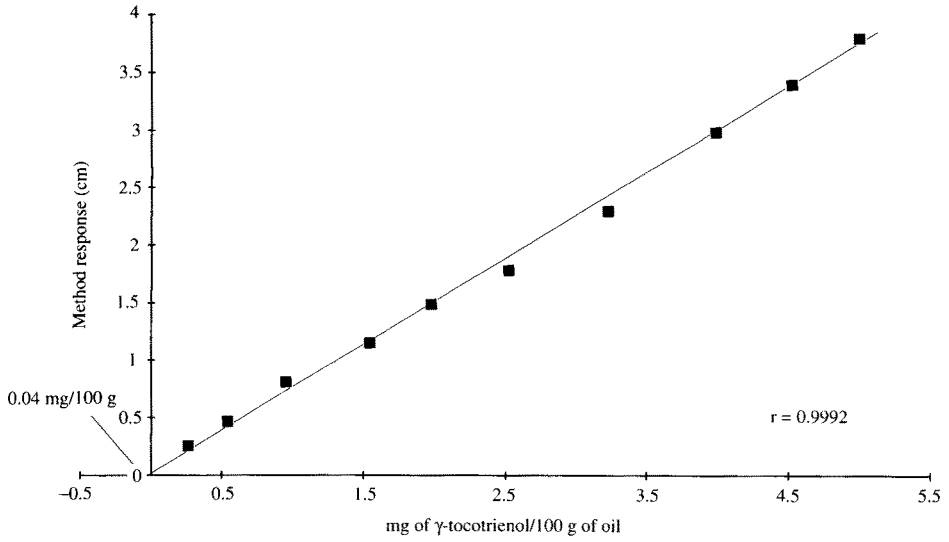

FIG. 2. Evaluation of matrix effect: reversed-phase high-performance liquid chromatography method response (cm) as function of increasing quantity of γ-tocotrienol added to a sample of extra virgin olive oil.

DL and influence of matrix. The DL of γ-T3 only was calculated with a statistical procedure (13), at a 95% confidence level, from the data obtained from the linearity control. The lowest quantity of γ-T3 detectable corresponded to 0.15 mg/100 g of oil. Consequently, the technique was much more sensitive than the NP–HPLC/fluorimetric detection method. This DL was determined with tocotrienol standard solutions without considering the matrix effect. To evaluate the influence of the matrix, a sample of extra virgin olive oil was spiked with increasing amounts of γ-T3 and analyzed with the RP–HPLC method (13) (Fig. 2). Linear regression gave a correlation coefficient of r = 0.9992 and an intercept at the x axis of 0.04 mg/100 g. It can be concluded that the matrix does not affect the determination of γ-T3. The reported data also were used to calculate the true limit of detection (13). A value of 0.20 mg of γ-T3/100 g oil was obtained, which is close to that calcu-lated with standard solutions and confirms the absence of a matrix effect.

Recovery experiment and repeatability. Known amounts (1 mg/100 g oil) of α-T3 and γ-T3 were added to a sample of extra virgin olive oil, containing no tocotrienols, and analyzed in triplicate by using the previously described method. Recoveries were good, 97.0% for α-T3 and 102.0% for γ-T3.

Repeatability. The repeatability of the method was determined with a sample of fractionated palm oil. The sample was analyzed ten times within the same day. RSD were 0.9% (α-T), 3.9% (β-T + γ-T), 1.4% (α-T3), 0.7% (β-T3 + γ-T3), and 2.5% (δ-T3), which showed good precision of the method, even for compounds at low concentrations.

Analysis of vegetable oils. The tocopherol and tocotrienol profiles of different vegetable oils were determined with the RP–HPLC/amperometric detection method (results are given in Tables 4–6). Extra virgin and virgin "lampante" olive oils were of different ages, ranging from a few months to four years old, and from different geographical origins. Results showed that δ-T and tocotrienols did not occur in olive oils, whatever their age, origin, and degree of refining (Table 4). α-T was the major tocopherol present, with concentrations ranging from 14.7 to 23.7 mg/100 g in extra virgin oil, whereas β-T and/or γ-T were present at low levels. Interestingly, no α-T, β-T, and/or γ-T were found in four nonedible virgin olive oils (lampante). All data obtained were in agreement with those already reported in the literature (8,10–12).

Fractionated palm oil exhibited a tocopherol profile close to that found in olive oil, but a different tocotrienol profile (Tables 5 and 6). β-T3 + γ-T3 ranged from 20.0 to 43.3 mg/100 g (Table 5), whereas lower quantities of α-T3 (4.9–18.4 mg/100 g) and δ-T3 (4.5–9.9 mg/100 g) were found. Fractionated palm oils were two years old and had been stored at room temperature in the presence of light. This means that the tocotrienol content of fractionated palm oil remained high, even after nonoptimal storage conditions. Considering these results and the DL for γ-T3 (0.20 mg/100 g), it should be possible theoretically to detect about 1% of palm oil in olive oil by using β-T3 + γ-T3 as markers.

A chromatogram of a sample of extra virgin olive oil, spiked with 1% of the palm oil sample containing the lowest β-T3 + γ-T3 concentration (20.0 mg/100 g), is given in Figure 3. The tocotrienols β-T3 + γ-T3 (peak 1) can still be clearly detected and identified. The proposed procedure is more sensitive than those previously reported. Indeed, the detection of this foreign oil in olive oil *via* the determination of myristic acid is about at 3%, whereas when using the triacylglyceride C_{48} as a marker, the DL is about 3–5% (14).

Three samples of palm oil of various fractionation degrees (mono-, bi-, and trifractionated) also were analyzed to evaluate the distribution of tocotrienols in the different fractions. Table 6 shows that tocotrienols increased during the fractionation process, suggesting that if highly unsaturated fractions

TABLE 4
Tocopherol and Tocotrienol Content in Olive Oils (mg/100 g)[a]

	Origin	α-T	β-T + γ-T
Extra virgin olive oil	Italy 1	22.7	1.3
	Italy 2	14.7	1.1
	Italy 3	18.0	1.0
	Italy 4	17.0	1.2
	Italy 5	22.2	1.6
	Italy 6	18.9	1.6
	Italy 7	19.9	1.1
	Italy 8	22.0	2.0
	Italy 9	23.7	1.9
	Spain 1	16.7	1.9
	Spain 2	18.3	1.6
	Spain 3	12.5	1.1
	Spain 4	20.5	1.6
	Spain 5	20.5	2.4
	Greece 1	19.2	1.6
	Greece 2	21.2	1.3
	Greece 3	22.1	1.2
	Greece 4	22.4	2.4
	Greece 5	19.7	1.7
Virgin lampante olive oil	Italy 1′	1.3	—
	Italy 2′	—	—
	Italy 3′	20.8	2.2
	Italy 4′	8.3	1.3
	Italy 5′	20.2	2.2
	Spain 1′	14.1	3.5
	Spain 2′	22.1	2.1
	Spain 3′	—	—
	Spain 4′	16.3	1.3
	Spain 5′	9.9	1.2
	Spain 6′	—	—
	Greece 1′	2.9	0.7
	Greece 2′	1.4	0.8
	Greece 3′	—	—
	Tunisia 1′	14.1	0.9
	Tunisia 2′	13.1	1.1
Refined olive oil	*Mixture 1*	12.5	1.1
	Mixture 2	9.6	0.8
	Mixture 3	10.6	1.1
	Mixture 4	10.9	0.9
	Mixture 5	14.4	1.2
	Mixture 6	14.1	1.1

[a]Reversed-phase high-performance/amperometric detection method. Abbreviations as in Table 1.

TABLE 5
Tocopherol and Tocotrienol Content in Vegetable Oils (mg/100 g)[a]

	α-T	β-T + γ-T	δ-T	α-T3	β-T3 + γ-T	δ-T3
Refined palm 1	16.0	1.9	—	5.9	22.0	5.8
Refined palm 2	20.7	3.2	—	12.0	28.1	6.9
Refined palm 3	20.9	3.2	—	11.5	30.5	8.2
Refined palm 4	21.3	2.6	—	12.2	29.4	8.1
Refined palm 5	15.7	3.0	—	9.0	20.0	4.5
Refined palm 6	21.9	2.0	—	8.0	37.1	6.8
Refined plam 7	17.3	2.8	—	17.1	41.7	8.9
Refined palm 8	17.3	3.2	—	15.8	43.3	9.1
Refined palm 9	20.7	3.6	—	15.1	37.5	8.0
Refined palm 10	17.8	3.6	—	14.3	33.7	6.4
Refined palm 11	18.8	3.6	—	18.4	32.0	9.9
Refined palm 12	17.8	3.2	—	13.6	31.5	6.0
Refined palm 13	16.9	3.2	—	14.3	37.6	7.6
Refined palm 14	6.3	1.2	—	4.9	20.5	4.8
Virgin grapeseed 1	5.8	1.9	—	8.8	27.1	0.7
Virgin grapeseed 2	6.4	2.1	—	9.3	27.8	0.7
Virgin grapeseed 3	5.8	1.9	—	9.4	28.4	0.9
Refined grapeseed 1	11.7	—	—	6.0	10.0	—
Refined grapeseed 2	17.0	—	—	8.0	22.1	—
Virgin hazel-nut 1	49.0	9.0	—	—	—	—
Virgin hazel-nut 2	32.0	1.2	—	—	—	—
Virgin hazel-nut 3	34.9	1.0	—	—	—	—
Virgin hazel-nut 4	36.5	13.1	0.5	—	—	—
Virgin hazel-nut 5	11.9	12.0	0.5	—	—	—
Virgin hazel-nut 6	40.2	3.5	0.8	—	—	—
Virgin hazel-nut 7	39.2	2.2	—	—	—	—
Virgin hazel-nut 8	44.2	2.6	—	—	—	—
Refined sunflower 1	44.1	2.2	0.3	—	—	—
Refined sunflower 2	62.5	7.7	0.7	—	—	—
Refined sunflower 3	58.2	4.2	0.8	—	—	—
Refined sunflower 4	58.0	4.0	0.9	—	—	—
Refined soy 1	9.6	66.9	26.4	—	—	—
Refined soy 2	8.7	75.8	33.1	—	—	—
Refined soy 3	3.4	45.6	16.6	—	—	—

[a]Method: reverse-phase high-performance liquid chromatography/amperometric detection. Abbreviations as In Table 1.

of palm oil were added to adulterate olive oil, the use of tocotrienols as markers is even more justified and the technique even more sensitive.

Grapeseed oil (Table 5) contained less α-T than extra virgin olive oil, but it had a total tocotrienol content of about two-thirds of that found in palm oil. Levels of β-T3 + γ-T3 ranged from 10.0 to 28.4 mg/100 g. Therefore, it should be possible to detect about 2% of grapeseed oil in olive oil using these tocotrienols as tracers.

Hazelnut and sunflower oils (Table 5) have two and three times, respectively, more α-T than extra virgin olive oil and do not contain any tocotrienols. Consequently, neither α-T nor tocotrienol can be considered as a good tracer for the presence of these two vegetable oils in olive oil.

No tocotrienols were detected in soy oil (Table 5), but large quantities of β-T + γ-T and γ-T were observed. This fact confirms the results obtained in previous studies (8,10–12). Allowing for their concentration ranges, these tocopherols could be used to detect a minimum of 10% of soy oil in olive oil. However, other analytical methods, which are more reliable and sensitive than this method, i.e., fatty acid and sterol composition, could be applied to trace lower amounts of soy oil.

The described RP–HPLC/amperometric detection method for the analysis of tocopherols and tocotrienols is accurate, repeatable, sensitive, and easy to perform. The simple sample preparation avoids saponification, and therefore, the risk of oxidation, and guarantees high recovery as well. Tocotrienols can be used as markers in olive oil for the presence of tocotrienol-containing oils, such as palm and grapeseed oils. Our results showed that the method allowed detection of 1%

TABLE 6
Tocotrienol Content in Fractionated Palm Oils (mg/100 g)[a]

	α-T3	β-T3 + γ-T3	δ-T3
Monofractionated	14.1	31.0	6.2
Difractionated	15.6	34.8	7.2
Trifractionated	19.3	39.5	7.8

[a]Method: reverse-phase high-performance liquid chromatography/amperometric detection. Abbreviations as in Table 1.

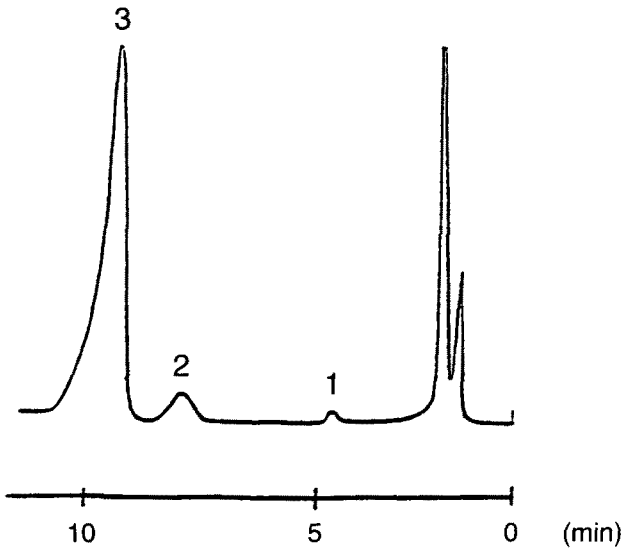

FIG. 3. Reversed-phase high-performance liquid chromatography/amperometric detection of 1% palm oil (β-tocotrienol + γ-tocotrienol content, 20.0 mg/100 g) in olive oil. Peak identification: 1, β-tocotrienol + γ-tocotrienol; 2, β-tocopherol + γ-tocopherol; and 3, α-tocopherol.

of palm oil and 2% of grapeseed oil in both virgin and refined olive oils. The method also could be extended to the detection of palm and grapeseed oil in other oils that do not contain tocotrienols (hazelnut, sunflower, soybean) as a tool to assess authenticity of vegetable oils.

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