

Analysis of Olive Oil and Seed Oil Triglycerides by Capillary Gas Chromatography as a Tool for the Detection of the Adulteration of Olive Oil

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Abstract

Individual triglyceride (TG) species of olive oil and several seed oils (corn, cottonseed, palm, peanut, soybean, and sunflower) are baseline separated on a WCOT TAP CB fused-silica capillary column by capillary gas chromatography (CGC) with a flame-ionization detector (FID) and either cold on-column or split injection. An adulteration of olive oil with a low content (< 5%) of these seed oils (except peanut oil) can be verified by the detection of the increasing levels of trilinolein or tripalmitin in olive oil in which these TG species are normally absent or present at very low levels (< 0.5%). An adulteration with over 20% peanut oil can be detected by the increasing levels of palmitodilinolein. TG species that can be coeluted with trilinolein in the reversed-phase high-performance liquid chromatographic (RP-HPLC) mode are baseline separated by the CGC technique, and their structures are identified by selective ion monitoring mass spectrometry. The following comparisons—the CGC-FID and RP-HPLC methods for detection of adulteration, cold on-column and split-injection modes for CGC-FID, and silylation or thin-layer chromatography pretreatment and simple dilution of one or more of the oil samples—are also presented. The normalized percentage area of the TG species is sufficient for the method limits used in this study. Mixtures of virgin olive oil with refined or residue olive oil could not be distinguished from the virgin type by the method used in this study.

Introduction

Olive oil is a valuable product that is traditionally produced in Mediterranean countries and now in the United States (particularly California) (1,2) with nutritional advantages concerning cardiovascular disease prevention. It is offered at a higher price than

other seed oils and for this reason is sometimes adulterated with other cheaper seed oils. This problem is addressed by developing reliable methods for the detection of adulteration in order to discourage this action.

A first approach to the detection of olive oil adulteration was made by a combination of two techniques—first the fractionation of the linoleic acid-rich triglyceride (TG) fraction of one or more oil mixtures by either column argentation chromatography (3) or low-temperature crystallization (4) and secondly by transesterification of the TG fatty acids followed by a gas chromatographic (GC) analysis of the resulting fatty-acid methyl esters (3,4). By these methods, the detection of adulteration was based on the quantitation of the linoleic-acid percentage in the mixture, which had the disadvantage of several complex step manipulations given that the detection limits could not be lower than approximately 5%. The attention of researchers was then focused on the composition and percentage of each TG present in the edible oils. Extensive identifications have been reported (5–23) for olive oil (7,13–17,19,21–23); high-trilinolein oils (i.e., cottonseed) (16,19); corn oil (6,8,16); soybean oil (7,8,11,13–16,18–20); sunflower oils (8,9,11,13,16,18); and low-trilinolein oils such as palm oil (11,16,18) and peanut (5,6,10–12,14) oils. These identifications were accomplished by reversed-phase (RP) high-performance liquid chromatographic (HPLC) methods.

The symbols LL (dilinoleoyl-glycerol or dilinolein), OLn (oleoyl-linolenoyl-glycerol), PSO (palmitoyl-stearoyl-oleoyl-glycerol), and OLLn (oleoyl-linoleoyl-linolenoyl-glycerol) are used for the different diglyceride or TG species in which each letter corresponds to each fatty-acyl moiety on the glycerol backbone sitting in increasing order on the carbon number chain and increasing unsaturation corresponding to A (arachidic acid) (20:0), Be (behenic acid) (22:0), Ga (gadoleic acid) (20:1, \emptyset^1), L (linoleic acid) (18:2, $\emptyset^{1,2}$), Ln (linolenic acid) (18:3, $\emptyset^{1,2,15}$), M (myristic acid) (14:0), O (oleic acid) (18:1, \emptyset), P (palmitic acid) (16:0), Po (palmitoleic acid) (16:1, \emptyset), and S (stearic acid) (18:0).

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This letter sequence for the TG species is followed because by the applied capillary gas chromatographic (CGC) technique, the exact location of each fatty-acyl moiety could not be determined even though these locations have already been proposed for several oil TGs after regiospecific lipase hydrolysis and mass spectrometry (MS) (36,37).

The RP-HPLC methods offered a new approach to the solution of the problem of adulteration based on the observation that the highly unsaturated TG species containing linoleic or linolenic acids (i.e., LLL, OLLn, PLLn, and SLnLn) or both are almost absent in olive oil (16), but some of them are predominant in the adulterant linoleic acid-rich oils. These TG species are coeluted on HPLC columns with a 10- μ m particle size packing and are called "critical pairs" because they share the same equivalent carbon number (ECN), namely ECN 42. The term ECN is defined by the equation:

$$\text{ECN} = \text{CN} - 2n \quad \text{Eq. 1}$$

where CN is the sum of the carbon atoms of the esterified fatty acids on the TG moiety and n is the sum of the double bonds (24). The quantitation by HPLC and the ultraviolet detection of the percentage of the ECN 42 fraction in the oil mixture have been effectively used for the detection of the olive oil adulteration within the levels of 2–5%, depending on the origin of the adulterant oil (13,15,25,26). The reproducibility of the ECN determination by HPLC with ultraviolet and refractive-index detection has been tested by the International Union of Pure & Applied Chemistry (IUPAC, Geneva, Switzerland) in circular analyses in 1986 (27) and adopted in 1991 by the European Communities (EC, Brussels, Belgium) (28). The RP-HPLC method for the determination of the ECN 42 fraction has the advantage that it requires only a one-step analysis with dilution of the oil, but it has the limitation of the coelution of the different TG species with the same ECN 42 in which TGs are present in different percentages in the different adulterant oils.

HPLC development in column stationary-phase packings with 5- μ m and 3- μ m particle sizes or silver ion packing (or both) resulted in improving the separation of the ECN 42 species. Therefore, the new ECN values were expressed in more specific terms as the theoretic carbon number (TCN) according to the equation:

$$\text{TCN} = \text{ECN} - U \quad \text{Eq. 2}$$

where U expresses the sum of the numerical factors specific for each individual fatty acyl moiety of the TG molecule (16,29,30). This is determined experimentally by U being considered 0.0 for palmityl, 0.6 for oleyl, and 0.7 for linoleyl resulting in POL having a TCN of 44.5 (29), 44.4 (16), or 43.65 (30) instead of the ECN 44. By these techniques, the ECN critical pairs LLL–PLLn and LLL–SLnLn can be satisfactorily separated, but no complete HPLC baseline separation could be achieved for the ECN critical pair LLL–OLLn (7,9,11,12,17–20) except in cases for the separation of LLL and OLLn having a comigration of OLLn with PLLn (11) or LLL with PLLn (31). Based on the quantitation of the LLL–OLLn fraction, the detection of olive oil adulteration has been effectively performed by using either 5- μ m column packing

with refractive-index detection (13) or 3- μ m column packing with light-scattering detection (15).

New data derived from olive oil samples produced in the North African Mediterranean countries Morocco and Tunisia showed that the contribution of LLL could exceed 0.7% and lead to a revision of the EC regulation (which appeared in 1997), thus introducing the ECN 42 difference between the ECN 42 value experimentally determined by HPLC analysis and the theoretical value calculated after a GC analysis of the main fatty acids of the oil (esterified or free) (32). This method is time consuming and requires the three steps of HPLC analysis of the oil TGs, isolation of oil fatty-acid methyl esters by transesterification, and GC analysis of the isolated fatty-acid methyl esters.

CGC, MS, or both CGC and MS have been successfully used for the analysis of edible oils (16,33–42). The separation and quantitation of the TG species for olive oil (16,35,36,40) and seed oils have also been reported (16,34,35,37,38,40,41). Different column coatings have been introduced (31,36,42–44), and the medium-polarity coatings of methyl-phenyl-silicone or phenyl-methyl-polysiloxane have resulted in the most sufficient separation (34–38,40). In these modes, the different TG species were eluted according to their equivalent chain length (ECL) (16) resulting in separation according to the molecular weight of the molecule and the degree of unsaturation (in most cases) with the same molecular weight. By this CGC technique, several ECN critical pairs of the HPLC mode were clearly separated such as LLL from PLLn (16,35) thus exhibiting distinct ECL values, and the separation of LLL and OLLn has also been reported (21–23) (the OLLn comigrating with PoPoL). Other CGC critical pairs also appeared such as OOO–SSL and AOO–SOGa (16) (the latter fortunately not present in olive oil).

In conclusion, the identified TG species in olive oil were LLL, OLLn, and PLLn for the relative ECN 42 and LLL and OLLn for the relative TCN 42, respectively, in RP-HPLC with LLL being the predominant. These TG species almost coeluted in RP-HPLC and can be used together as an individual fraction for the detection of adulteration with LLL-rich oils. Other TG species almost absent from olive oil but present in relatively high levels in palm oil and peanut oil were the PPP and PLL species, respectively. These TG species have not yet been used (either by HPLC or CGC) for the detection of olive oil adulteration with these two seed oils, which have a very low LLL content. All of the previously mentioned TG species were clearly baseline separated by the CGC technique, thus a good approach to the solution of the olive oil adulteration with seed oils with either high-LLL or low-LLL content seems to be the percent quantitation of LLL or PPP and PLL, respectively, in the oil mixture through their clear separation from the other possibly interfering TG species.

In this study, a CGC method for the estimation of olive oil adulteration in low levels with certain seed oils is reported for the first time. The method is based on the quantitation of the percent relative composition of certain individual TG species. Also, the baseline separation of the TG species of olive oil and four common adulterant seed oils (corn, cottonseed, soybean, and sunflower oils) with CGC is shown together with two other seed oils (palm and peanut oils) that are not usually used as adulterants, indicating the possibilities and limitations of this method. Finally, a comparison between different sample treatment techniques such as dilution,

silylation, or thin-layer chromatography (TLC) and between different CGC injection techniques (split or cold on-column) is presented. The relatively large number of references provide a brief review of the HPLC and CGC methods used for the separation of the TG species of the most common edible oils together with the methods used for the detection of olive oil adulteration.

Experimental

Materials

The CGC, HPLC, MS, and TLC solvents used were of HPLC grade from Rathburn Chemicals (Walkerburn, Peebleshire, U.K.). All other reagents and chemicals were of analytical grade and obtained from Merck (Darmstadt, Germany) except otherwise noted.

The vegetable oils of guaranteed quality that were supplied by the Food Division of the General State Laboratories of Greece (Athens, Greece) were corn oil, cottonseed oil, linseed oil, olive oil ("ordinary" or "pure", a commercial mixture usually composed of two-thirds the refined olive oil and one-third virgin olive oil), palm oil, peanut oil, soybean oil, sunflower oil, residue (kernel) olive oil, and virgin olive oil. The oils examined were dissolved in *n*-hexane to form 0.2% and 0.02% solutions.

Fatty-acid methyl ester standards were purchased from Sigma (St. Louis, MO). The AAA, BeBeBe, MMM, PPP, and SSS TG standards were purchased from AccuStandard (New Haven, CT) and Supelco (Bellefonte, PA) and prepared as 0.2% and 0.02% mixtures each in *n*-hexane. The other TG standards (shown in Figure 1) obtained from Chrompack (Middelburg, The Netherlands) were provided with the capillary column as a mixture in *n*-hexane of unknown concentration and was used after a 1:9 dilution in *n*-hexane.

Silylation of oils

Selected vegetable oils or olive and seed oil mixtures were silylated by accurately weighing one or more oils (100–200 mg) in screw-capped glass tubes, dissolving them in 10–20 mL pyridine, and then vortexing 1.0 mL of this solution with 0.25 mL

N,O-bis-(trimethylsilyl)-trifluoroacetamide (Aldrich, Stenheim, Germany) and 0.15 mL trimethylchlorosilane (Supelco) (a modification of that which can be found in reference 39). The mixture was allowed to react for 20 min at 80°C in a waterbath with a nitrogen atmosphere and then (after cooling at an ambient temperature for 20 min) diluted with pyridine to form 0.2% and 0.02% solutions.

TLC of oils

Total TG from selected oil samples were separated from the other lipid fractions by diluting one or more oils (1000 mg) in iso-propanol-*n*-hexane (1:4, v/v) in order to form a 10% w/v solution (a modification of reference 45). A quantity of this solution (200 μ L) was applied on a 20- × 20-cm TLC plate, impregnated with silica gel 60- and 70-230 mesh, and developed with petroleum ether (40°C–60°C boiling point), diethyl ether, and 1% acetic acid (70:30:1, v/v/v) as eluting solvents together with TG standards on a separate line. The separated standards were visualized with iodine vapors and the silica correspondent band with the purified oil TGs was scraped off, suspended in 4 mL of chloroform-methanol-water (1:2:0.8, v/v/v), vortexed, and then centrifuged for 5 min at 3000 rpm in screw-capped tubes. The solvent layer was removed, and by the addition of 2 mL chloroform and 2 mL water, a biphasic system was formed. After vortexing and equilibration for 30 min at 4°C, the chloroform layer was removed and evaporated under nitrogen, and the TGs were redissolved in *n*-hexane to form 0.2% and 0.02% final solutions.

Preparation of fatty-acid methyl esters

Fatty-acid methyl esters were prepared from one or more oil samples using boron-trifluoride in methanol (14:86, w/w) (Sigma) according to the derivatization method ISO 5509 (46).

CGC-FID for TGs

CGC of intact TGs was performed using a PerkinElmer (PE) (Norwalk, CT) Auto System Model GC equipped with cold on-column and split-splitless injectors (PE) and an FID. All TGs were separated on a medium-polarity open-tubular (WCOT) fused-silica capillary column (30-m × 0.25-mm i.d.) coated with a 0.1- μ m layer of methyl-65% phenyl-silicone TG CB-type phase (TAP) (Chrompack). The carrier gas was helium at either 2.2 mL/min (cold on-column) or 0.65 mL/min (split 1:20), and the injector and detector temperatures were set at 360°C and 375°C, respectively. In the cold on-column mode, the oven temperature was programmed from 70°C to 280°C at 40°C/min, raised to 352°C at 4°C/min, and then held isothermally for 20 min with 0.5- μ L injections of 0.02% sample solutions in *n*-hexane. In the split mode, the analyses were accomplished isothermally at 352°C with 3- μ L injections of 0.2% sample solutions in *n*-hexane or pyridine for the silylated samples. The percentage of each TG species (referring to the total TG area) was calculated by the PE computer system's software Turbochrom Version 4.

Each oil sample analysis was followed by a respective analysis of one or more TG standard mixtures under the same conditions, and each analysis was performed in triplicate. For the PPP, PLL, and LLL species, the recovery was over 90% with a coefficient of variation being less than 5%, which has been previously reported (39).

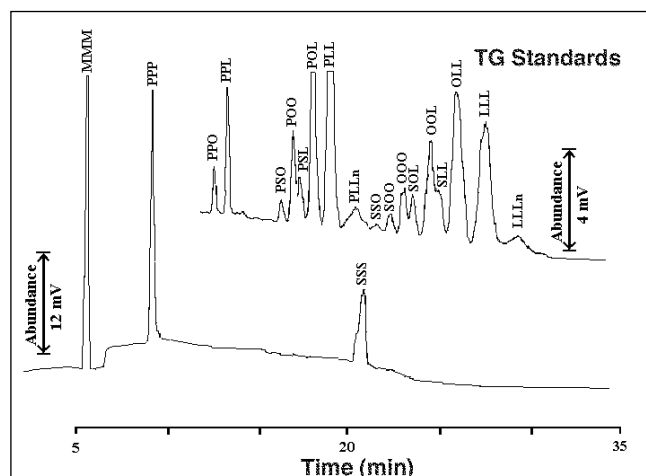


Figure 1. CGC-FID chromatograms of two TG standard mixtures with split (1:20) injections.

It should be noted that even though the FID response might not be equal for all the TG species (and thus the compositions quoted in this study not absolute compositions), these compositions can be used for comparative purposes because reproducible responses were obtained that can also be found in previously reported studies (15).

RP-HPLC for TGs

TG species of authentic and adulterated virgin olive oil of one or more samples (5% in acetone, w/v) were analyzed on a Novapack stainless steel column (30-cm \times 4.6-mm i.d.) packed with 5- μ m C18 bonded phase particles purchased from Waters (Milford, MS). The HPLC unit consisted of a Jasco (Tokyo, Japan) Model PU980 liquid chromatograph with a 20- μ L loop injector (Autosampler AS950) coupled with a Jasco 930 refractive-index detector. Acetone-acetonitrile (50:50, v/v) was used isocratically as the eluting solvent at a flow rate of 1.5 mL/min with a 36°C column temperature. The percentage of the ECN 42 fraction was calculated for both EC methods (28,32) by Jasco data software.

CGC-MS-SIM for TGs

A VG Biotech (Altrincham, U.K.) Model VG TRIO 2000 MS was used in the selective ion monitoring (SIM) mode with an ion-source temperature of 270°C. The carrier gas was helium at 0.65 mL/min. The same WCOT TAP CB capillary column was used as in the CGC-FID analysis but with a splitless injector system and a column temperature of 352°C held isothermally for 35 min. The [M]⁺, [RCO]⁺, and [M-OCOR]⁺ fragments (in which M is the molecular mass and R is the saturated or unsaturated carbon chain) of the TG species obtained were monitored by a Labase acquisition software system.

CGC-MS detector for fatty-acid methyl esters

Fatty-acid methyl esters from one or more oil samples were analyzed on a BPX70 SGE (Austin, TX) capillary column (50-m \times 0.25-mm i.d., 0.1-mm thickness) with helium as the carrier gas at a flow rate of 1.0 mL/min. A Hewlett-Packard (Palo Alto, CA) Model 6890 GC was used coupled to an HP MS detector Model 6890 (800 amu). The oven was programmed from 120°C to 220°C at a rate of 2.5°C/min and then held isothermally for 10 min. Injections of 1 μ L from fatty-acid methyl ester standards (0.1% in *n*-hexane) and samples (in *n*-hexane) were used with a split injector (1:25) at 230°C. Percentages of each fatty-acid methyl ester species were calculated by an HP data system, and the identification of each peak was accomplished by the aid of a Wiley MS library.

Results and Discussion

Peak identification

A CGC analysis of two TG standard mixtures was performed on a WCOT TAP CB column with FID detection for either split (as shown in Figure 1) or cold on-column injections (data not shown). From the chromatograms obtained, the split-injection technique was considered as the most suitable for revealing peaks of almost the same sharpness but with a reduced time of analysis

(35 min as compared with the 45-min interval of the cold on-column injections); therefore, this was applied to the analyses of the oil samples (Tables I and II) (Figures 2–4).

As shown in Figure 1, there were four groups of peaks corresponding to a CN of 48, 50, 52, and 54. In accordance with the ECL of the constituents, the elution order of the respective peaks of each group followed the degree of unsaturation with the more unsaturated eluting after the less unsaturated ones (i.e., PLL eluted after POL). For cases in which unsaturation and molecular weight were the same, the TG species with the higher amounts of double bonds on the same fatty-acid moiety exhibited higher retention time (i.e., SLL eluted after OOL and SLO eluted after OOO).

The OLL_n, PLL_n, and LLL species were originally reported to have similar ECN and TCN values in HPLC (24,29,30), but by the present CGC method, they were clearly separated and identified. As shown in Figure 1, PLL_n and LLL had a wide difference in retention times and could not interfere with each other. Although the OLL_n standard was not available, its presence was confirmed during the analyses of olive oil samples (as will be discussed). Other HPLC critical pairs that were clearly separated by CGC-FID were PLL-OOL and PPP-OOO. These separations were proved useful for the detection of olive oil adulteration with peanut and palm oil (as will be discussed), which have low LLL contents.

It has been stated that during CGC or GC-MS or both thermal degradations of polyethylenic TGs take place (16,35). These phenomena were observed for the LnLnLn standard in this study. For

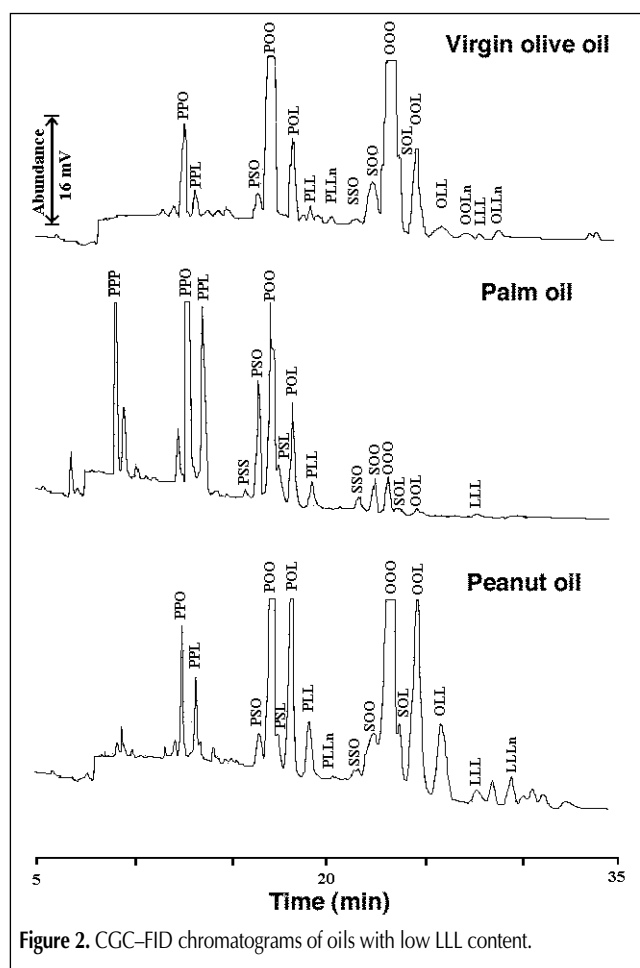


Figure 2. CGC-FID chromatograms of oils with low LLL content.

this reason, a clear chromatogram of the highly unsaturated TGs of linseed oil could not be achieved by this method (data not shown), although an LLLn standard and LLLn species derived from soybean oil were clearly detected as previously reported (34,41,47). The AAA and BeBeBe standards were not eluted by the conditions and technique used.

The peak identification of TG species from the oil samples examined was performed by a comparison of the retention times to those obtained from the available TG standards. The confirmation of the other peaks was deduced from the relative retention times of the standards and from the elution order of the TGs from different oil types that have been previously reported (16,33–42). Identification of the main peaks was also achieved by CGC–MS–SIM analysis of the oil samples, as also previously reported (34) (data not shown).

Features of olive oil

As shown in Figure 2, the CGC chromatographic patterns of virgin olive oil with split injection (1:20) and FID detection could be divided into three groups corresponding to a CN of 50, 52, and 54 with POP, POO, and OOO predominating in each group, respectively. These three main components showed good agreement with percentages in the oil TG mixture that have been previously reported (35,36) and good correlation to the respective retention times of the standards.

The CN 54 group of peaks consisted of two subgroups—the first including the main components SOO, OOO, SLO, OOL, OLL, and the minor SSO and the second including three minor peaks with higher retention times. For the latter three minor peaks, it can be stated that they corresponded to the OOLn, LLL, and OLLn species already reported for olive oil (16,21–23) as concluded from the comparison with their retention time distances from the main peaks of the first subgroup and the retention times of the LLL and LLLn standards (Figure 1). An effort for the direct iden-

tification of these three later-appearing peaks was made by CGC–MS–SIM analysis with the splitless injection of the virgin olive oil sample, but the very low content of these species in the oil did not allow for a direct and positive estimation. It is also noteworthy that PPP was absent (or present in traces) in the olive oil samples examined and PLL was found in very low levels (Table I and Figure 2) in accordance with previously reported values (21–23) almost within the higher range found for LLL (16,26). Finally, the OOA and OOGa species that have been previously identified in olive oil (16,22,23) were not recognized in this study, but their retention times could be predicted to be after the retention time of LLLn. The LLL content in the olive oil samples examined was found to be approximately 0.1–0.2% (Table I), which does not exceed the 0.5% limit of the EC 1986 regulations (28). The range of 0.1 to 0.7% has previously been reported (21–23,26), and in two cases (16,19), the 1.0% level has also been reported.

The possibility of improving the sensitivity of the detection was tested by the silylation of the oil sample in order to eliminate the possible influence of the oil polar fraction on the TG quantitation apart from its influence on the column stability. For the same reason, the purification by TLC of the total TG fraction of the oil was tested. As shown in Table I, no remarkable differences in the percentage range of the LLL fraction were observed after CGC–FID injections of these purified samples, and the percent content of PLL was slightly increased after silylation and slightly decreased after TLC purification. These results could also be considered as an advantage of the method because a simple dilution of the oil sample was sufficient without the requirement of manipulation and pretreatment of the sample.

As expected, the different edible olive types (virgin, pure, and residue) revealed similar CGC–FID chromatographic patterns and thus could not be distinguished by the CGC–FID technique in this study (data not shown). Adulteration of virgin olive oil with other refined oil types (including refined, pure, and residue olive) could be defined by the ultraviolet second-derivative technique (48). Because the most valuable oil is the virgin grade, the virgin olive oil type was used throughout this work.

Features of seed oils

As shown in Figure 3 and Table I, a common feature of the CGC–FID patterns of the four LLL-rich oils examined in this study (corn, cottonseed, soybean, and sunflower) was that the PPP species was absent and the LLL and PLL species could be considered as the main fractions. All of the above findings were in accordance with the values previously reported (5–23). Besides olive oil, there were also three groups of peaks in other oils corresponding to a CN of 50, 52, and 54 with the predominating species of PPL, PLL (or PLO or both), and OLL (or LLL or both), respectively, and the overall patterns of olive oil and these four seed oils (with the exceptions of LLL and PLL) remaining almost the same. The same relative patterns (shown in Figure 2) were observed in the CGC–FID analysis of the low levels in LLL content oils (palm and peanut oils). However, in palm oil, LLL and PLL were found at very low levels, but PPP consisted of approximately 10% of the total TGs (Table I). In peanut oil, PPP was absent and LLL and PLL species were found in low levels (Table I). The percentages of these TG species found by the method in this study (Table I) were also within the ranges that have been previously reported for palm oil

Table I. CGC–FID* Percentages of LLL, PLL, and PPP Triglyceride Species in Authentic Oil Samples

	LLL	PLL	PPP
Authentic olive oils			
Virgin	0.10	0.67	tr [†]
Virgin [‡]	0.10	0.65	nd [§]
Virgin (silylated)	0.11	0.71	tr
Virgin (total triglycerides after TLC)	0.09	0.60	tr
Pure	0.19	1.13	nd
Residue	0.10	0.74	nd
Authentic seed oils			
Corn	16.16	16.55	tr
Cottonseed	10.95	25.70	tr
Soybean	20.66	19.04	tr
Sunflower	12.41	9.99	tr
Palm	0.08	1.08	9.73
Peanut	0.72	2.89	nd

* Split-injection mode.

[†] tr, traces.

[‡] Cold on-column injection mode.

[§] nd, not detected.

(11.6% for PPP, 3.1% for PLL, and 0.0% for LLL) (16) and peanut oil (0.0% for PPP, 5.1% for PLL, and up to 3% for LLL) (11,14,20).

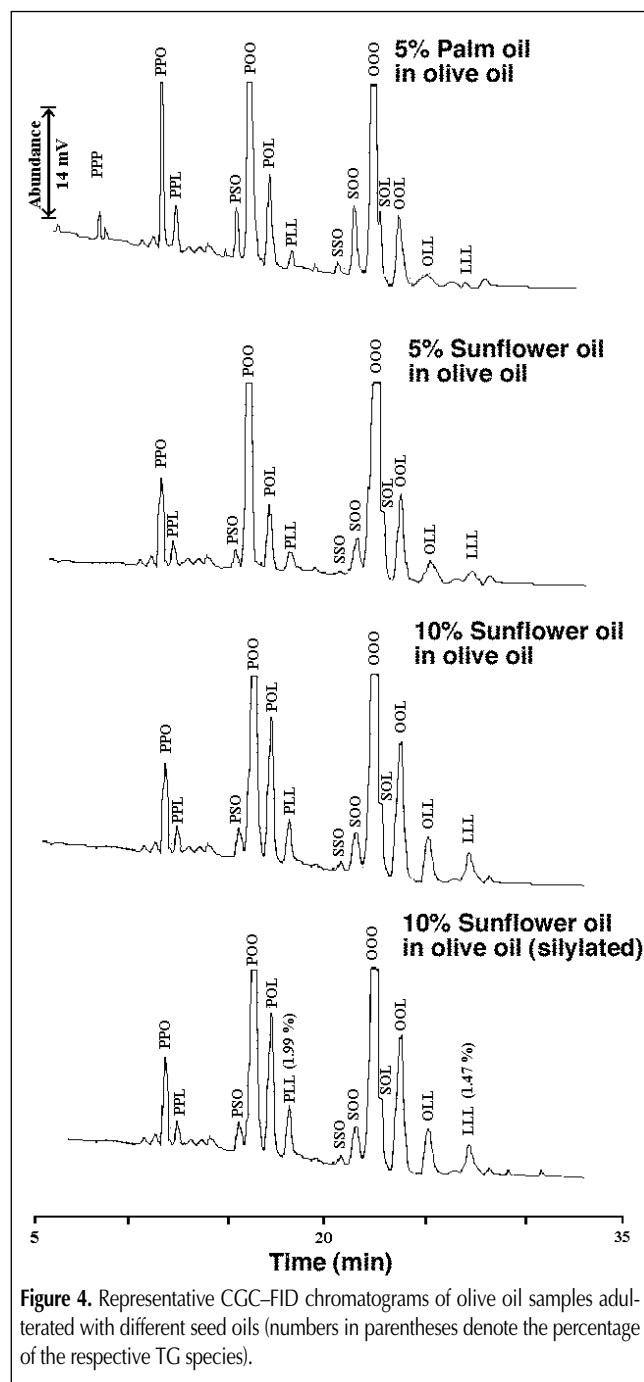
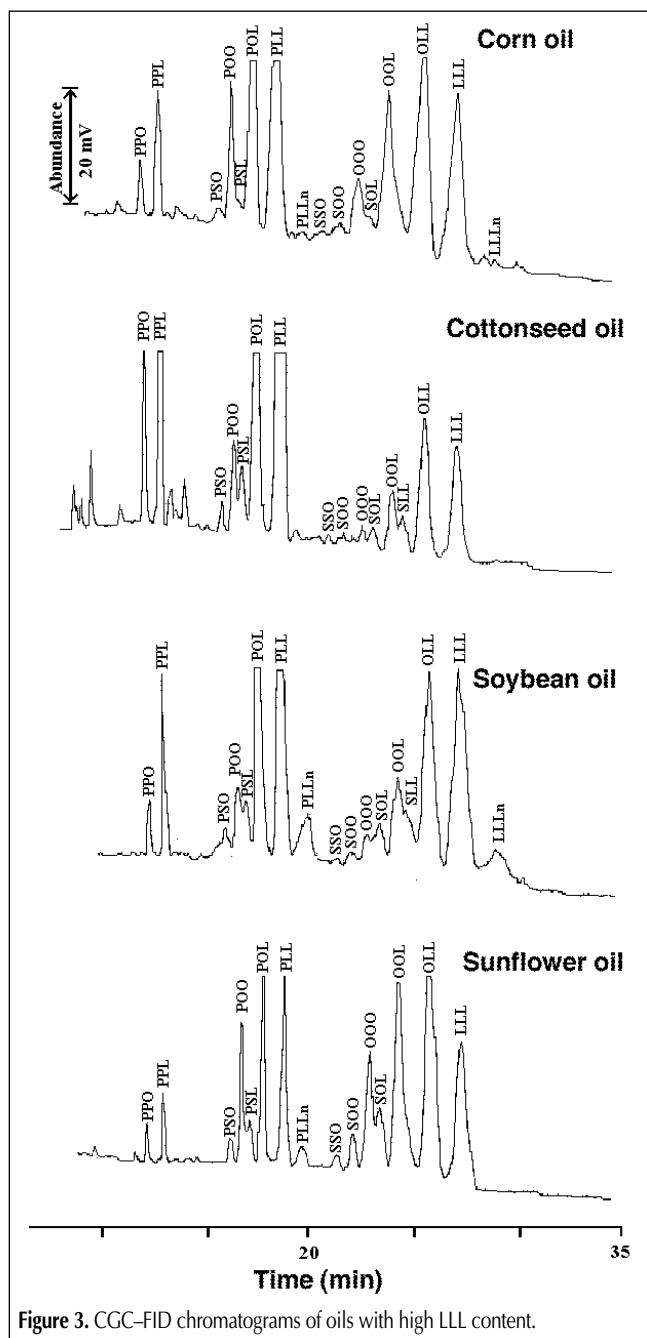
Another common feature for all the seed oils was that the HPLC critical pairs of ECN 42 (LLL, OLLn, and PLLn), ECN 44 (OLL and PLL), and ECN 48 (OOO, POO, and PPP) were clearly distinguished in CGC-FID, as was also shown in the case of olive oil (Figures 2 and 3).

Detection of adulteration

From the aforementioned CGC features of olive and seed oils, it can be concluded that the detection of adulteration can be performed by the CGC-FID quantitation of the LLL or PLL and PPP species or both in the adulterated samples. It is noteworthy to mention that the absolute intensities of the other fractions of the adulterant oils are quite close to those of olive oil. Therefore, the

addition of other LLL-, PLL-, or PPP-rich oils in olive oil will not appreciably change the respective part of the CGC pattern of the adulterated olive oil. An exception should be noted for the OLL species that were found at high levels in the LLL-rich oils (Figure 3) and in very low levels in the examined virgin olive oil (Figure 2), but this difference could not be suggested for the detection of adulteration because the range of 8.3 to 13.9% was reported for Italian olive oils (21). However, in other reports (13,16,19,22, 23,36), the calculated percentage was found to be between 0.8 and 5.8%.

As can be seen in Figure 4 and Table II, adulteration of olive oil with 5% LLL-rich seed oils (corn, cottonseed, soybean, or sunflower oils) resulted in an increment of the percent LLL and percent PLL levels above 0.7% and 1.0%, respectively. These figures



were very close to the higher levels reported for olive oil, and 10% adulteration resulted in at least 1.25% LLL and 1.52% PLL, clearly reflecting an adulteration. Adulteration with 2% could not be unequivocally detected, indicating the limits of the present method.

Adulteration with palm oil (having a very low content of LLL and PLL) can be detected by the presence of the PPP species in the adulterated olive oil. As shown in Figure 4 and Table II, adulteration with a 5% and 10% increase of the PPP fraction to 0.62% and 1.13%, respectively, permits a positive detection of the adulteration. An adulteration with a 2% increase resulted in 0.25% PPP and could not be unequivocally detected because a PPP content of 0.4% has previously been reported for olive oil (7,16).

Adulteration with peanut oil (in which PPP is absent and LLL and PLL exist in low levels) could not be detected even at a 10% adulteration result (Table II), which was within the ranges that have been reported for olive oil (16,21–23). Adulteration with 20% resulted in 0.25% LLL and 1.18% PLL in the adulterated olive oil, which was also within the aforementioned ranges. Determination of adulteration can be achieved at levels of approximately 30%. The same high detectable levels of adulteration have been reported for hazelnut oil (49), which has the same components with olive oil and in similar amounts (0.0% PPO, 0.7–1.1% PLL, and 0.9–1.9% LLL) (35–40). However, peanut and hazelnut oils are not common adulterants of olive oil, and their addition in olive oil at approximately 20% could be identified only for olive oils with an almost zero content in PLL or LLL or both.

CGC–FID analyses of silylated mixtures of sunflower oil and olive oil have not revealed remarkable differences in the percent

LLL range (Figure 4) as compared with those obtained from the same nonsilylated samples (Table II), but for PLL the percentage was increased (Figure 4) as in the case of the silylated virgin olive oil (Table I). Nevertheless, it can be stated that the silylation step could also be avoided. Silylations of the polar fraction of the oil is usually applied for extending the column life by minimizing the influence of the polar fractions on the stability of the column coating material.

The normalized percent area of the TG species proved to be sufficient for the detection of adulteration within the level of 5 to 10% or higher with commercial interest, and the absolute quantities of TGs might be useful for more accurate calculations in lower percentages of adulteration. In practice, a visual inspection of the CGC–FID patterns obtained can reveal the presence of 10% corn, cottonseed, soybean, sunflower, and palm oils, and in some cases, 5% adulteration could also be visually detected (i.e., soybean and palm oils).

Comparison of CGC–FID with RP–HPLC

For comparative reasons, the adulterated olive oil sample with 5% sunflower oil was also analyzed by RP–HPLC with refractive-index detection according to EC 1991 regulation (28) and the ECN 42 fraction was found to be 1.76%. This figure was quite different from the 0.74% that was found by CGC–FID (Table II) for the individual LLL content denoting the influence of the other TG species comigrating with the LLL species (the most probable being the OLLn species). The coelution of LLL and OLLn species in HPLC is a limiting factor for this method of the detection of olive oil adulteration. This coelution was confirmed by the CGC–MS–SIM analysis of a collected ECN 42 fraction of an olive oil sample, which was the very same oil that was used for the CGC experiments. A sufficient amount was collected after ten successive RP–HPLC analyses (100 μ L each injection) according to the official EC method for the detection of olive oil adulteration (28,32). This RP–HPLC fraction revealed two distinct peaks in CGC–FID and CGC–MS–SIM analysis with retention times and *m/z* fractions identical to those for LLL and OLLn. The presence of LLL was verified by the 861, 599, and 261 MS–SIM fragments corresponding to the molecular ion [M]⁺ (or [LLL]⁺), the diglyceride moiety [M–OCOR]⁺ (or [LL]⁺), and the linoleyl moiety [COR]⁺ (or [L]⁺), respectively, and the OLLn species was identified respectively by [M]⁺ = 859, [OLn]⁺ = 599, [LnLn]⁺ = 595, [O]⁺ = 265, and [Ln]⁺ = 261 fragments, as was also previously reported (34). The same adulterated sample analyzed by the EC 1997 method (32) resulted in 1.35% as the difference between the ECN 42 content (calculated by HPLC) and by the theoretical value of ECN 42 (calculated after fatty-acid methyl ester analysis), which was much higher than the 0.2% maximum limit for the adulteration. The latter figures allow for a much more positive estimation of the adulteration than with this study's CGC method, but this CGC method has the advantage of the one-step analysis.

Conclusion

The method described is a one-step rapid method (35 min) requiring only the dilution of the oil (as in the RP–HPLC method)

Table II. CGC–FID Percentages of LLL, PLL, and PPP Triglyceride Species in Adulterated Virgin Olive Oil Samples

	LLL	PLL	PPP
At 2% level with the seed oils			
Corn	0.41	0.80	tr [†]
Cottonseed	0.38	1.29	tr
Soybean	0.34	0.99	tr
Sunflower	0.37	0.77	tr
Palm	0.10	0.68	0.25
Peanut	0.12	0.34	tr
At 5% level with the seed oils			
Corn	0.89	1.39	tr
Cottonseed	0.71	2.03	tr
Soybean	0.99	1.54	tr
Sunflower	0.74	1.05	tr
Palm	0.10	0.69	0.62
Peanut	0.15	0.72	tr
At 10% level with the seed oils			
Corn	1.69	2.19	tr
Cottonseed	1.25	3.28	tr
Soybean	2.08	2.46	tr
Sunflower	1.35	1.52	tr
Palm	0.11	0.71	1.13
Peanut	0.18	0.84	tr

* Split-injection mode.

[†] tr, traces.

but without the shortcomings of the comigration of the HPLC critical pairs. This method offers the possibility of detecting and determining the adulteration of olive oil with common adulterant seed oils, but mixtures of virgin olive oil with refined or residue olive oil or both could not be distinguished from the virgin type. Adulteration of olive oil (virgin, pure, or residue) with seed oils could be detected in the range of 5% or higher and in some particular cases 2% or higher based on the percent quantitation of LLL or PPP-PLL species or both that are normally absent or in very low levels in olive oil but present in relatively high levels in the adulterant oils.

The method is reproducible and capable of providing detailed separation of the component TGs for a range of different types of oils containing a CN-*n* ratio of up to 54:7 using the common split-splitless injection system without the requirement of the more expensive cold on-column system. The method is also reliable as proved by the CGC-MS-SIM and HPLC analysis of coupled samples.

An improvement of the method presented in this study could be achieved by calculating the peak response factors of exactly calculated TG standards and determining the range of the LLL species alone for a large number of virgin olive oil samples of different origins and productions.

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