Detection of Olive Oil Adulteration with Canola Oil from Triacylglycerol Analysis by Reversed-Phase High-Performance Liquid Chromatography

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The objective of this study was to explore the use of reversed-phase high-performance liquid chromatography (RP-HPLC) as a means to detect adulteration of olive oil with less expensive canola oil. Previously this method has been shown to be useful in the detection of some other added seed oils; however, the detection of adulteration with canola oil might be more difficult due to similarities in fatty acid composition between canola oil and olive oil. Various mixtures of canola oil with olive oils were prepared, and RP-HPLC profiles were obtained. Adulteration of olive oil samples with less than 7.5% (w/w) canola oil could not be detected.

KEY WORDS: **Adulteration of olive oil, canola oil, composition, fatty acids, GLC, "low-linolenic" canola varieties, olive oil, reversed-phase HPLC, seed oils, triacylglycerol analysis, virgin olive oil.**

Olive oil is gaining in popularity in countries such as Canada and the United States due to increased consumer preference for its taste and aroma as well as to recent findings pertaining to potential health benefits. A diet rich in olive oil has been shown to cause a specific fall in non-high-density lipoprotein cholesterol, while leaving high density lipoprotein cholesterol and triacylglycerols unchanged (1).

Olive oil requires a clear identification of its purity because, due to its high market price, there have been, and will most likely continue to be, reports of adulteration in the industry (2,3).

The International Olive Oil Council defines olive oil products as follows: "Virgin olive oil is the oil obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions that do not lead to alterations in the oil. The oil has not undergone any treatment other than washing, decantation, centrifugation and filtration. Olive oil or pure olive oil (or 100% pure olive oil) is the oil consisting of a blend of virgin olive oil fit for consumption as it is and refined olive oil" (4). Refined olive oil is obtained from virgin olive oils by means of alkali refining, bleaching and deodorization.

In olive oil-producing countries, illegal additions to virgin olive oil could include refined olive oil and olive residue (solvent-extracted and refined) oils. This can be accomplished by either the small olive oil producer or by large-scale industries that blend and pack olive oil. On the other hand, in countries that import oil, especially in bulk amounts, common adulterants used during packaging could include inexpensive and highly available seed oils.

International organizations have suggested the use of physical and chemical constants (*e.g.*, refractive index, iodine value) as well as the absorption in ultraviolet (UV) radiation and the fatty acid composition carried out by gas-liquid chromatography (GLC), as standards for checking the purity of olive oil (4,5). However, as early as 1969, Gracian (6) stated that it is still easy to prepare fraudulent mixtures, even with large proportions of adulterants, which remain within the limits characteristic for olive oil. Today, GLC analysis of the fatty acid composition is an official method (4) considered as the most widely accepted test. It is used after simpler and cheaper analytical procedures have indicated the possible presence of foreign oils. Nevertheless, GLC analysis of the fatty acid composition of a natural vegetable oil does not always lead to indications regarding its purity, because most vegetable oils contain similar fatty acids in sometimes similar amounts (7).

Recently, the use of reversed-phase high-performance liquid chromatography (RP-HPLC) in the detection of olive oil adulteration has gained much attention (8–11). This method, which allows the separation and analysis of triacylglycerols (TAGs) enables the detection of low levels of seed oils (such as sunflower, soybean, cottonseed and corn oil) rich in linoleic acid. Preparation of the sample for analysis is uncomplicated, and RP-HPLC of TAGs has the advantage that natural variations in fatty acid composition do not affect the characteristic triacylglycerol profile (8).

Most studies utilizing this technique have been based either on seed oils with a high content of linoleic acid (8,11), and therefore a high content of trilinoleoylglycerol (a triacylglycerol that is absent or present in traces in olive oil) or in model mixtures that contain rather high amounts of the foreign oil (9,10). Canola oil is characterized by contents of oleic and linoleic acid that could be within the limits for olive oil (12), and this characteristic makes the detection of added canola oil difficult.

Data on RP-HPLC triacylglycerol analysis of olive oil mixed with canola oil are reported in this study, as well as the minimum level of adulteration with canola oil that could be detected by RP-HPLC.

EXPERIMENTAL PROCEDURES

Oil samples and solvents. Virgin olive oil samples of guaranteed purity were obtained from a number of different sources. Samples G1–10 were all of Greek origin, whereas S1–3 were Spanish olive oils and I1 and I2 were Italian olive oils. They were all donated by Elaiourgiki SYNPE (Athens, Greece). Samples GC1 and GC2 were obtained from a producer, from the island of Crete, Greece, and they were obtained from olives grown locally. Samples CA1–5 were from California, purchased from Nick Sciabica & Sons, Modesto, CA. Refined, bleached and deodorized canola oil was donated by CSP Foods Ltd. (Altona, MB, Canada). Solvents used were of "Omnisolv" grade as supplied by BDH Chemicals (Edmonton, AB, Canada).

Triacylglycerol analysis. All samples were analyzed by RP-HPLC in a Beckman model 342 system (Beckman Instruments Inc., Berkeley, CA) containing a 20-μL injection loop, a micro-guard ODS-5S (4.6×40 mm) pre-column (Bio-Rad Laboratories, Richmond, CA) and a Supelcosil LC-18 (5 micron) ($25 \text{ cm} \times 4.6$ mm i.d.) column (Supelco Inc., Bellefonte, PA). An Altex model 156 refractive index (RI) detector (Altex Scientific Inc., Berkeley, CA) was used. A mobile phase of acetone/acetonitrile (93:7, vol/vol)

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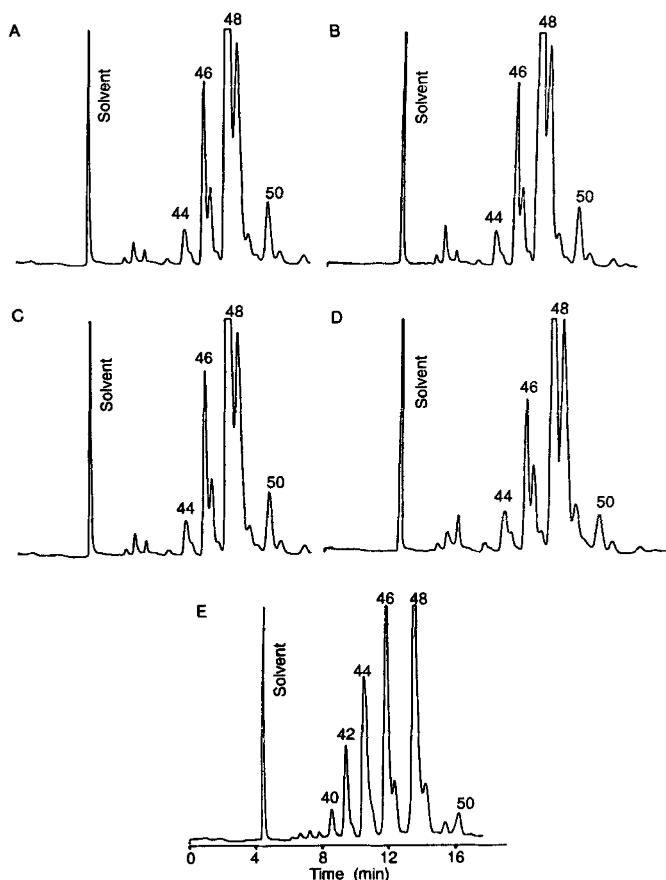

FIG. 1. Representative RP-HPLC profiles of vegetable oil samples. (A): Greek olive oil; (B): Spanish olive oil; (C): Italian olive oil; (D): Californian olive oil; (E): canola oil. ECN = carbon number − 2 × number of double bonds. For conditions see Experimental Procedures section.

TABLE 1

Triacylglycerol Composition, as Determined by Direct RP-HPLC Analysis[a], According to ECN[b], of Olive Oil Samples with Guaranteed Purity

	Area% of peaks with ECN					
Sample	42	44	46	48	50	52
G1	trace[c]	3.5	13.6	76.5	6.5	trace
G2	trace	3.9	16.9	73.2	6.0	trace
G3	trace	5.8	24.8	65.0	4.3	trace
G4	trace	5.8	26.1	65.2	3.0	trace
G5	trace	3.7	18.7	72.5	5.2	trace
G6	trace	3.7	18.3	72.2	5.7	trace
G7	trace	4.8	19.7	70.5	5.0	trace
G8	trace	5.0	22.4	69.5	3.2	trace
G9	trace	3.3	15.4	75.0	6.3	trace
G10	trace	4.8	21.1	68.9	5.3	trace
GC1	trace	4.5	20.6	66.9	7.1	0.8
GC2	trace	3.8	17.5	72.1	5.8	0.9
S1	trace	2.9	14.1	74.0	9.0	trace
S2	trace	5.3	25.9	66.0	2.8	trace
S3	trace	3.8	17.9	72.2	5.5	0.7
I1	trace	4.0	19.5	72.5	4.1	trace
I2	trace	4.8	22.6	68.2	4.4	trace
CA1	0.8	4.8	19.8	70.2	4.2	trace
CA2	trace	4.0	18.8	71.6	5.6	trace
CA3	trace	3.6	16.9	73.8	5.7	trace
CA4	trace	3.3	15.3	75.6	5.9	trace
CA5	0.9	4.0	19.3	70.9	4.9	trace
Mean	<0.5	4.2	19.3	71.0	5.2	<0.5
Min	trace	2.9	13.6	65.0	2.8	trace
Max	0.9	5.8	26.1	76.5	9.0	0.9
SD	—	0.8	3.5	3.3	1.4	—

[a]Mean of three determinations.
[b]ECN = carbon number − 2 × number of double bonds.
[c]<0.5%. Abbreviations: G: Greece; GC: Crete (Greece); S: Spain; I: Italy; CA: California (U.S.A.).

was pumped isocratically at a flow rate of 1 mL per minute. All samples were 7.5% (wt/vol) solutions in acetone and were filtered prior to injection through a 0.45-μm nylon filter. Triacylglycerols standards were purchased from Sigma Chemical Co. (St. Louis, MO). Quantitation was based directly on area% because separated peaks represented complex mixtures of TAGs with similar equivalent carbon numbers (ECNs), where ECN = actual carbon number (without glycerol) minus two times the number of double bonds per triacylglycerol molecule (13), a term that has also been defined as partition number (PN) according to Petersson *et al.* (14). Shoulders were included with the main peaks, and results are reported as area% of the individual ECNs.

RESULTS AND DISCUSSION

Typical RP-HPLC chromatograms of virgin olive oil and canola oil are presented in Figure 1. All of the samples of virgin olive oil with guaranteed purity had similar triacylglycerol profiles. Triacylglycerols were eluted according to their ECNs in approximately 15 min. Olive oil was characterized by four major clusters of triacylglycerol peaks with ECNs of 44, 46, 48 and 50. Triacylglycerols with ECN of 40 were absent from olive oil, which agrees with previous studies (15). The main clusters of peaks in canola oil are those representing ECNs of 48 and 46 as in olive oil. However, canola oil contains appreciable amounts of triacylglycerols with an ECN of 42 as well as small amounts of triacylglycerols with an ECN of 40.

Table 1 includes quantitative data on the triacylglycerol composition of 22 virgin olive oil samples of guaranteed purity as determined by RP-HPLC. The peak for ECN of 42 was present only in minute amounts (<1%) with a range from traces up to 0.9%. Therefore, it was decided to establish an upper limit of 1% for the ECN 42 peak in olive oils. A good correlation was found between the area% of the peaks for ECN 44 and 46 ($R = 0.941$), 44 and 48 ($R = 0.921$) and 46 and 48 ($R = 0.951$). More specifically, samples with a high content in triacylglycerols with ECN 44 also had higher amounts of triacylglycerols with ECN 46, whereas the same samples had a lower content in triacylglycerols with ECN 48. All possible ratios between the area% of the eluting peaks were calculated to establish an alternative rule helpful in detecting adulteration of olive oil with canola oil. The area% of the peaks for ECN 48 was the least variable (Table 1), but its ratio with the area% of the peaks representing ECN 44 was more variable (significant at 95%) than the ratio of the area% of the peaks for ECN 46 to that for ECN 44 (Table 2). The latter ratio was found to range between the values 3.9 and 5.1 (Table 2), and therefore, any olive oil sample with a value for this ratio of less than 3.9 should be considered

TABLE 2

Area% Ratios of the Triacylglycerols Peaks Representing ECN[a] of 44, 46 and 48 as Determined by Direct RP-HPLC[b] Analysis for Olive Oils with Guaranteed Purity

Sample	Area% ratio of peaks with ECN 46:44	48:44
G1	3.9	21.9
G2	4.3	21.9
G3	4.3	11.2
G4	4.5	11.2
G5	5.1	19.6
G6	4.9	19.5
G7	4.1	14.7
G8	4.5	13.9
G9	4.7	22.7
G10	4.4	14.4
GC1	4.6	14.9
GC2	4.6	18.8
S1	4.9	25.5
S2	4.9	12.5
S3	4.7	19.0
I1	4.9	18.1
I2	4.7	14.2
CA1	4.1	14.6
CA2	4.7	17.9
CA3	4.7	20.5
CA4	4.6	22.9
CA5	4.8	17.7
Mean	4.6	17.5
Min	3.9	11.2
Max	5.1	25.5
SD	0.3	3.9

[a]ECN = carbon number − 2 × number of double bonds.
[b]Mean of three determinations. Abbreviations as in Table 1.

TABLE 3

Area% of the Triacylglycerols Peaks for ECN[a] 42 and Ratio of 46:44 Peaks as Determined by Direct RP-HPLC Analysis for Olive Oil with Guaranteed Purity and Its Admixtures with Different Proportions of Canola Oil

Sample	% Area of peaks ECN 40	ECN 42	Ratio of peaks 46:44
Olive oil[b]	—[c]	<0.5	4.6 (0.30)[d]
Canola oil	2.1 (0.03)	8.1 (0.05)	1.4 (0.01)
2.5%[e] Canola oil	—	<0.5	4.1 (0.38)
5% Canola oil	—	0.7 (0.46)	3.9 (0.35)
7.5% Canola oil	—	1.1 (0.28)	3.7 (0.26)

[a]ECN = carbon number − 2 × number of double bonds.
[b]Average of 22 olive oil samples (from Tables 1 and 2).
[c]Not detected.
[d]Mean of three determinations (number in brackets equals the standard deviation).
[e]w/w Admixture with the olive oil sample.

TABLE 4

Percentage of Olive Oil Samples (n=22) Mixed with Canola Oil That Were Detected as Adulterated Using the Area% of Peak for ECN[a] 42 or the Ratio of Area% of Peak for ECN 46 to Peak for ECN 44 After Direct RP-HPLC Analysis of the Triacylglycerols

% (w/w) of canola oil in olive oils	% of samples detected as adulterated using Area% of peak 42	Area% ratio of 46:44 peaks
2.5	5	18
5	18	32
7.5	64	77
10	100	100

[a]ECN = carbon number − 2 × number of double bonds.

as adulterated because incorporation of seed oils in olive oil results in reduction of this ratio (Table 3).

The genuine olive oil samples were then mixed with 2.5% to 30% (w/w) in (2.5% increments) canola oil. The area percentage of the peaks consisting of triacylglycerols with ECN 42 and the ratio of the area percentage of the peaks for ECN 46 to that for ECN 44 were obtained by direct RP-HPLC analysis.

As shown in Table 3, by using the above-mentioned factors, adulteration of the genuine olive oil samples with canola oil at less than 7.5% (w/w) proportion could not be detected (% for peaks representing ECN 42 was <1% and ratio ECN 46/44 peaks was >3.9). The area of the ECN 40 peaks was not useful as a tool for detecting adulteration of the olive oil samples with canola oil because it starts to appear in the chromatograms only when olive oil was mixed with 30% (w/w) canola oil.

However, the ratio (46:44) proved to be consistently more useful than the area% of the peaks for ECN 42 in detecting the presence of canola oil in olive oil samples. This is shown in Table 4, which contains the percentage of the samples detected as adulterated by using both factors. At the low levels of adulteration (2.5%, 5% and 7.5% w/w canola in olive oil), the calculation of the ratio (46:44) confirmed the results found with the ECN 42 data but also revealed a larger number of adulterated samples. From the data presented here, it can be seen that any olive oil sample with a ratio of the area% of the peaks for ECN 46 to those for ECN 44 of less than 3.9 and/or with a content in triacylglycerols represented by the peaks for ECN of 42 greater than 1% (and/or the appearance of a peak for ECN of 40 for high levels of canola) must be considered as an adulterated sample.

It is finally concluded that canola oil presents special difficulties in detection when it is incorporated in olive oil because of its low content in triacylglycerols that are either absent or present in traces in olive oil (*i.e.*, species with ECNs of 40 and 42). A further difficulty lies in the continuing genetic modifications that canola is presently undergoing. As a result of such manipulations, different varieties exist, such as the "low-linoleic" spring variety. This seed contains only approximately 3% linolenic acid, and the total percentages of the triacylglycerols with ECNs of 40 and 42 have been decreased, while triacylglycerols with ECNs of 44 and 46 have been increased (16). Such properties will probably make the detection of new varieties of canola oil in olive oil even more difficult.

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