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Short communication

Detection of olive oil adulteration by measuring its authenticity factor using reversed-phase high-performance liquid chromatography

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Abstract

Addition of as little as 1% of linoleic-rich vegetable oils to olive oil can be detected easily and quantitatively by reversed-phase high-performance liquid chromatography on an octyl-bonded silica stationary phase (Supelcosil-LC 8). The mobile phase was acetone–acetonitrile (70:30, v/v), used isocratically. The chromatogram of pure olive oil was compared with those of mixtures of soybean, sunflower and corn oils with olive oil. The results indicate the possibility of the detection of adulteration by less than 1% of linoleic-rich vegetable oils in olive oil qualitatively and quantitatively in less than 15 min. An olive oil authenticity factor was established as a rapid indicator of adulteration and a simple equation for determining the extent of adulteration was derived.

1. Introduction

In Libya, olive oil is the main oil used in food preparation, cooking and frying and large volumes are imported every year. However, recently other oils such as corn, sunflower and soybean oils have also been imported. The Libyan Secretariat of Agriculture buys local olive oil at a higher price than imported olive oil, to encourage farmers not to neglect olive trees. However, frequent adulteration of both imported and local olive oil with the cheaper oils high in linoleic acid required a rapid method for the detection of adulteration to protect the economy and the consumer. Fatty acids have been used as indicators of adulteration [1–6], but their wide range in the adulterant and adulterated oils make them unsuitable for this purpose. Unsaponifiables have also been used as adulteration indicators [3–8], but extraction and processing operations make them unreliable. As fatty acids are distributed on glycerol molecules according to certain position-specific patterns, triacylglycerols are considered as fingerprints of natural oils. A combination of chemical, physical and/or chromatographic methods [9–17] has been used to determine the triacylglycerol composition of oils as a means of detecting possible adulteration. Peak ratios of triacylglycerols separated by HPLC have been used as a measure of olive oil adulteration [16].

This work was undertaken to develop a simple, rapid method for the detection of oils high in linoleic acid in olive oil by reversed-phase

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high-performance liquid chromatography (RP-HPLC) and a simple authenticity factor and a derived equation to determine the extent of adulteration with a one short chromatographic step, completed in less than 15 min.

2. Experimental

Commercial vegetable oils (soybean, sunflower and corn oils) high in linoleic acid were used as adulterants and mixed with a virgin olive oil sample.

2.1. RP-HPLC

The HPLC system consisted of a Model 2249 gradient pump (LKB, Bromma, Sweden) connected to two 150 × 4.5 mm I.D. stainless-steel columns packed with an octyl-bonded silica stationary phase (Supelcosil-LC 8) (Supelco, Bellefonte, PA, USA). Samples were injected through a Rheodyne (Cotati, CA, USA) Model 7125 injector equipped with a 20-ml sample loop and an LKB differential refractometric detector connected to an LKB Model 2221 integrator recorder. The isocratic mobile phase was acetone–acetonitrile (70:30, v/v). Samples were dissolved in the mobile phase and injected without any prior treatment.

3. Results and discussion

Fig. 1 shows typical chromatograms of olive, corn, soybean and sunflower oil triacylglycerols separated according to their equivalent carbon number (*ECN*). Soybean oil contains 1.2% of triacylglycerols with *ECN* 38 and 7.0% of *ECN* 40 triacylglycerols. The *ECN* 42 triacylglycerol contents in corn, sunflower and soybean are 24.2 ± 0.04, 22.4 ± 0.10 and 24.9 ± 0.11%, respectively (Table 1), while that in olive oil is 1.0 ± 0.02%. The *ECN* 42 triaclyglycerol group was used as an indicator of adulteration because it shows the greatest difference in triacylglycerol content between olive oil and the high linoleic acid oils.

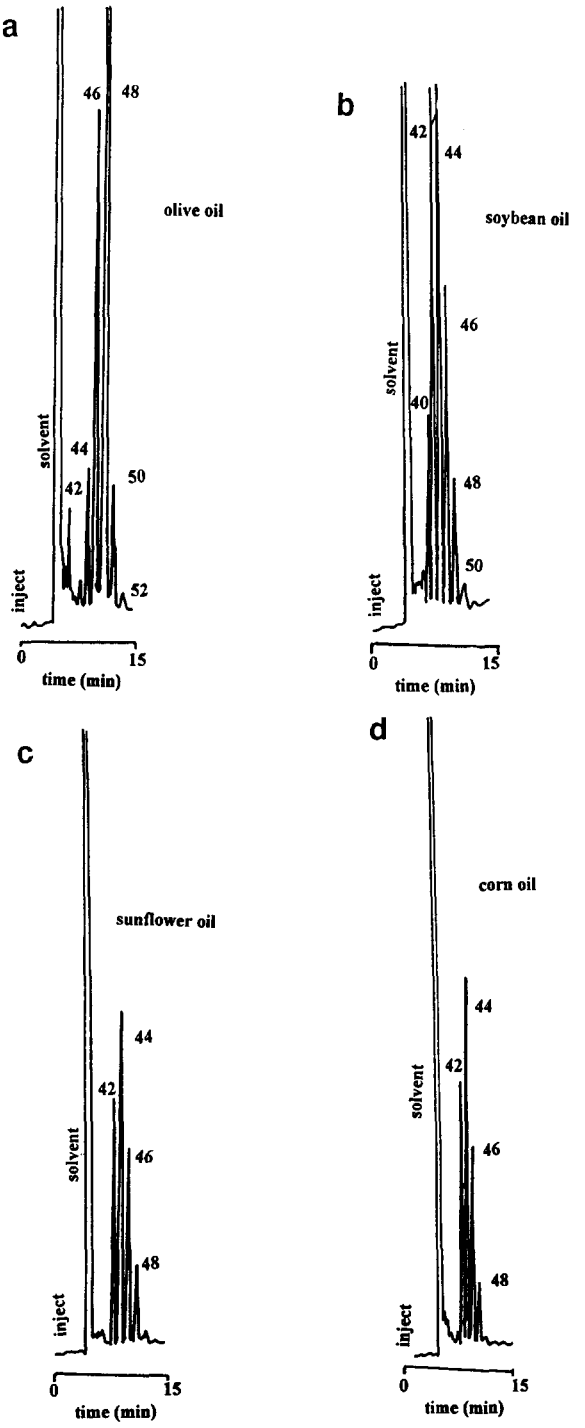

Fig. 1. Separation of triacylglycerols on Supelcosil-LC 8 with acetone–acetonitrile (70:30, v/v) as the mobile phase and refractive index detection. Flow-rate, 1.0 ml/min. (a) Olive oil; (b) soybean oil; (c) sunflower oil; (d) corn oil.

Table 1
Triacylglycerol composition (%) (± S.D., $n = 9$) and authenticity factors (Au) of olive, corn, soybean and sunflower oils separated by RP-HPLC

Triacylglycerol *ECN*	Oil			
	Corn	Sunflower	Soybean	Olive
38	nd[a]	nd	1.2 ± 0.04	nd
40	nd	nd	7.0 ± 0.02	nd
42	24.2 ± 0.04	22.4 ± 0.10	24.9 ± 0.11	1.0 ± 0.02
44	38.2 ± 0.40	35.2 ± 0.16	30.8 ± 0.32	5.7 ± 0.51
46	23.6 ± 0.21	25.0 ± 0.16	21.6 ± 0.54	23.2 ± 0.37
48	9.2 ± 0.04	12.7 ± 0.03	10.5 ± 0.13	60.1 ± 1.25
50	1.4 ± 0.01	1.1 ± 0.09	2.7 ± 0.22	6.8 ± 0.19
52	1.5 ± 0.10	2.2 ± 0.11	0.9 ± 0.12	0.9 ± 0.13
Au	3.2 ± 0.05	3.5 ± 0.03	3.1 ± 0.03	100.0 ± 2.73

[a] Not detected.

The presence of vegetable oils of high linoleic acid content in olive oil can be detected by measuring its authenticity factor (Au) as follows

$$Au = \frac{100 - ECN\ 42(\%)}{ECN\ 42(\%)} \quad (1)$$

Virgin olive oil separated by RP-HPLC has $Au = 98.2 \pm 3.86$. The authenticity factors of corn, sunflower and soybean oils are 3.2 ± 0.02, 3.5 ± 0.06 and 3.2 ± 0.19, respectively. Fig. 2 shows that addition of as little as 1% of corn, sunflower and soybean oils decreased the olive oil Au to 81.6 ± 2.5, 80.3 ± 4.05 and 79.0 ± 3.54, respectively, and additions of 5% of these oils decreased Au to 46.2 ± 1.38, 48.3 ± 1.26 and 46.4 ± 1.70, respectively.

Table 2
Change in authenticity factor (Au) and *ECN* 42 triacylglycerol content due to change in added high linoleic acid oils

Oil (%)[a]	*ECN* 42 ± S.D.[b] (%)	Au ± S.D.[b]
0.0	1.0 ± 0.02	95.2 ± 3.86
1.0	1.3 ± 0.04	78.4 ± 2.56
2.0	1.5 ± 0.04	66.5 ± 1.94
3.0	1.7 ± 0.05	57.8 ± 1.62
4.0	1.9 ± 0.05	51.0 ± 1.43
5.0	2.1 ± 0.06	45.6 ± 1.31
6.0	2.4 ± 0.07	41.3 ± 1.23
7.0	2.6 ± 0.08	37.7 ± 1.16
8.0	2.8 ± 0.09	34.6 ± 1.10
9.0	3.0 ± 0.10	32.0 ± 1.05
10.0	3.2 ± 0.11	39.8 ± 1.00
100.0	23.1 ± 1.08	3.3 ± 0.20

[a] Percentage of oils high in linoleic acid in olive oil.
[b] $n = 27$.

Plotting the percentage of added high linoleic acid oil versus the percentage of *ECN* 42 triacylglycerol group (Fig. 3) showed the possibility of measuring the extent of olive oil adulteration by the simple equation

$$\text{added oil}(\%) = \frac{ECN\ 42(\%) - b}{a} \quad (2)$$

where a and b are constants. The constants a and b differ slightly according to the oil added. Thus, corn, sunflower and soybean oils added to olive oil can be calculated using the following equations:

$$\text{corn oil}(\%) = \frac{ECN\ 42(\%) - 0.9820}{0.2326} \quad (3)$$

$$\text{sunflower oil}(\%) = \frac{ECN\ 42(\%) - 0.9954}{0.2142} \quad (4)$$

$$\text{soybean oil}(\%) = \frac{ECN\ 42(\%) - 0.9801}{0.2388} \quad (5)$$

The overall equation for oils added to olive oil is

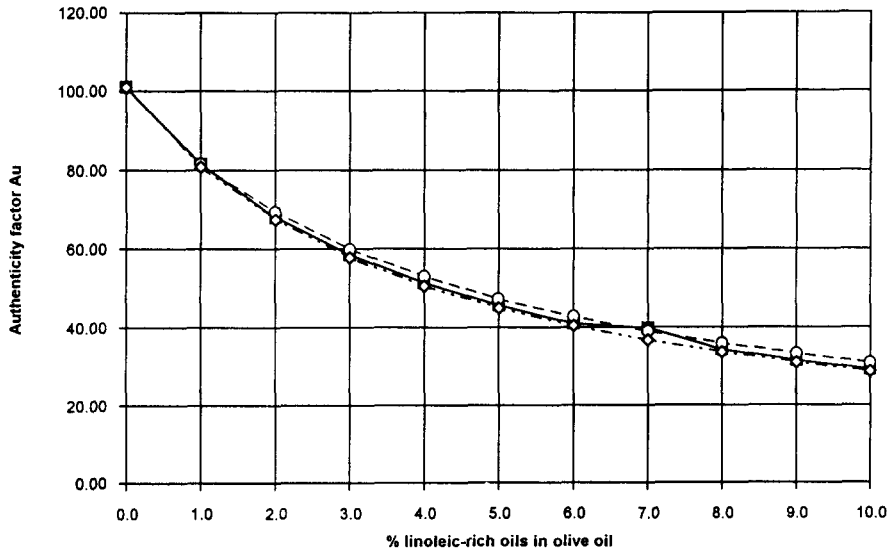

Fig. 2. Change of authenticity factors (*Au*) of olive oil as a result of addition of vegetable oils of high linoleic acid content. ■ = Corn oil; ○ = sunflower oil; ◇ = soybean oil.

$$\text{added oil}(\%) = \frac{ECN\,42(\%) - 0.9850}{0.2285} \qquad (6)$$

This study indicates the possibility of detecting and determining as little as 1% of oils high in linoleic acid in olive oil, and suggests the use of an authenticity factor as a simple and rapid indicator of adulteration. The whole analysis requires less than 15 min. However, oleic acid rich oils such as residue or re-esterified olive oils require emphasis on triacylglycerol groups other than *ECN* 42. A similar detection method for

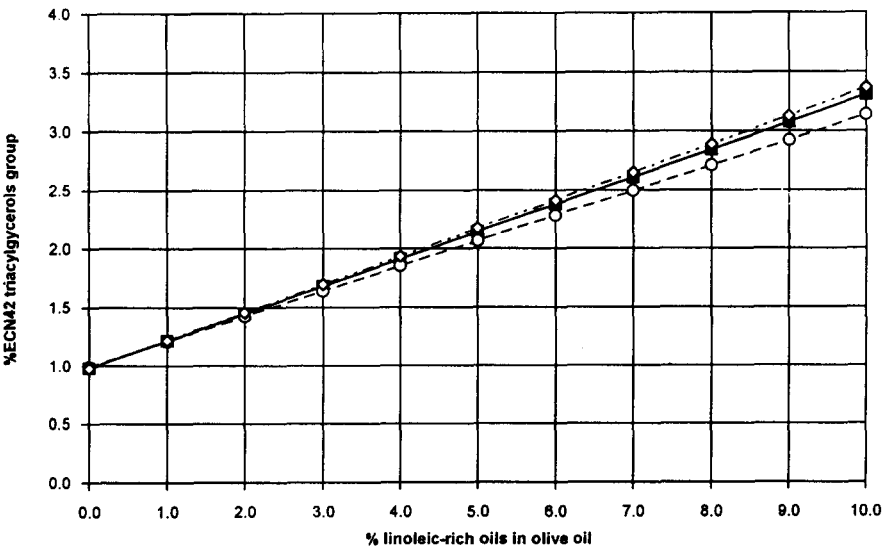

Fig. 3. Change of *ECN* 42 triacylglycerol content in olive oil due to change of added high linoleic acid vegetable oils. ■ = Corn oil; ○ = sunflower oil; ◇ = soybean oil.

such oils is under development using an octadecyl-bonded phase.

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