ELSEVIER

Food Chemistry 84 (2004) 463–474

Food Chemistry

www.elsevier.com/locate/foodchem

Effectiveness of determinations of fatty acids and triglycerides for the detection of adulteration of olive oils with vegetable oils

E. Christopoulou[a], M. Lazaraki[a], M. Komaitis[b,*], K. Kaselimis[b]

[a]*Chemical Laboratories, Ministry of Development, Kannigos Square, Athens 101 81, Greece*
[b]*Department of Food Science and Technology, Agricultural University of Athens, Iera Odos 75, Athens 118 55, Greece*

Received 4 December 2002; received in revised form 12 May 2003; accepted 12 May 2003

Abstract

This paper investigates the effectiveness of the determinations of fatty acids and triglycerides in the detection of adulteration of olive oil with certain vegetable oils. Detection of adulteration up to the level of 5% was possible. The use of the established limits of fatty acid contents could detect the adulteration of olive oil with the six of the investigated vegetable oils. The established limits of the ΔECN42 could be used to detect the adulteration of olive oil with the nine of the examined vegetable oils. Certain other parameters, based on differences of triglyceride and fatty acid compositions between olive oil and vegetable oils, could be used as discriminating factors between the olive oil and eight of the examined vegetable oils. However, no single known parameter could detect the presence of hazelnut and almond oils in olive oil, in percentages lower than or equal to 5%.

Keywords: Adulteration; Olive oil; Vegetable oils; Fatty acids; Triglycerides; ΔECN42

1. Introduction

Olive oil is a product of great importance because its nutritional value has been acknowledged internationally. Due to the entire procedure for its production, olive oil is a food of high price and so, it is important to safeguard it from adulteration.

A lot of methods and limits were introduced into the International Olive Oil Council (IOOC) trade standard, into EC Regulation 2568/91 and into the Codex Alimentarius Standard for controlling product authenticity and quality. Among the established methods for the control of authenticity of olive oil, the determinations of fatty acids and triglycerides seem to be very useful (Aparicio, Morales, & Alonso, 1997; Aparicio et al., 1994; Aparicio & Alonso, 1994b; Dennis, 1998; Elhamdy & Elfizga, 1995; Felatzarrouck, Buteiller, & Maurin, 1988; Gigliotti, Daghetta, & Sidoli, 1993; Salivaras & McCurdy, 1992, 1993; Synouri, Frangiscos, Christopoulou, & Lazaraki,1995).

Fatty acid analysis is performed by GC. The official bodies have established limits with regard to the content of fatty acids in olive oil. These limits are used for the discrimination between genuine olive oil and other vegetable oils.

The determination of triglycerides is carried out by HPLC. It is noteworthy that, out of the entire chromatogram achieved by the HPLC analysis of triglycerides, the only peaks which are taken into consideration are those of trilinolein (LLL) and Equivalent Carbon Number 42 (ECN42).

Until recently, the trilinolein (LLL) content was used for the detection of adulteration of olive oil with other vegetable oils. Nowadays, the trilinolein content has been replaced by the ΔECN42, since significant deviations from the established limit (0,5%) have been reported, notably in the trilinolein content of Tunisian olive oil samples. The parameter ΔECN42 is calculated from the difference between the theoretical ECN42 (calculated by a special computer programme based on the GC determination of fatty acid composition) and

* Corresponding author. Tel.: +30-210-529-4681; fax +30-210-529-4681.
E-mail address: achem@aua.gr (M. Komaitis).

0308-8146/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0308-8146(03)00273-5

the experimental ECN42 (determined by HPLC). (Elhamdy & Elfizga, 1995; Fellatzarrouck et al., 1988)

The present work studies the effectiveness of the use of fatty acid and triglyceride content for the detection of adulteration of olive oil with the most common vegetable oils. More specifically, the aim of this study was to define, among the parameters (for which official limits have been established), the effective ones that can be used in the detection of vegetable oils added to olive oil. Another important issue was to pinpoint the differences between olive oil and other vegetable oils with regard to the overall triglyceride composition and to search for other parameters different from those set by the official bodies and which may be used for a satisfactory interpretation of suspected fraud. It should also be noted that this work aims to facilitate the work of analysts by providing a simple guide for the detection of adulteration of olive oil with the most common vegetable oils.

2. Materials and methods

2.1. Chemical reagents and samples

All reagents used were of analytical grade. The analyzed samples were: extra virgin olive oil, sunflowerseed oil, soybean oil, cotton oil, corn oil, walnut oil, sesameseed oil, safflowerseed oil, canola oil, rapeseed oil, hazelnut oil, almond oil, peanut and mustardseed oil. Sampling of extra virgin olive oil took place in the olive oil mills, sunflower, soybean, cottonseed and corn oil samples were obtained from local refineries. The samples of walnut, sesame, safflower, canola, rapeseed, hazelnut, almond peanut and mustard oils were purchased from delicatessen stores. To avoid any changes in the chemical composition, samples were analyzed immediately after their arrival in the laboratory.

For the investigation of detection of adulteration of the olive oil with vegetable oils, mixtures of the sample extra virgin olive oil with each one of the vegetable oils were prepared. For each vegetable oil, five mixtures were prepared with percentages 1, 2, 3, 4 and 5% of the respective oil in the genuine olive oil sample. Altogether, 65 admixtures were prepared. These admixtures were analyzed immediately after their preparation.

2.2. Methods

The analyses performed for the purpose of this study were carried out in the laboratory of the Ministry of Development which applies the EC official methods of analysis, including the precision values of each method, for both the determination of fatty acid methyl esters (FAME) and the triglycerides with ECN42 (HPLC) in oils (Commission Regulation 2568/91 Annexes VIII, XA, XB XVIII). All tests were carried out in duplicate and the results presented are the averages of the values obtained.

Furthermore, this laboratory has been holding the IOOC certificate of recognition for olive oil testing laboratories since 1990, achieving acceptable results every year: The International Olive oil Council (IOOC) annually organizes a competence check test of olive oil testing laboratories. In these tests, the participating labs are requested to perform certain determinations (including the FAME and HPLC analyses). The results are then statistically evaluated for the assessment of laboratory competence.

Moreover, it is labaratory policy to evaluate performance using as reference materials control samples of known mean values ($\bar{x}$) and standard deviation (s), provided annually by the IOOC. The results are acceptable if they lie within the confidence level of 95% ($\bar{x} \pm 2s$).

The gas chromatographic analysis of FAMEs was performed on an AutoSystem Gas Chromatograph equipped with a FID detector. The column used was a capillary Supelco SP-2340 (length 60 m, ID 0.32 mm and film thickness 0.20 μm). The conditions for the analysis were: (a) injector 230 °C, (b) oven 150 °C for 18 min, rate 2.0 °C/min 175 °C for 5 min, rate 5.0 °C/min 200 °C for 10.5 min and (c) detector 230 °C. The determination of triglycerides was carried out in a LC-10Advp Shimadzu Liquid Chromatograph, equipped with a CT0-10Asvp Shimadzu column oven and a RID-10A refractive index detector. The column used was a Macherey-Nagel Nucleosil 100-5 C18, length 250 mm, ID 4.6 mm.). The conditions for the analysis were: solvent: acetone/acetonitrile 50:50 v/v, flow rate 1.5 ml/min, oven temperature 42 °C. Identification of triglycerides was identical to that reported by the official method and by Gigliotti et al. (1981).

The theoretical value of ECN42 triglycerides was calculated by the computer programme of the EC Regulation 2568/91 method.

3. Results and discussion

3.1. Fatty acids and triglycerides in vegetable oils

Tables 1–7 present the results of the analysis of the olive oil, vegetable oils and their admixtures with olive oil. These Tables show only the values of those parameters which are essential for the aims of this study. Comparison between the olive oil and the other vegetable oils, revealed that there are significant differences with regard to triglyceride and fatty acid compositions.

The determined values of the fatty acid composition were the normal ones encountered in olive oils and

Table 1
Fatty acids and triglycerides of olive oil, sunflower oil, soyabean oil and the mixtures of olive oil with sunflower or soyabean oils

Sample	Extra virgin olive oil	Sunflower oil	Extra + 1% sunflower	Extra + 2% sunflower	Extra + 3% sunflower	Extra + 4% sunflower	Extra + 5% sunflower	Soyabean oil	Extra + 1% soya	Extra + 2% soya	Extra + 3% soya	Extra + 4% soya	Extra + 5% soya
Composition of fatty acids (% of total fatty acids)													
C18:3 linolenic acid	0.89	0.08	0.74	0.71	0.70	0.72	0.71	7.22	0.90	1.00	1.02	1.10	1.19
C20:0 arachidic acid	0.48	0.29	0.45	0.43	0.44	0.42	0.42	0.49	0.45	0.46	0.47	0.46	0.48
C20:1 gadoleic acid	0.25	0.09	0.20	0.20	0.21	0.20	0.21	0.05	0.22	0.23	0.22	0.24	0.23
C22:0 behenic acid	0.15	0.90	0.15	0.16	0.17	0.17	0.20	0.33	0.15	0.15	0.17	0.16	0.16
C22:1 erucic acid	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C24:0 lignoceric acid	0.06	0.22	0.08	0.07	0.06	0.06	0.06	0.53	0.06	0.07	0.06	0.08	0.07
Composition of triglycerides (% of total triglycerides)													
LLL	0.11	27.7	0.38	0.66	0.93	1.21	1.48	25.2	0.37	0.63	0.84	1.15	1.38
ECN42	0.50	27.7	0.81	1.12	1.43	1.75	1.91	26.1	0.75	1.05	1.29	1.50	1.80
ECN44	3.67	37.9	4.07	4.44	5.03	5.27	5.65	31.2	3.97	4.28	4.53	4.82	5.00
ECN46	17	20.5	16.8	17.2	17.4	17.5	17.7	21.6	17.2	17.2	17.3	17.4	17.4
Parameters calculated by the triglycerides													
ΔECN42	−0.10	−0.50	−0.45	−0.76	−1.05	−1.35	−1.50	−2.21	−0.32	−0.56	−0.75	−0.89	−1.10
(LLL/ECN42)*100	22.0	100	46.9	58.9	65.0	69.1	77.5	96.7	49.3	60.0	65.1	76.7	76.7
ECN46/LLL	156	0.74	44.2	26.1	18.7	14.4	11.9	0.86	46.5	27.4	20.6	15.1	12.6
(ECN44 + ECN46)/LLL	189	2.11	54.9	32.8	24.1	18.8	15.8	2.10	57.2	34.1	26.0	19.3	16.2

Table 2
Fatty acids and triglycerides of olive oil, cotton oil, corn oil and the mixtures of olive oil with cotton or corn oils

Sample	Extra virgin olive oil	Cotton oil	Extra +1% cotton	Extra +2% cotton	Extra +3% cotton	Extra +4% cotton	Extra +5% cotton	Corn oil	Extra +1% corn	Extra +2% corn	Extra +3% corn	Extra +4% corn	Extra +5% corn
Composition of fatty acids (% of total fatty acids)													
C18:3 linolenic acid	0.89	0.13	0.85	0.84	0.85	0.84	0.83	0.44	0.85	0.86	0.86	0.86	0.84
C20:0 arachidic acid	0.48	0.27	0.45	0.46	0.45	0.44	0.44	0.52	0.46	0.48	0.46	0.46	0.49
C20:1 gadoleic acid	0.25	0.05	0.24	0.24	0.23	0.24	0.23	0.20	0.24	0.24	0.23	0.24	0.25
C22:0 behenic acid	0.15	0.15	0.15	0.14	0.15	0.15	0.14	0.12	0.15	0.14	0.14	0.15	0.14
C22:1 erucic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C24:0 lignoceric acid	0.06	0.05	0.05	0.06	0.05	0.05	0.05	0.12	0.06	0.06	0.05	0.05	0.05
Composition of triglycerides (% of total triglycerides)													
LLL	0.11	21.4	0.36	0.56	0.72	0.91	1.15	23.2	0.36	0.59	0.83	1.00	1.29
ECN42	0.50	21.4	0.75	0.95	1.10	1.30	1.53	23.3	0.77	0.99	1.21	1.45	1.69
ECN44	3.67	38.5	4.10	4.40	4.75	5.09	5.45	37.8	4.08	4.45	4.61	5.00	5.32
ECN46	17.1	28.9	17.3	17.3	17.4	17.7	17.6	26.3	17.2	17.3	17.5	17.6	17.6
Parameters calculated by the triglycerides													
ΔECN42	−0.10	−5.69	−0.33	−0.51	−0.63	−0.80	−1.02	−4.75	−0.35	−0.54	−0.73	−0.94	−1.16
(LLL/ECN42)*100	22.0	100	48.0	59.0	65.5	70.0	75.2	99.5	46.8	59.6	68.6	69.0	76.3
ECN46/LLL	156	1.35	48.1	30.9	24.2	19.4	15.3	1.14	47.6	29.2	21.0	17.6	13.6
(ECN44 + ECN46)/LLL	189	3.15	59.4	38.8	30.8	25.0	20.1	2.76	59.0	36.8	26.6	22.6	17.8

Table 3
Fatty acids and triglycerides of olive oil, walnut oil, sesame oil and the mixtures of olive oil with walnut or sesame oils

Sample	Extra virgin olive oil	Walnut oil	Extra +1% walnut	Extra +2% walnut	Extra +3% walnut	Extra +4% walnut	Extra +5% walnut	Sesame oil	Extra +1% sesam	Extra +2% sesam	Extra +3% sesam	Extra +4% sesam	Extra +5% sesam
Composition of fatty acids (% of total fatty acids)													
C18:3 linolenic acid	0.89	11	1.02	1.10	1.19	1.29	1.41	0.24	0.87	0.87	0.86	0.86	0.86
C20:0 arachidic acid	0.48	0.10	0.45	0.45	0.44	0.43	0.46	0.50	0.48	0.48	0.48	0.48	0.48
C20:1 gadoleic acid	0.25	0.34	0.25	0.24	0.25	0.24	0.24	0.13	0.25	0.25	0.25	0.25	0.25
C22:0 behenic acid	0.15	0.03	0.15	0.14	0.14	0.14	0.14	0.32	0.15	0.15	0.16	0.16	0.16
C22:1 erucic acid	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
C24:0 lignoceric acid	0.06	0.01	0.06	0.06	0.05	0.05	0.05	0.15	0.06	0.06	0.06	0.06	0.06
Composition of triglycerides (% of total triglycerides)													
LLL	0.11	31.6	0.42	0.72	1.00	1.32	1.64	18.7	0.29	0.47	0.65	0.83	1.02
ECN42	0.50	36.5	0.84	1.20	1.48	1.90	2.25	18.8	0.66	0.85	1.02	1.21	1.39
ECN44	3.67	28.7	3.96	4.07	4.52	4.57	4.97	32.0	3.98	4.27	4.56	4.83	5.12
ECN46	17.1	13.0	17.2	17.2	17.2	16.9	16.8	28.2	17.3	17.4	17.4	17.6	17.6
Parameters calculated by the triglycerides													
ΔECN42	−0.10	−0.77	−0.35	−0.63	−0.83	−1.16	−1.39	−4.31	−0.24	−0.41	−0.55	−0.71	−0.86
(LLL/ECN42)*100	22.0	86.7	50.0	60.0	67.6	69.5	72.9	99.7	43.9	55.3	63.7	68.6	73.4
ECN46/LLL	156	0.41	41.0	23.8	17.2	12.8	10.3	1.50	59.6	37.0	26.8	21.2	17.3
(ECN44 + ECN46)/LLL	189	1.32	50.4	29.5	21.7	16.2	13.3	3.22	73.3	46.1	33.8	27.0	22.3

vegetables oils and within the official limits established for olive oil and the limits that are referred in the Codex Alimentarius standards for the named vegetable oils (International Olive Oil Council: Trade standard applying to olive oils and olive pomace oils, Official Journal of the European Community-Commission Regulation 2568/91, Codex Alimentarius standard for olive oils and olive pomace oils, Codex Alimentarius standard for Named Vegetable Oils). The maximum limits of fatty acids in olive oils are: arachidic acid (C20:0) 0.6%, gadoleic acid (C20:1) 0.4%, behenic acid (C22:0) 0.2%, lignoceric acid (C24:0) 0.2% and linolenic acid (C18:3) 1.0%.

The vegetable oils, canola, mustard and peanut, exhibited considerably higher arachidic contents than the normal values encountered in the olive oil The vegetable oils, canola, rapeseed, mustard and peanut, exhibited considerably high gadoleic acid contents. The vegetable oils, sunflower, canola, mustard and peanut, had high amounts of behenic acid. Peanut oil had a

Table 4
Fatty acids and triglycerides of olive oil, safflower oil, canola oil and the mixtures of olive oil with safflower or canola oils

Sample	Extra virgin olive oil	Safflower oil	Extra +1% safflower	Extra +2% safflower	Extra +3% safflower	Extra +4% safflower	Extra +5% safflower	Canola oil	Extra +1% canola	Extra +2% canola	Extra +3% canola	Extra +4% canola	Extra +5% canola
Composition of fatty acids (% of total fatty acids)													
C18:3 linolenic acid	0.89	0.13	0.88	0.87	0.87	0.86	0.86	2.43	0.95	0.96	0.96	0.97	1.00
C20:0 arachidic acid	0.48	0.32	0.48	0.48	0.47	0.48	0.47	1.61	0.47	0.50	0.51	0.53	0.54
C20:1 gadoleic acid	0.25	0.15	0.25	0.24	0.24	0.24	0.24	1.71	0.25	0.29	0.30	0.32	0.33
C22:0 behenic acid	0.15	0.26	0.16	0.16	0.16	0.16	0.16	0.57	0.15	0.16	0.16	0.17	0.17
C22:1 erucic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C24:0 lignoceric acid	0.06	0.10	0.06	0.05	0.06	0.06	0.06	0.45	0.06	0.08	0.08	0.09	0.09
Composition of triglycerides (% of total triglycerides)													
LLL	0.11	44.2	0.54	0.97	1.41	1.85	2.30	10.4	0.23	0.35	0.40	0.50	0.65
ECN42	0.50	45.2	0.93	1.37	1.82	2.31	2.74	10.4	0.63	0.74	0.79	0.85	0.96
ECN44	3.67	31.1	3.96	4.19	4.51	4.73	5.07	22.2	3.85	4.00	4.25	4.38	4.64
ECN46	17.1	13.4	17.2	17.1	17.1	16.9	16.9	29.2	17.3	17.4	17.5	17.7	17.7
Parameters calculated by the triglycerides													
ΔECN42	−0.10	−5.29	−0.50	−0.90	−1.30	−1.75	−2.14	−6.80	−0.20	−0.29	−0.33	−0.38	−0.47
(LLL/ECN42)*100	22.0	97.9	58.1	70.8	77.5	80.1	83.9	100	36.5	47.3	50.6	58.8	67.7
ECN46/LLL	156	0.30	31.8	17.6	12.1	9.15	7.36	2.81	75.2	49.6	43.7	35.3	27.2
(ECN44 + ECN46)/LLL	189.2	1.00	39.1	21.9	15.3	11.7	9.57	4.96	92.0	61.0	54.3	44.1	34.4

Table 5
Fatty acids and triglycerides of olive oil, hazelnut oil, almond oil and the mixtures of olive oil with hazelnut or almond ois

Sample	Extra virgin olive oil	Hazelnut oil	Extra +1% hazelnut	Extra +2% hazelnut	Extra +3% hazelnut	Extra +4% hazelnut	Extra +5% hazelnut	Almond oil	Extra +1% almond	Extra +2% almond	Extra +3% almond	Extra +4% almond	Extra +5% almond
Composition of fatty acids (% of total fatty acids)													
C18:3 linolenic acid	0.89	0.12	0.87	0.86	0.85	0.85	0.84	0.03	0.87	0.86	0.85	0.85	0.84
C20:0 arachidic acid	0.48	0.11	0.48	0.47	0.47	0.47	0.46	0.11	0.47	0.47	0.46	0.46	0.45
C20:1 gadoleic acid	0.25	0.14	0.25	0.25	0.24	0.24	0.24	0.07	0.25	0.25	0.24	0.23	0.23
C22:0 behenic acid	0.15	0.07	0.15	0.15	0.15	0.14	0.15	0.03	0.15	0.15	0.15	0.15	0.14
C22:1 erucic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C24:0 lignoceric acid	0.06	0.01	0.06	0.06	0.06	0.06	0.06	0.03	0.06	0.05	0.06	0.06	0.05
Composition of triglycerides (% of total triglycerides)													
LLL	0.11	2.02	0.12	0.14	0.16	0.18	0.20	1.51	0.12	0.15	0.16	0.18	0.19
ECN42	0.50	2.42	0.51	0.52	0.53	0.56	0.58	1.56	0.52	0.54	0.55	0.57	0.59
ECN44	3.67	6.41	3.75	3.76	3.77	3.80	3.83	10.7	3.78	3.84	3.89	3.98	4.04
ECN46	17.1	21.6	17.1	17.2	17.2	17.2	17.3	28.1	17.3	17.3	17.5	17.5	17.7
Parameters calculated by the triglycerides													
ΔECN42	−0.10	−2.09	−0.11	−0.13	−0.14	−0.16	−0.18	−0.90	−0.12	−0.14	−0.15	−0.16	−0.18
(LLL/ECN42)*100	22.0	83.5	23.5	26.9	30.2	32.1	34.5	96.8	23.1	27.8	29.1	31.6	32.2
ECN46/LLL	156	10.7	143	123	108	96.1	86.6	18.6	144	116	109	97.4	92.9
(ECN44 + ECN46)/LLL	189.2	13.9	174	150	131	117	106	25.7	176	141	133	120	114

considerably high lignoceric content. Mustard oil had a very high erucic acid (C22:1) content, whereas the rapeseed oil had high erucic acid content. The oils, soybean, canola, rapeseed, walnut, mustard had very high linolenic acid contents, much more than that of the maximum value in olive oil (1.0%). The linolenic acid content of the other vegetable oils was lower than that of olive oil. These differences, related to the linolenic acid content, are depicted in Fig. 1. The compositions of fatty acids in hazelnut and almond oils were very similar to that of olive oil.

Trilinolein (LLL) content, in all vegetable oils, was higher than that of the reference samples of the olive oils. Large differences were observed with regard to trilinolein contents between olive oils and sunflower, soybean, cotton, corn, canola, walnut, sesame and safflower oils. The trilinolein content of the other vegetable oils were slightly higher than that of olive oil. These differences are also depicted in Fig. 2. The differences between theoretical and experimental ECN42 contents (ΔECN42) in all vegetable oils were higher than that in

olive oil. It ranged from 22.9 in mustard oil to 0.33 in rapeseed oil. These differences are also depicted in Fig. 3. At this point, it would be useful to remember that, according to the established limits, the ΔECN42 value must not exceed 0.2 in the case of edible virgin olive oil, 0.3 in the case of lampante and olive oil, 0.5 in the case of olive pomace oil and 0.6 in the case of crude olive pomace oil.

The above results revealed that the correlation between the linolenic acid, trilinolein content and ΔECN42 has some shortcomings. To put it in other words, certain vegetable oils have high linolenic acid, low LLL content and high ΔECN42. Certain others exhibit low linolenic acid, high LLL content and low ΔECN42, whereas some others have high linolenic acid, low LLL content and low ΔECN42.

3.2. Detection of the presence of vegetable oils in an olive oil sample

In the present study, detection of adulteration of olive oil, up to the level of 5%, was investigated. The results of the analyses of the fraudulent mixtures of olive oil with vegetable oils are presented in Tables 1–7. Based on these results, the parameters which are efficient in the detection of fraud were examined. The percentage of

Table 6
Fatty acids and triglycerides of olive oil, rapeseed oil, peanut oil and the mixtures of olive oil with rapeseed or peanut oils

Sample	Extra virgin olive oil	Rapese oil	Extra +1% rapeseed	Extra +ed2% rapeseed	Extra +3% rapeseed	Extra +4% rapeseed	Extra +5% rapeseed	Peanut oil	Extra +1% peanut	Extra +2% peanut	Extra +3% peanut	Extra +4% peanut	Extra +5% peanut
C18:3 linolenic acid	0.89	8.28	0.93	1.00	1.09	1.13	1.23	0.10	0.84	0.84	0.84	0.83	0.82
C20:0 arachidic acid	0.48	0.56	0.45	0.48	0.48	0.48	0.48	1.49	0.48	0.51	0.51	0.53	0.53
C20:1 gadoleic acid	0.25	1.37	0.26	0.27	0.28	0.29	0.31	1.04	0.26	0.26	0.27	0.27	0.28
C22:0 behenic acid	0.15	0.35	0.15	0.16	0.16	0.16	0.16	2.56	0.17	0.19	0.21	0.25	0.26
C22:1 erucic acid	0.00	0.35	0.00	0.01	0.01	0.01	0.02	0.07	0.00	0.00	0.00	0.00	0.00
C24:0 lignoceric acid	0.06	0.13	0.05	0.05	0.06	0.05	0.06	1.28	0.07	0.07	0.09	0.10	0.11
Composition of triglycerides (% of total triglycerides)													
LLL	0.11	0.50	0.12	0.12	0.12	0.12	0.13	1.82	0.14	0.15	0.17	0.19	0.21
ECN42	0.50	8.47	0.59	0.69	0.78	0.85	0.94	1.90	0.52	0.55	0.56	0.59	0.61
ECN44	3.67	21.2	3.87	4.07	4.15	4.32	4.59	15.2	3.74	3.97	4.07	4.17	4.29
ECN46	17.1	28.8	17.2	17.4	17.5	17.7	17.7	33.7	17.2	17.39	17.7	17.9	17.9
Parameters calculated by the triglycerides													
ΔECN42	−0.10	−0.33	−0.16	−0.23	−0.28	−0.32	−0.36	−0.69	−0.13	−0.15	−0.15	−0.16	−0.18
(LLL/ECN42)*100	22.0	5.90	20.3	17.4	15.4	14.1	13.8	95.8	26.9	27.3	30.4	32.2	34.4
ECN46/LLL	156	57.6	143	145	146	147	136	18.5	123	115.9	104	94	85.2
(ECN44 + ECN46)/LLL	189	100	176	179	181	183	171	26.9	150	142.4	128	116	106

Table 7
Fatty acids and triglycerides of olive oil, mustard oil and the mixtures of olive oil with mustard oil

Sample	Extra virgin olive oil	Mustard oil	Extra +1% mustard	Extra +2% mustard	Extra +3% mustard	Extra +4% mustard	Extra +5% mustard
Composition of fatty acids (% of total fatty acids)							
C18:3 linolenic acid	0.89	14.6	1.05	1.18	1.28	1.41	1.56
C20:0 arachidic acid	0.48	1.56	0.48	0.50	0.51	0.52	0.52
C20:1 gadoleic acid	0.25	8.76	0.34	0.42	0.53	0.60	0.69
C22:0 behenic acid	0.15	1.24	0.15	0.17	0.18	0.19	0.19
C22:1 erucic acid	0.00	36.5	0.38	0.73	1.07	1.42	1.81
C24:0 lignoceric acid	0.06	0.62	0.07	0.07	0.08	0.08	0.09
Composition of triglycerides (% of total triglycerides)							
LLL	0.11	0.47	0.12	0.12	0.12	0.13	0.13
ECN42	0.50	2.00	0.54	0.55	0.56	0.57	0.59
ECN44	3.67	5.64	3.71	3.73	3.76	3.78	3.80
ECN46	17.1	9.20	17.0	17.0	16.9	16.8	16.7
Parameters calculated by the triglycerides							
ΔECN42	−0.10	22.9	−0.06	−0.01	0.03	0.09	0.16
(LLL/ECN42)*100	22.0	23	22	21.8	21.4	22.8	22.0
ECN46/LLL	156	19.6	142	141	140	129	129
(ECN44 + ECN46)/LLL	189	31.6	173	172	172	158	158

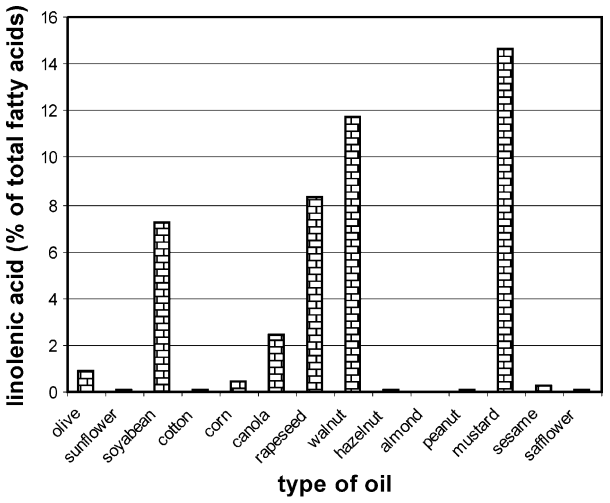

Fig. 1. Linolenic acid content of certain vegetable oils.

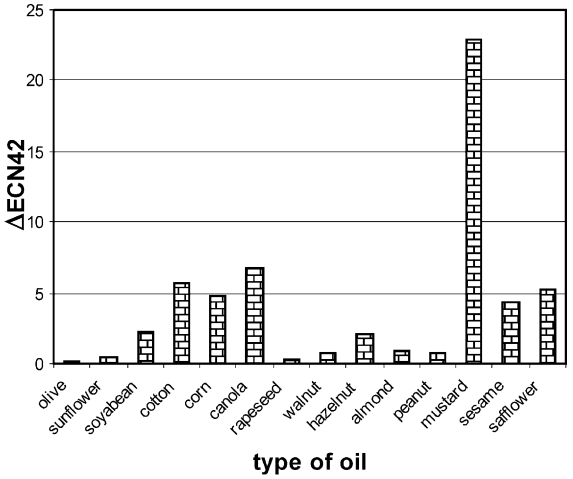

Fig. 3. ΔECN42 content of certain vegetable oils.

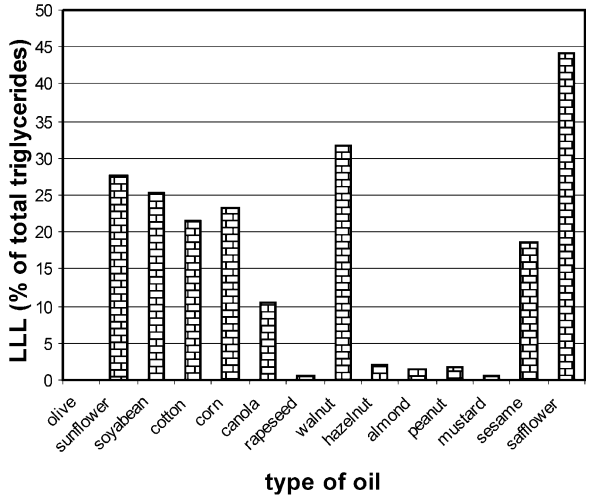

Fig. 2. Trilinolein (LLL) content of certain vegetable oils.

added vegetable oil, that can be detected by these parameters was also examined. The effectiveness of a parameter was based on the comparison of the determined value in each fraudulent sample with the official one set for this parameter for olive oil. In the case of new parameters (for which there are no official limits), the effectiveness of a parameter was based on the comparison of the determined value in each fraudulent sample with the maximum or the minimum value of this parameter in olive oil. These maximum or minimum values were extracted from statistical data on a large number of authentic virgin olive oils.

3.2.1. Use of the composition of fatty acids for the detection of fraud

Taking into account the results presented in Tables 1–7, it could be concluded that the analysis of fatty acids does not produce satisfactory results with regard to the levels of adulteration investigated in this study. The most effective parameters for the detection of adulteration are mentioned below.

The linolenic acid content could be used as a parameter for the detection of fraud of olive oil with the following vegetable oils: 2% soybean, 5% canola, 2% rapeseed, 1% walnut and 1% mustard. The gadoleic acid content could be used as a parameter for the detection of fraud of olive oil with 2% mustard oil. The behenic acid content could be used as a parameter for the detection of fraud of olive oil with 3% peanut oil. The erucic acid content could be used as a parameter for the detection of fraud of olive oil with the oils: mustard and rapeseed. No single one of the other fatty acids is effective in the detection of added vegetable oil, up to the level of 5%, in an olive oil.

Based on the data and on the above mentioned observations, the following conclusions can be drawn. Although the composition of fatty acids, in the examined seed oils, is different from that of olive oils, the fatty acids could not be satisfactorily used as discriminatory parameters between olive oil and the respective vegetable oil, in most cases. This means that fatty acids determination can not be used for the detection of the adulteration of olive oil with the following vegetable oils: sunflower, cotton, corn, sesame, hazelnut, almond and safflower oils.

3.2.2. Use of the ΔECN42 for the detection of fraud

The use of the ΔECN42 proved to be more effective in detecting even low levels of adulteration of olive oil with most of the examined vegetable oils. According to the data on the fraudulent mixtures presented in Tables 1–7, the determination of the ΔECN42 can be used as a parameter for the detection of fraud of olive oils with each one of the oils, 1% sunflower, 1% soybean, 1% cotton, 1% corn, 3% canola, 4% rapeseed, 1% walnut, 1.5% sesame and 1% safflower.

The ΔECN42 can not be used as a parameter for the detection of fraud of olive oil with the following vegetable oils: mustard, hazelnut, almond and peanut, up to the level of 5%.

Generally speaking, it is expected that parameters which differentiate sufficiently between olive oil and other vegetable oils could be very effective in the detection of the adulteration of olive oil with these vegetable oils. However, the results of the fraudulent mixtures led to the following observations. Although the value of the ΔECN42 in sunflower oil is low (0.50), that is close to the value of the ΔECN42 in olive oil, this parameter is very effective for the detection of addition, even at levels of 1% of this oil in olive oil. On the other hand, although the value of the ΔECN42 in mustard oil is very high (22.9) that is much higher than the value of the ΔECN42 in olive oil itself, this parameter is not at all effective for the detection of the addition, even at levels of 5% of this oil, in olive oil.

To interpret this contradictory finding, the following considerations should be taken into account: it is easy to calculate the composition of fatty acids and that of triglycerides in a mixture of olive oil with a vegetable oil, since it depends on their values in the initial samples used for the preparation of the mixture. For example, if a mixture consists of 98% of olive oil (with 0.89% linolenic acid content) and 2% of soyabean oil (with 7.22% linolenic acid content), it will be expected that the linolenic acid content of the mixture will have an intermediate value, close to the theoretical value of 1.02%. In fact, in this example, the determined linolenic acid content of the mixture was 1.00%.

On the other hand, the value of the ΔECN42 in a mixture is not the expected one from the values of this parameter in the initial samples. For example, if a mixture consists of 98% of olive oil (with 0.10 ΔECN42) and 2% of sunflower oil (with 0.50 ΔECN42), it will be expected that the ΔECN42 of the mixture will be close to the theoretical value of 0.11. However, in this example, the determined ΔECN42 of the mixture was 0.76.

This finding can be attributed the fact that the ΔECN42 is a number calculated by a combination of fatty acids and triglyceride composition. So, the differences in the composition of the triglycerides and the six fatty acids (which are taken into account for the calculation of the theoretical ECN42) between the initial samples and their mixtures are expected. However, these differences do not have the expected effect on the values of the theoretical ECN42 and ΔECN42. A consequence of this is the complete lack of correlation between linolenic acid or LLL and ΔECN42 of vegetable oils and their admixtures with olive oil. This claim was certified by the results of the present study.

From these results, the following conclusions can be drawn: when a parameter, calculated by a combination of other parameters (e.g. ΔECN42), is used for the detection of the adulteration, the effectiveness of this parameter does not depend on the values of these parameters in the initial samples.

The parameters used for the detection of adulteration of olive oil with vegetable oils as well as the percentages of detectable vegetable oil in olive oil mixtures are summarized in Table 8. It should be noted that the conclusions for the detection of fraud are extracted from the analyses performed on the samples examined in this study. It could be argued that, if, for the detection of fraud, different samples had been used, the conclusions might have been different. However, as has already been mentioned, the effectiveness of a parameter was based on comparison of the determined value in each fraudulent sample with the maximum established limit of this parameter in olive oil. On the other hand, the examined samples of vegetable oils exhibited the normal values for each parameter encountered in vegetable oils. These values are approximately in the middle of the range that is referred in the Codex Alimentarius standard for this particular parameter. Consequently, the findings of this study can be applied to the detection of adulteration of olive oil with the only reservation that the percentage of vegetable oil that is detectable in olive oil mixtures may be somewhat different from that proposed in the present study. Overall this Table could be used as a simple guide for analysts working on olive oil analysis.

On the basis of the results of Table 8, a very important conclusion can be drawn. The determinations of fatty acids and ΔECN 42 can not be used for the detection of hazelnut and almond oils in olive oil

Table 8
Used parameters for the detection of adulteration of olive oil with vegetable oils

Type of vegetable oil	Used parameter for the detection of adulteration	Percentage of detectable vegetable oil
Sunflower	ΔECN42	1
Soyabean	Linolenic acid	2
	ΔECN42	1
Cotton	ΔECN42	1
Corn	ΔECN42	1
Walnut	Linolenic acid	1
	ΔECN42	1
Sesame	ΔECN42	1.5
Safflower	ΔECN42	1
Canola	Linolenic acid	5
	ΔECN42	3
Rapeseed	Linolenic acid	2
	Erucic acid	1
	ΔECN42	4
Hazelnut	None	
Almond	None	
Peanut	Behenic acid	3
Mustard	Linolenic acid	1
	Erucic acid	1

admixtures. Consequently, it is absolutely necessary to find another effective parameter for the detection of the presence of hazelnut and almond oil in an olive oil. For this purpose, the examination of some other parameters, possibly different from those set by the official bodies, should be investigated. So, we proceeded to the actions referred in Sections 4.2.3.1–4.2.3.3.

3.2.3. Use of other parameters for the detection of fraud

In Tables 1–7 parameters calculated by the triglycerides are also reported. The investigated parameters are as follows.

*3.2.3.1. (LLL/ECN42)*100.* Certain researchers have pointed out the importance of this factor in foretelling the geographical origin of the olive oil, as well as for the detection of adulteration of olive oil with certain vegetable oils (Giliotti et al., 1993; Synouri et al., 1995). This parameter is based on the low LLL content of the olive oil in comparison to that of the other vegetable oils. The value of this parameter in the vegetable oils, sunflower, soya bean, cotton, corn, walnut, sesame, safflower, canola, hazelnut, almond and peanut, is higher than that in olive oil. The value of this parameter in mustard oil is similar to that in olive oil and the value of this parameter in rapeseed oil is lower than that in olive oil. On the basis of the analysis of over 500 samples of genuine olive oils, the value of this parameter in olive oils was found to range from 10.0 to 61.0 (mean value of 34).

3.2.3.2. ECN46/LLL. This parameter is based on the low LLL content of the olive oil in comparison to that of the other vegetable oils and the similar ECN46 value of the olive oil to that of the other vegetable oils. So, the value of this parameter in the vegetable oils, sunflower, soya bean, cotton, corn, walnut, sesame, safflower and canola, is much more lower than that in olive oil. The value of this parameter in rapeseed oil is similar to that in olive oil and the value of this parameter in hazelnut, almond, peanut and mustard oils is slightly lower than that in olive oil. On the basis of the analysis of over 500 samples of genuine olive oils, the value of this parameter in olive oils was found to range from 31.0 to 750 (mean value of 176).

3.2.3.3. (ECN44 + ECN46)/LLL. This parameter is based on the low LLL content of the olive oil in comparison to that of the other vegetable oils and the similar ECN44 + ECN46 values in the olive oil and other vegetable oils. So, the value of this parameter in the vegetable oils, sunflower, soya bean, cotton, corn, walnut, sesame, safflower and canola, is much lower than that in olive oil. The value of this parameter in rapeseed oil is similar to that in olive oil and the value of this parameter in hazelnut, almond, peanut and mustard oils is slightly lower than that in olive oil. On the basis of the analysis of over 500 samples of authentic olive oils, the value of this parameter in olive oils was found to range from 39 to 890 (mean value of 214).

Figs. 4–6 show the correlation of the three parameters in olive oil and in mixtures of olive oil with 1 and 5% of vegetable oils. Comparing the maximum or minimum values of the three parameters in olive oil to the values of the same parameters in the admixtures, the effectiveness of each parameter can be extracted. That is, if the value of the parameter in the fraudulent sample is higher than the maximum value or lower than the minimum value of this parameter in the olive oil, then this parameter is effective for the detection of that vegetable oil in olive oil. Figs. 4–6, it is obvious that the use of the three parameters (LLL/ECN42)*100, ECN46/LLL and (ECN44 + ECN46)/LLL in the dis-

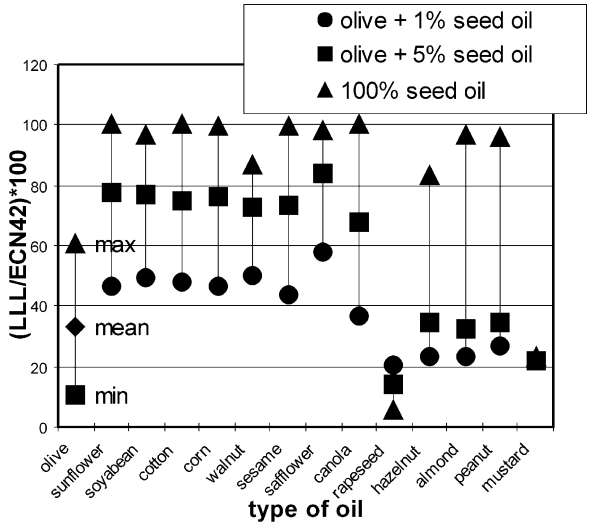

Fig. 4. Values of the parameter (LLL/ECN42)*100 in olive oils, vegetable oils and their mixtures.

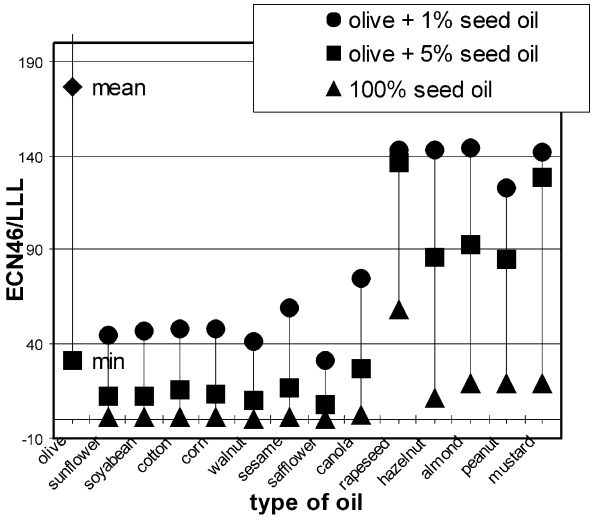

Fig. 5. Values of the parameter ECN46/LLL in olive oils, vegetable oils and their mixtures.

crimination between genuine and fraudulent samples of olive oil leads to similar conclusions. The use of the three parameters (LLL/ECN42)*100, ECN46/LLL, (ECN44 + ECN46)/LLL is very effective in the detection of eight vegetable oils in percentages lower than 5%. This applies to the following vegetable oils: sunflower, soya bean, cotton, corn, walnut, sesame, safflower and canola. The use of the three parameters (LLL/ECN42)*100, ECN46/LLL, (ECN44 + ECN46)/LLL is not effective for the detection of five vegetable oils in percentages lower than 5%. This applies to the following vegetable oils: rapeseed, hazelnut, almond, peanut and mustard.

In conclusion the use of the three parameters (LLL/ECN42)*100, ECN46/LLL, (ECN44 + ECN46)/LLL produced very satisfactory results with regard to eight of the examined vegetable oils, even in percentages lower than 5%. However, this investigation proved that the detection of hazelnut and almond oils at levels below 5% in an olive oil continues to be a major problem.

3.2.4. Use of the plotting of certain parameters for the detection of fraud

Many workers have used the plotting of suitably selected parameters for the discrimination between two categories. For overcoming the problem of discriminating between the olive oil and the hazelnut and almond oils, the following plots were investigated:

*3.2.4.1. (LLL/ECN42)*100 = f(ECN46).* In Fig. 7 the values of the parameters (LLL/ECN42)*100 are plotted versus the values of the parameter ECN46 in the examined samples of the olive oil and the other vegetable oils.

*3.2.4.2. (LLL/ECN42)*100 = f(ECN44 + ECN46).* In Fig. 8 the values of the parameters (LLL/ECN42)*100 are plotted versus the values of the parameter ECN44 + ECN46 in the examined samples of the olive oil and the other vegetable oils.

The Figs. 7 and 8 have similar profiles. All vegetable oils, except for mustard and rapeseed oils, are very different from olive oil. Taking into account that the hazelnut and almond oils are placed far from the olive oil in the Figs. 7 and 8, it could be assumed that the use of these plots is effective for the detection of these two "difficult" oils.

In Fig. 9, the values of the parameter (LLL/ECN42)*100 are plotted versus the values of the parameter ECN46 in the examined samples of the olive oil and the fraudulent mixtures of the olive oil containing 1% of each one of the vegetable oils. In Fig. 10, the values of the parameters (LLL/ECN42)*100 are plotted versus the values of the parameter ECN44 + ECN46 in the examined samples of the olive oil and the fraudulent mixtures of the olive oil with 1% of each one of the vegetable oils.

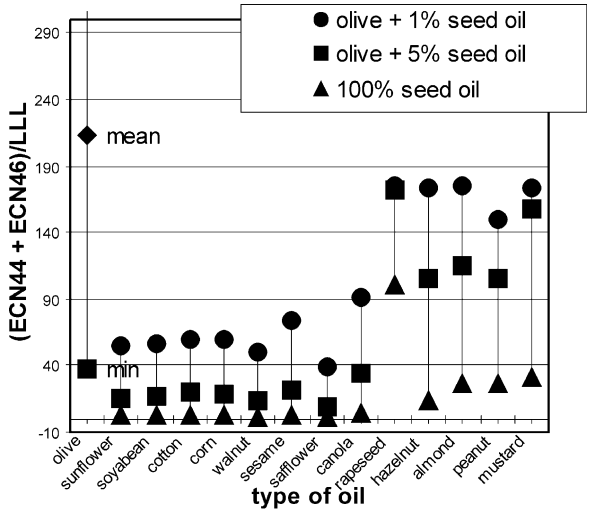

Fig. 6. Values of the parameter (ECN44 + ECN46)/LLL in olive oils, vegetable oils and their mixtures.

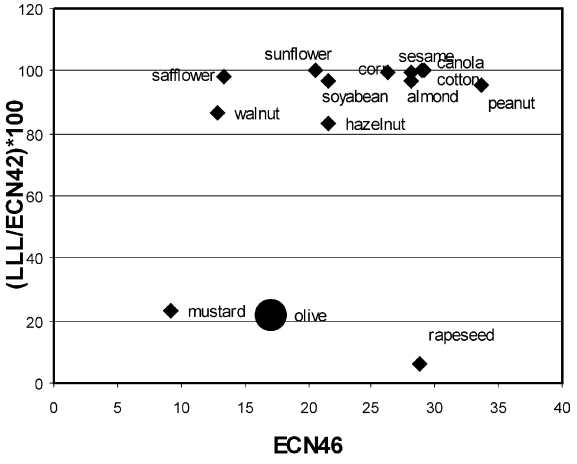

Fig. 7. Plotting of the values (LLL/ECN42)*100 versus ECN46 in olive oil and in certain vegetable oils.

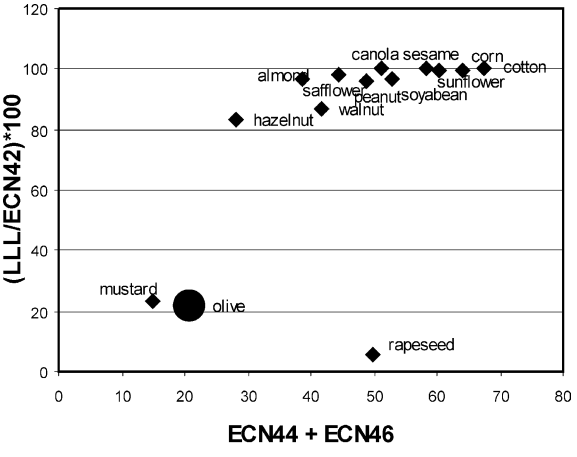

Fig. 8. Plotting of the values (LLL/ECN42)*100 versus ECN44 + ECN46 in olive oil and in certain vegetable oils.

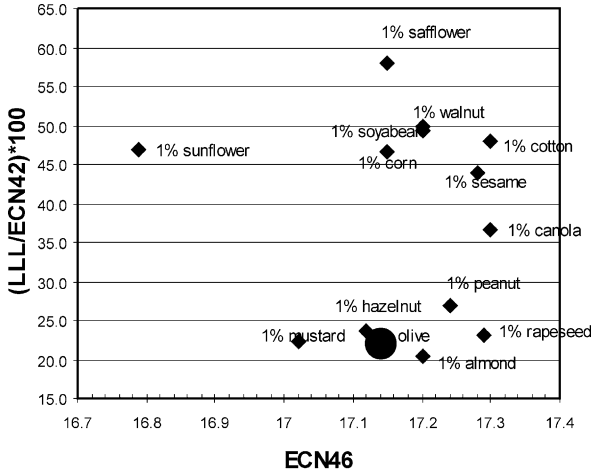

Fig. 9. Plotting of the values (LLL/ECN42)*100 versus ECN46 in olive oil and in the mixtures of olive oil with 1% of certain vegetable oils.

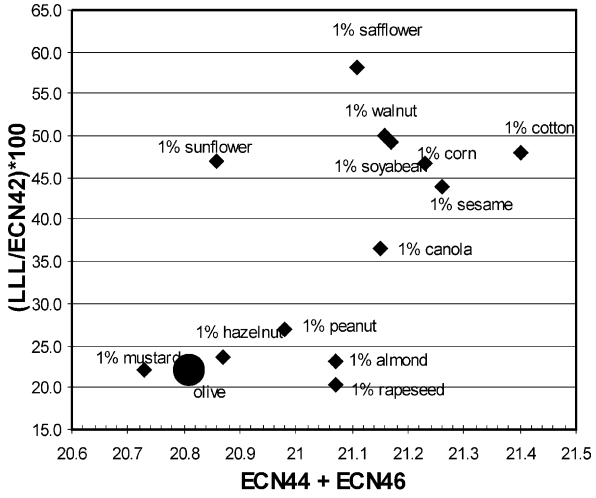

Fig. 10. Plotting of the values (LLL/ECN42)*100 versus ECN44 + ECN46 in olive oil and in the mixtures of olive oil with 1% of certain vegetable oils.

As can be seen from Figs. 9 and 10, all the fraudulent mixtures, except of those with rapeseed, hazelnut, peanut, almond and mustard oils, are very distinct from the olive oil. So, these parameters could not be used for the discrimination between genuine and fraudulent (with peanut, rapeseed, mustard, hazelnut and almond oils) samples of olive oils at the 1% level of adulteration. The detection of fraud was based on the values of the examined samples. Clearly, data on a large number of samples would provide more reliable conclusions.

Consequently, the use of the plotting (LLL/ECN42)*100 = f(ECN46) and (LLL/ECN42)*100 = -f(ECN44 + ECN46) leads to almost the same conclusions, extracted by the use of the three parameters referred to in Sections 4.2.3.1–4.2.3.3. That is, their use is effective for the detection of fraud in olive with the following vegetable oils: sunflower, soya bean, cotton, corn, walnut, sesame, safflower and canola, even at low levels of adulteration, less than 1%. However, it is not effective for the detection of fraud with the following vegetable oils: rapeseed, hazelnut, almond, peanut and mustard.

4. Conclusions

The established limits for the fatty acids are useful for the detection of fraud of an olive oil with the following vegetable oils: soyabean, walnut, canola, rapeseed, peanut and mustard, even at levels of adulteration (below 5%). However, these could not be used to detect percentages lower or equal to 5% of sunflower, cotton, corn, sesame, safflower, hazelnut and almond oils in mixtures with olive oil.

The parameter ΔECN42 is a very useful and effective tool in the detection of the presence of the most common vegetable oils. More specifically, the established limit for the ΔECN42 in olive oil is satisfactory for the detection of adulteration of an olive oil with the following vegetable oils: sunflower, soyabean, cotton, corn, walnut, sesame, safflower, canola and rapeseed. The use of this limit allows the detection of even very low levels of adulteration.

The established limit for the ΔECN42 is not satisfactory for detecting percentages lower than or equal to 5% of hazelnut, almond, peanut and mustard oils in mixtures with olive oil.

The parameters (LLL/ECN42)*100, ECN46/LLL and (ECN44 + ECN46)/LLL, which are based on the differences in triglyceride and fatty acid composition between the olive oil and vegetable oils, can be used as a discriminator factor between the olive oil and the eight of the examined vegetable oils: sunflower, soyabean, cotton, corn, walnut, sesame, safflower and canola. However, the use of these parameters is not satisfactory for detecting percentages lower than or equal to 5% of rapeseed, hazelnut, almond, peanut and mustard oils in mixtures with olive oil.

No single one of the official parameters, or the proposed ones, can detect the presence of percentages lower than or equal to 5% of hazelnut and almond oils in olive oil, since the fatty acid and triglyceride compositions of these oils are very similar to that of olive oil. The detection of hazelnut and almond oils in olive oil is a very difficult issue and more research is needed.

References

Aparicio, R., Morales, M. T., & Alonso, V. (1997). Authentication of European virgin olive oils by their chemical compounds, sensory

attributes and consumers attitudes. *Journal of Agricultural and Food Chemistry*, *45*, 1076–1083.

Aparicio, R., Ferreiro, L., & Alonso, V. (1994). Effect of climate on the chemical composition of virgin olive oil. *Analytica Chimica Acta*, *292*, 235–241.

Aparicio, R., & Alonso, V. (1994). Characterization of virgin olive oil by SEXIA expert system. *Progress in Lipid Research*, *33*, 29–38.

Codex Alimentarious Standard for olive oils and olive pomace oils.

Codex Alimentarius Standard for named vegetable oils.

Dennis, M. J. (1998). Recent developments in food authentication. *Analyst*, *123*(9), 151R–156R.

Elhamdy, A. H., & Elfizga, N. K. (1995). detection of olive oil adulteration by measuring its authenticity factor using reversed phase high-performance liquid chromatography. *Chromatography A*, *708*(2), 351–355.

Fellatzarrouck, K., Bouteiller, J. C., & Maurin, R. (1988). Detection of olive oil adulteration by HPLC in seed oils. *Revue Francaise des Corps Gras*, *35*(10), 383–386.

Gigliotti, C., Daghetta, A. A., & Sidoli, A. (1993). Indagine concoscitiva sul contenuto trigliceridico di oli extra vergini di oliva di varia provenienza. *Rivista Italiana Sostanze Grasse*, *70*, 483–489.

International Olive Oil Council: Trade Standard applying to olive oil and olive pomace oils.

Official Journal of the European Community-Commission Regulation 2568/91 of 11th July 1991 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis, L. 248, 5.9.91.

Salivaras, E., & McCurdy, A. R. (1992). Detection of olive oil adulteration with canola oil from triacylglycerol analysis be reversed phase high-performance liquid-chromatography. *Journal of the American Oil Chemists' Society*, *69*(9), 935–938.

Salivaras, M., & McCurdy, A. R. (1993). Adulteration of olive oil: detection through reversed-phase high performance liquid chromatography. In G. Charalambous (Ed.), *Food flavors, ingredients and composition* (pp. 279–300). Elseviers Science.

Synouri, S., Frangiscos, S. E., Christopoulou, E., & Lazaraki, E. M. (1995). Influence of certain factors on the composition of olive–pomace oil. *Rivista Italiana Sostanze Grasse, LXXII*, 483–491.