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Evaluation of Authenticity of Iranian Olive Oil by Fatty Acid and Triacylglycerol Profiles

Z. Piravi-Vanak · M. Ghavami · H. Ezzatpanah · J. Arab · H. Safafar · Jahan B. Ghasemi

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Abstract For evaluation of the authenticity of Iranian olive oil, samples from many Iranian olive oil producers especially north of Iran in the production year 2007 were collected. The fatty acid and triacylglycerol compositions were measured. The most recent calculation methods including ΔECN the difference between the actual and theoretical ECN42 (equivalent carbon number), triglyceride content and R of olive oils according to IOOC methods were applied. On the basis of our results, we were able to classify the olive oils into the extra virgin, virgin olive and olive oil categories. The important fatty acids are oleic, palmitic and linoleic acids and their main triacylglycerols are OOO, POO, OOL, PLO, SOS plus POP, and OLL, respectively. On the basis of the triacylglycerol results, experimental ECN48, ECN46, ECN50, ECN44 and ECN42 were obtained. By using the fatty acids results and a computer program, the theoretical ECN42 and ECN44 were calculated. Then *R* values, being the ratio of *r* ECN42/*r* ECN44 for authenticity of all olive oils and ΔECN for determining categories of olive oils, were defined. The results of olive oil samples were in the accepted limits of Codex and IOOC. Finally we suggest that the *R* and ΔECN can be used in identification of adulteration of olive oils and also they are useful from the point of view of authenticity and classification.

Keywords Olive oil · Fatty acid · Triacylglycerol · ECN · Adulteration · Codex and IOOC

Z. Piravi-Vanak · M. Ghavami · H. Ezzatpanah
College of Food Science and Technology,
Islamic Azad University, Science and Research Branch,
Tehran, Iran

J. Arab
Oilve Office, Ministry of Jehad and Agriculture,
Tehran, Iran

H. Safafar
Olive Oil Section, Techno Azma Laboratory,
Tehran, Iran

J. B. Ghasemi (✉)
Chemistry Department, Faculty of Sciences,
KNT University of Technology, Tehran, Iran
e-mail: jahan.ghasemi@gmail.com

Introduction

Olive trees (*Olea europaea*) are among the oldest known cultivated trees in the family Oleaceae, native to coastal areas of the eastern Mediterranean region, from Lebanon and the maritime parts of Asia Minor and northern Iran at the southern end of the Caspian Sea [1]. Iranian Olive oil has received prominent attention over the last few decades, as it is a major constituent in the Mediterranean diet. It is mostly produced in the north of Iran (Gilan, Gholestan, Zanjan, and Qhazvin provinces) and produced to a lesse extent in the west and south west including the Kermanshah and Fars provinces. It enjoys the protection of several regulations and trademarks issued by the International Olive Oil Council, Codex alimentarius and the European Commission [1, 2]. The International Olive Oil Council (IOOC) has established criteria [2] for its categorization into various grades, namely virgin olive oil, refined olive oil and pure olive oil. The oil with the best quality is extra virgin olive oil, because it is free of artificial processing except for its mechanical extraction. Refined olive oil is obtained from virgin olive oil using refining methods that do not lead to alterations in the initial glyceridic structure, whereas pure olive oil is formed by blending of the two former types [1, 2]. In addition, olive-pomace oil is defined

as that obtained by extracting olive-pomace with authorized solvents. Its constituents exhibit a protective effect against different types of cancer and significantly reducing mortality caused by heart disease [3–6].

As a result of its benficial health effect, olive oil is more expensive than other types of oils, making it a target for adulteration [7–15]. Adulteration has been a problem in the oil and fat trade for a long time [16–19]. This is especially true for olive oil products. The most common adulterants in virgin olive oil have included refined olive oil, olive pomace oil, and esterified oil prepared by re-esterifying low-grade olive oils with glycerol. Other current adulterants are tea seed oil and other less expensive seed oils such as corn oil, cottonseed oil, rapeseed oil, sunflower oil, and soybean oil.

Although different techniques have been proposed for the characterization of oils and for the detection of adulterants, none of them has been universally accepted for the determination of the authenticity of the different types of vegetable oils [20, 21]. Since the chemical composition reflects the authenticity of the oil, the development of sensitive and selective methods for olive oil analysis is desirable [22, 23]. The technique most widely used is chromatography [24, 25]. The IOOC has developed a global method for the detection of extraneous oils in olive oils. High linoleic vegetable oils such as sunflower and colza, and some high oleic vegetable oils such as hazelnut, high oleic sunflower and olive pomace oils are detected indicating whetrher an olive oil is genuine or not [26]. In this method the triacylglycerol (TAG) composition is determined by reverse phase high resolution liquid chromatography using a refractive index detector and fatty acids are identified by capillary gas chromatography using high polar columns. The theoretical TAG composition is calculated from the fatty acids composition by a computer program assuming a 1, 2 and 3-random distribution of fatty acids in the TAG.

Several mathematical algorithms have been proposed according to theoretical and experimental (HPLC results) results for the TAG compositions. The ECN42 and ECN44 are calculated and the following r ratios can be calculated:

$$r\ \text{ECN42} = \text{ECN42 HPLC}/\text{ECN42 theoretical}$$

$$r\ \text{ECN44} = \text{ECN44 HPLC}/\text{ECN44 theoretical}$$

The authenticity of all olive oils, virgin and refined (except for olive- pomace oils) is defined by the ratio R:

$$R = r\ \text{ECN42}/r\ \text{ECN44}$$

According to the IOOC method, R for different kinds of virgin olive oils and refined olive oils must be as follow:

$R \leq 0.95$, for olive oils with an oleic acid/linoleic acid ratio ≤ 5

$R \leq 1.05$, for olive oils with an oleic acid/linoleic acid ratio $>5 \leq 15$

$R \leq 1.10$, for olive oils with an oleic acid/linoleic acid ratio ≥ 5

Determination of the ΔECN (difference between the actual and theoretical ECN42 triglyceride content) is applicable to the detection of the presence of small amounts of seed oils (rich in linoleic acid) in every class of olive oils. The Codex and IOOC standards for olive oil products [1, 2] specify a value ≤ 0.2 for virgin olive oils and ≤ 0.3 for refined olive oils.

In this study, we want to evaluate the Iranian olive oils with regard to fatty acids and triacylglycerols and demonstrate the usefulness of applying the global method along with ΔECN to distinguish genuine or nongenuine oils and to categorize olive oils according to the Codex alimentarius and the IOOC, respectively.

Materials and Methods

Samples

Thirty Olive oil samples were randomly collected from all the districts where Iranian olive oil producers are situated especially north of Iran in the production year 2007, these included: 19 extra virgin olive oil samples, 4 virgin olive oil samples, and 6 refined olive oil samples. The sampled regions are shown in blue in the geographical map of Iran, Fig. 1. The sampled regions are four Northern provinces of Iran, Gilan, Golestan, Zanjan and Qazvin and one province

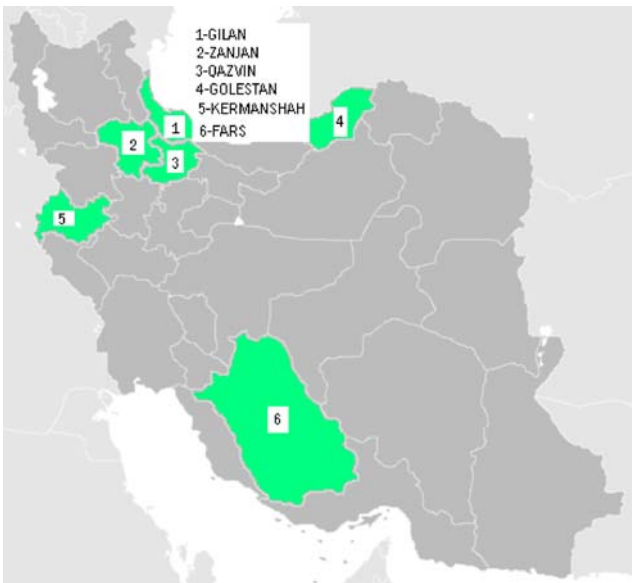


Fig. 1 Sample regions of Iranian olive oils

in the west; Kermanshah and one in the south west; Fars province.

Reagents

Silica gel cartridges, a solvent mixture of hexane/diethyl ether (87:13, v/v), *n*-heptane, acetone, methanol containing not more than 0.5% water, propionitrile, heptanes, and a 2-N solution of potassium hydroxide in methanol (all from Merck) were used as received. All other reagents were of analytical grade and were purchased from Merck or Aldrich.

Apparatus

A Hewlett Packard gas chromatograph instrument (model 6890), equipped with a flame ionization detector (FID), a HP-5 (Cross linked 5% PH ME Siloxane) capillary column (120 m × 0.25 mm I.D., particle size 0.25 μm) an automatic injector and the Chemastation software package. A Younglin HPLC instrument model Acme 9000 with a degasser, quaternary pump, manual injection valve, refractive index detector and the Autochro software package for instrument control, data acquisition and data analysis. A Spherisorb RP- 100 (25 cm × 4.6 mm I.D., particle size 4 μm) analytical column was used.

Procedures for the Analysis of Triacylglycerols and Fatty Acids

The analytical methods for the determination and detection of extraneous oils in olive are described in the global method IOOC (COI/T.20/Doc. no. 25, 2006). The procedure describing the method for the determination of trans-unsaturated fatty acids was used (COI/T.20/Doc. no. 17). The analysis of triglycerides was performed according to the global method IOOC for the detection of extraneous oils in olive I (COI/T.20/Doc. no. 25, 2006). The triacylglycerols in the olive oils were separated according to equivalent carbon number (ECN), often defined as CN-2n, where CN is the carbon number and n is the number of double bonds.

Calculation and Expression of Experimental and Theoretical ECN (Equivalent Carbon Number) Triacylglycerol Composition, ΔECN and R

Experimental ECN of Triacylglycerol

The area normalization method was used, i.e., it was assumed that the sum of the areas of the peaks corresponding to TAGs from ECN42 up to ECN52 is equal to 100%. The relative percentage of each triacylglycerol was calculated using the formula:

% triacylglycerol = area of peak × 100/sum of peak areas. It is an experimental ECN

Theoretical Triacylglycerols with ECN42

The triacylglycerols with ECN42 were calculated according to equations COI T.20/Doc. 20 and the ECN42 amounts were given by the sum of the nine triacylglycerols including their positional isomers.

Theoretical Triacylglycerols with ECN44

The triacylglycerols with ECN44 were calculated according to the same mathematical equations and were then given in order of the expected elution in HPLC. The triacylglycerols with ECN44 were given by the sum of the eleven triacylglycerols including their positional isomers.

ΔECN

This is the difference between the actual and theoretical ECN42 triglyceride content

R

The theoretical composition of the triacylglycerols was calculated from the composition of the C16 and C18 fatty acids, palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid, according to method COI/T.20/Doc. no. 20.

The calculated theoretical content and the content determined by the HPLC analysis for ECN42 and ECN44 were compared. The following ratios must then be calculated:

$$r\ \text{ECN}\ 42\ = \text{ECN}\ 42\ \text{HPLC}/\text{ECN}\ 42\ \text{theoretical}$$
$$r\ \text{ECN}\ 44\ = \text{ECN}\ 44\ \text{HPLC}/\text{ECN}\ 44\ \text{theoretical}$$

The authenticity of all olive oils, virgin and refined (except for olive-pomace oils), was defined by the ratio *R*, that is, $R = r$ ECN42/r ECN44

The oil was genuine when:

the oils had an oleic acid/linoleic acid ratio ≤ 5 $R \leq 0.95$
the oils had an oleic acid/linoleic acid ratio ≥ 15 $R \leq 1.10$

Statistical Analysis

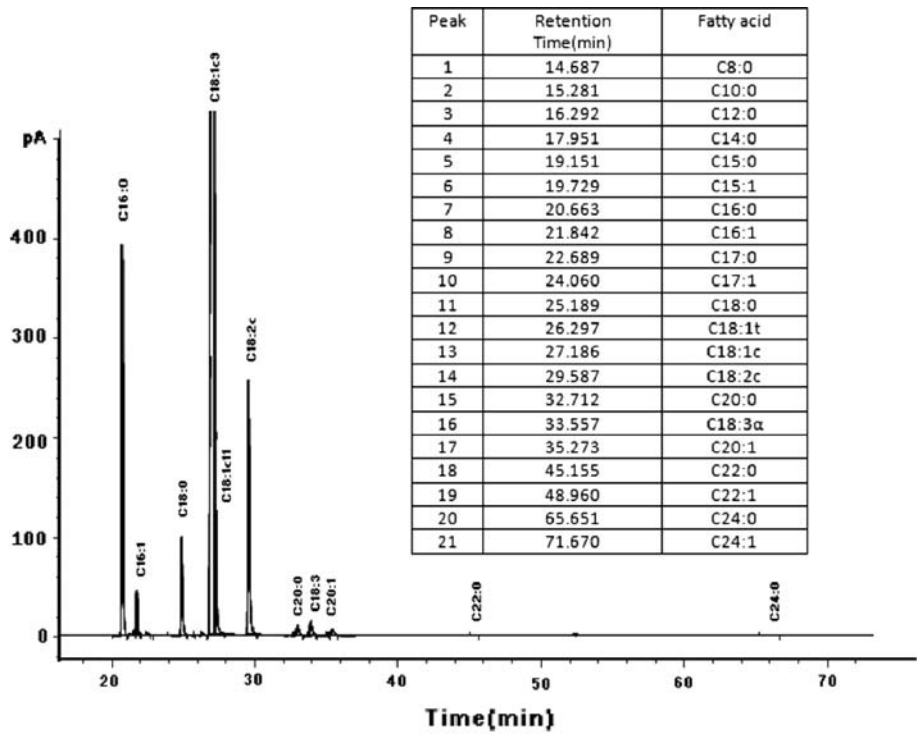
For the results showed in all Tables the confidence intervals were calculated at 95%. In the case of the comparison

of the two mean values of fatty acids or other parameters the Student's *t*-statistic was used at appropriate confidence levels.

Results and Discussion

The gas chromatographic analysis of the whole set of olive oils samples yielded a complete fatty acid profile (data not reported) peak areas of 16 fatty acids (C14:0, C 15:0, C16:0, C16:1, C17:0, C17:1, C18:0, C18:1, C18:1t, C18:2, C18:2t, C18:3, C20:0, C20:1, C22:0, C24:0) were obtained. A sample chromatogram of the fatty acids of one of the collected Iranian extra virgin olive oil sample is shown in Fig. 2. The fatty acids and their levels in the analyzed oils are shown in Table 1. Oleic acid (C18:1) was the most important fatty acid in the three kinds of Iranian olive oil (71.67% for extra virgin olive oil, 69.55% for virgin olive oil and 69.54% for refined olive oil). The oleic value in the Iranian extra virgin olive oil was more than virgin and refined olive oils ($p \leq 0.06$ at 90% of confidence level). The next important fatty acid is linoleic acid that stands right after the oleic acid in the unsaturated fatty acids (9.50% for extra virgin olive oil, 10.72% for virgin olive oil and 9.96% for refined olive oil). Palmitic acid (C16:0) was the major saturated fatty acid in the olive oils and the content ranged between 13.00 and 13.72 in the three kinds. We compared the results of the fatty acid compositions of olive oils with those published and confirmed by the IOOC and Codex Alimentarius [1, 2] with which they showed good agreement. The variability found was found in the fatty acid composition of the olive oils could be due to important factors such as the nature of the cultivar, soil characteristics, climatic conditions, olive maturity, etc. (Table 2).

The triacylglycerol compositional data are valuable indicators for giving a quantitative measure of the quality and purity of vegetable oils. The high specificity and precision of the compositional data for the different kinds of fats and oils, made researchers to use it increasingly in the food industries to confirm authenticity, despite the laborious nature of the analysis. A representative HPLC chromatogram of TAGs of an Iranian virgin olive oil is shown in Fig. 3. For the analyzed olive oil sample, the main components of the chromatographic peaks are the mean values of triacylglycerols (TAGs) which belong to the five theorical equivalent carbon numbers from ECN42 to ECN50 as shown in Table 3. The most probable combination representatives of the TAGs containing OOO + PoPP + PLP are between 30.90 and 35.44% for the three kinds of olive oils and for the most common European olive oils according to Table 3, POO (between 20.35 and 25.36% in the three kinds of olive oils), OOL + LnPP (between 10.66 and 12.06% in the three kinds of olive oils), SLL + PLO (between 6.04 and 7.04% in the three kinds of olive oils), SOO (between 4.82 and 6.01% in the three kinds of olive oils), POP (between 3.50 and 4.56% in the three kinds of olive oils) and OLL (between 2.47 and



Peak	Retention Time(min)	Fatty acid
1	14.687	C8:0
2	15.281	C10:0
3	16.292	C12:0
4	17.951	C14:0
5	19.151	C15:0
6	19.729	C15:1
7	20.663	C16:0
8	21.842	C16:1
9	22.689	C17:0
10	24.060	C17:1
11	25.189	C18:0
12	26.297	C18:1t
13	27.186	C18:1c
14	29.587	C18:2c
15	32.712	C20:0
16	33.557	C18:3α
17	35.273	C20:1
18	45.155	C22:0
19	48.960	C22:1
20	65.651	C24:0
21	71.670	C24:1

Fig. 2 Chromatogram and the fatty acid composition of Iranian extra virgin olive oil

Table 1 Fatty acid compositions (%) of olive oil samples

Fatty acids	Extra virgin olive oil	Virgin olive oil	Refined olive oil	IOOC regulation	Codex alimentarius regulation	
					Virgin olive oil	Refined olive oil
C14:0	0.01 ± 0.002	0.008 ± 0.01	0.01 ± 0.004	<0.05	0.0–0.05	0.0–0.05
C15:0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–	–	–
C15:1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–	–	–
C16:0	13.00 ± 0.61	13.46 ± 3.18	13.72 ± 0.28	7.5–20.0	7.5–20.0	7.5–20.0
C16:1	0.94 ± 0.11	0.87 ± 0.37	0.84 ± 0.24	0.3–3.5	0.3–3.5	0.3–3.5
C17:0	0.06 ± 0.02	0.10 ± 0.1	0.15 ± 0.008	<0.3	0.0–0.3	0.0–0.3
C17:1	0.10 ± 0.002	0.13 ± 0.11	0.19 ± 0.004	<0.3	0.0–0.3	0.0–0.3
C18:0	3.10 ± 0.23	3.39 ± 0.95	3.75 ± 0.31	0.5–5.0	0.5–5.0	0.5–5.0
C18:1t	0.02 ± 0.005	0.03 ± 0.03	0.03 ± 0.004	–	0.0–0.05	0.0–0.20
C18:1c	71.67 ± 1.27	69.55 ± 8.44	69.54 ± 0.52	55.0–83.0	55.0–83.0	55.0–83.0
C18:2t	0.00 ± 0.00	0.01 ± 0	0.018 ± 0.0007	0	0.0–0.05*	0.0–0.30*
C18:2c	9.50 ± 0.86	10.72 ± 5.07	9.96 ± 0.51	3.5–21.0	3.5–21.0	3.5–21.0
C20:0	0.48 ± 0.04	0.57 ± 0.16	0.59 ± 0.04	<0.6	0.0–0.6	0.0–0.6
C18:3alpha	0.60 ± 0.04	0.61 ± 0.17	0.61 ± 0.04	<1.0	–	–
C20:1	0.27 ± 0.01	0.29 ± 0.17	0.29 ± 0.01	0.24	0.0–0.4	0.0–0.4
C21:1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	<0.4	–	–
C22:0	0.13 ± 0.01	0.14 ± 0.02	0.14 ± 0.01	<0.2	0.0–0.2	0.0–0.2
C23:0	0.00 ± 0.00	0.01 ± 0	0.00 ± 0.00	–	–	–
C24:0	0.06 ± 0.01	0.07 ± 0.03	0.06 ± 0.004	<0.2	0.0–0.2	0.0–0.2
Others	0.05 ± 0.01	0.10 ± 0.11	0.12 ± 0.03	*C18:2 T + C18:3 T		
Number	$n = 19$	$n = 4$	$n = 6$			

The mean values are given with confidence intervals at the 95% confidence level

Table 2 Triacylglycerol compositions (%) of the three kinds of olive oil sample

TAG	Extra virgin olive oil	Virgin olive oil	Refined olive oil	Spain (mean)	Italy-Greece (mean)	Tunisia (mean)
LLL	0.17 ± 0.04	0.12 ± 0.11	0.19 ± 0.03	–	0.09	0.8
OLLn + PoLL	0.19 ± 0.04	0.14 ± 0.14	0.25 ± 0.06	0.9	0.26	0.6
PLLn	0.09 ± 0.04	0.06 ± 0.06	0.07 ± 0.02	–	–	–
OLL	2.47 ± 0.71	2.60 ± 2.37	2.94 ± 0.52	0.3	–	5.8
OOLn + PoOL	1.10 ± 0.16	1.05 ± 1.48	1.06 ± 0.26	1.0	1.04	1.5
PLL + PoPoO	0.93 ± 0.24	0.50 ± 0.27	0.81 ± 0.33	0.5	0.27	2.8
POLn + PPoPo + PPoL	0.58 ± 0.1	0.33 ± 0.37	0.54 ± 0.17	0.3	–	1.1
OLL + LnPP	12.06 ± 0.72	10.66 ± 3.08	12.04 ± 1.04	10.4	16.48	18.2
PoOO	1.26 ± 0.2	0.96 ± 0.64	1.06 ± 0.08	1.1	–	–
SLL + PLO	6.80 ± 0.75	6.04 ± 3.4	7.04 ± 0.28	4.5	4.41	12.3
PoOP + SPoL + SPoPo	0.69 ± 0.13	0.59 ± 0.17	0.73 ± 0.25	0.4	–	1.2
OOO + PoPP + PLP	30.90 ± 2.1	30.90 ± 10.97	35.44 ± 0.56	43.8	44.17	23.9
SLO	–	–	–	–	1.12	–
POO	24.10 ± 0.98	20.35 ± 2.69	25.36 ± 0.84	23.1	17.68	20.0
POP	4.17 ± 0.52	3.50 ± 1.2	4.56 ± 0.65	2.9	1.77	–
SOO	5.31 ± 0.56	4.82 ± 2	6.01 ± 0.51	3.6	4.47	3.7
POS + SLS	1.30 ± 0.25	1.19 ± 1.02	1.70 ± 0.09	0.4	0.9	1.2
PPS	–	–	–	0.6	–	0.5
Number or reference	$n = 19$	$n = 4$	$n = 6$	(27)	(27)	(27)

The mean values are given with confidence intervals at the 95% confidence level

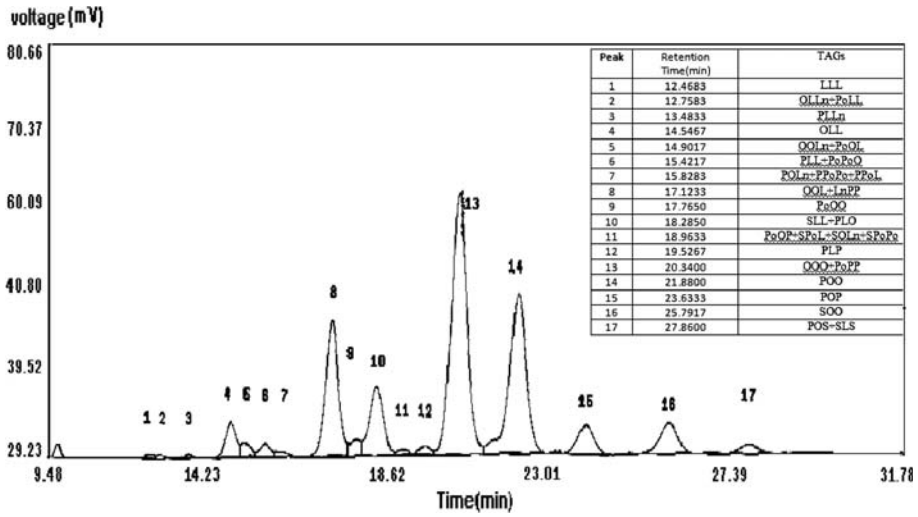


Fig. 3 HPLC profile of TAGs of a virgin olive oil. Main components of chromatographic peaks: (1) LLL; (2) OLLn + PoLL; (3) PLLn; (4) OLL; (5) OOLn + PoOL; (6) PLL + PoPoO; (7) POLn + PPoPo + PPoL; (8) OOL + LnPP; (9) PoOO; (10) SLL + PLO; (11) PoOP + SPoL + SOLn + SPoPo; (12) PLP; (13) OOO + PoPP; (14) POO; (15) POP; (16) SOO; (17) POS + SLS

Table 3 Experimental ECN42, ECN44, ECN46, ECN48 and ECN50 compositions (%) of different kinds of olive oil samples

Experimental ECN	Extra virgin olive oil	Virgin olive oil	Refined olive oil
ECN42	0.45 ± 0.11	0.45 ± 0.16	0.57 ± 0.21
ECN44	5.07 ± 0.73	5.28 ± 2.97	4.57 ± 0.89
ECN46	20.82 ± 1.32	22.14 ± 6.04	20.87 ± 1.36
ECN48	62.84 ± 2.12	60.31 ± 6.49	60.80 ± 2.4
ECN50	10.81 ± 0.83	11.83 ± 2.73	10.65 ± 0.99
Number	19	4	6

The mean values are given with confidence intervals at the 95% confidence level

Table 4 Chemical parameters of kinds of olive oil samples

Chemical parameters	Extra virgin olive oil	Virgin olive oil	Refined olive oil
ECN 42 (experimental)	0.45 ± 0.11	0.45 ± 0.16	0.57 ± 0.21
ECN42 (theoretical)	0.46 ± 0.07	0.61 ± 0.5	0.71 ± 0.39
ECN 44 (experimental)	5.07 ± 0.73	5.28 ± 2.97	4.57 ± 0.89
ECN44 (theoretical)	4.26 ± 0.46	5.27 ± 3.02	6.01 ± 2.65
ΔECN 42	0.12 ± 0.04	0.17 ± 0.33	0.30 ± 0.31
O/L	7.83 ± 0.82	6.26 ± 2.48	5.88 ± 1.88
R	0.72 ± 0.16	0.77 ± 0.25	1.050 ± 0.18
Number	19	4	6

The mean values are given with confidence intervals at the 95% confidence level

2.94% in the three kinds of olive oils). They account for about 90% of the total triacylglycerols for different kinds of olive oils. A total of 16 other triacylglycerols, LLL, OLLn, PoLL, PLLn, OOLn, PoOL, PLL, PoPoO, POLn, PPoPo, PoOO, PoOP, SPoL, SPoPo, POS, SLS, were also observed in all samples. As we did not have any international certified values for the triacylglycerols of different olive oils, we compared our values with three of the most famous olive oil producing countries of the world, Spain, Italy and Greece, Table 3 [27]. Although analysis conditions were different (mobile phase) the main triacylglycerol values (OOO, POO, LOO, PLO) are comparable to those of the most common Spanish, Italian and Greek olive oils but have some differences in comparison with Tunisian olive oils.

The results of five experimental equivalent carbon numbers, from ECN42 to ECN50 for three kinds of olive oils are also are shown in Table 3. The descending order of the experimental value of ECN's are ECN48 (between 60.8 and 62.84% in the three kinds of olive oils), ECN46

(between 20.82 and 22.14% in the three kinds of olive oils), ECN50 (between 10.65 and 11.83% in the three kinds of olive oils), ECN44 (4.57 and 5.28% in the three kinds of olive oils) and ECN42 (0.45 and 0.57%).

The important chemical parameters *R* and ΔECN42 are calculated according to both the experimental and theoretical ECN42 and ECN44 of the three different kinds of olive oils, the results are shown in Table 4. The results of ΔECN42 were 0.12 and 0.17 for the extra virgin and virgin olive oil, respectively, that was less than 0.2 and in the refined olive oil is 0.30 that these values are according to the codex criteria for extra virgin, virgin oil and refined olive oil. *R* values of the three olive oils were in accordance with the IOOC criteria and this shows that all of the olive oil samples are genuine.

The results of the evaluation show that Iranian olive oils are in accordance with the Codex and IOOC criteria. So we suggest that both *R* and ΔECN can be used for the identification of olive oils with regard to authenticity and classification. These indicators are very useful in comparison to existing methods that are generally time consuming and have severe limitations in sensitivity and selectivity.

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