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Detection and Quantification of Adulteration in Olive Oils by Global Method and Extinction Coefficient

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Abstract: The global method for detection of extraneous oils in different kinds of olive oils as a new method for identification of adulterations is applied using HPLC and GC data by calculations ECN (equivalent carbon number) and R (which is the ratio r ECN42/ r ECN44 for genuineness of all olive oils) according to IOOC method. In this research the global method and ECN42 (difference between the actual and theoretical ECN42 triglyceride content) has been applied for identification of adulterations in olive oils with sunflower, colza and soybean oils. Also the extinction coefficient was calculated from spectrophotometric data and used as a supplementary method in detection of the adulterations and classification of olive oils. The synthetic mixtures were made by 1, 5, 7 and 10 percent of sunflower, colza and soybean oil in olive oil respectively. The results showed the detection of adulteration in the 1 % of the seed oils can be detected.

Key words: Adulteration, Olive Oil, HPLC, Spectrophotometry, ECN, Global Method, IOOC.

INTRODUCTION

Olive oils are marketed according to the process used for their extraction (2003). Classification of olive oils in base of IOOC is Virgin olive oils which are produced using only cold pressing techniques, refined olive oil and pomace olive oil (IOOC 2001). They are more expensive than other seed oils in due to account of their organoleptic for virgin olive oil and their nutritional properties.[3] For this reason, they are a potential target for adulteration. The main adulteration issue involves addition of other cheaper oils, such as sunflower oil, soybean and other seed oils for long time. Therefore clearly stress on finding a way for detecting adulterations is very important (Moreno JJ and M.T. Mitjavila, 2003; Firestone D., 2001). There are several different methods used in the detection of adulteration (Firestone D., 2001; Marini F, 2004; Aparicio R., 2000). They include the iodine value, saponification value, colorimetric reaction as well as refractive index, density and viscosity measurements. Furthermore UV and fluorescence spectrometry have also be used (Ballabio D, 2006; Haddada FM, 2007). The method of analysis spectrophometric examination in the ultraviolet can provide information on the quality of a fat, its state of preservation and changes brought about in it by technological processes.

The absorption at the wavelengths specified in the method is due to the presence of conjugated diene and triene systems. These absorptions are expressed as specific extinctions $E_{1cm}^{1\%}$ (the extinction of 1% solution of the fat in the specified solvent, in a thickness of 1 cm) conventionally indicated by K (also referred to as "extinction coefficient"). As mentioned above the suppliers can be adultered olive oils. Current methodology for analysis of olive oil products includes use of a variety of techniques including GC, liquid chromatography (LC), mass spectrometry (MS), infrared and near infrared (IR and NIR) spectroscopy, Raman and nuclear magnetic resonance (NMR) spectroscopy and the use of statistical methods, but determination of triglyceride content by LC is especially useful for verifying the presence of small quantities of other vegetable oils in olive oil (A Cert *et al, 2000; Andrikopoulos N.K., 1986*).Comparison of the triglyceride composition calculated from its fatty acid composition (theoretical) with the triglyceride composition determined by LC (actual) provides a reliable means of detecting seed oils in olive oil (Nagy K., D. Bongiorno, 2005). The Codex standard for olive oil products specify that seed oils are detected by observing the maximum difference between the actual and theoretical ECN42 (equivalent carbon number) triglyceride (0.2 for virgin olive oil) that is named ECN.

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Frequently IOOC has developed a global method for the detection of extraneous oils in olive oils.[1] 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 and finally indicates a typical olive oil is genuine or not genuine (IOOC 2006). Several mathematical algorithms according to theoretical and experimental (HPLC) triacylglycerol composition and ECN42 and ECN44 are presented. The following ratios then are calculated:

r ECN42= ECN42 HPLC/ ECN42 theoretical
r ECN44= ECN44HPLC/ ECN44 theoretical

The genuineness of all olive oils, virgin and refined (except for olive- pomace oils) is defined by the ratio R: R= r ECN42/ r ECN44. In the present study the main goal is to show that the results of the spectrophometric examination, global method and ECN are precisely and specifically enable to detect the presence of sunflower oil, colza and soybean oil in olive oil at low percentages.

MATERIALS AND METHODS

Samples:

12 mixture samples of olive oil with seed oils contain sunflower oil, colza and soybean were prepared. The Iranian extra virgin oil sample olive oil for mixing was collected from Iranian olive oil producer north of Iran in the year of production 2007. The percentage of seed oils include, sunflower oil, colza and soybean were varied from 1 to 10% (1, 5, 7, and 10%).

Reagents:

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

Apparatus:

A Hewlett Packard instrument model 6890 gas chromatograph, 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) a automatic injector and software chemastation package is used. A Younglin HPLC instrument model Acme 9000 consisted by a degasser, quaternary pump, manual injection valve, refractive index detector, and Autochro software package for instrument control, data acquisition, and data analysis. Sphrisorb RP- 100 (25 cm × 4.6 mm I.D, particle size 4 μm) analytical column was used. A Carry 50 Varian spectrophotometric with ability measuring extinction in the ultraviolet between 220 and 360nm. Rectangular quartz cuvvetts, with covers, having an optical length of 1 cm were used.

Analytical Methods:

The analytical methods for the determination of extinction coefficient (K) by spectrophometric investigation in the ultraviolet region are according to COI/T20/Doc. no. 19/Rev.1, 2001. The analytical methods for the determination of fatty acid composition are described in the global method IOOC for the detection of extraneous oils in olive I (COI/T.20/Doc. no. 25, 2006). The procedure for the determination of trans-unsaturated fatty acids is described in COI/T.20/Doc. no. 17, 2001. The analysis of triglycerides is performed according to the global method IOOC for the detection of extraneous oils in olive I (COI/T.20/Doc. no. 25, 2006). Triacylglycerols in 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.

RESULTS AND DISCUSSION

The parameters K_{232}, K_{270} and K for mixtures with sunflower oils, colza and soybean are shown in the Table 1. The parameters K_{232}, K_{270} and K of the extra virgin olive oil for mixing is according to limitations of codex, IOOC and EC regulations. The results of the mixtures at 1% of soybean oil showed higher values (2.67) that corresponding IOOC value 2.6. These findings probably are due to good status of selected sample extra virgin olive oil. The results of K_{270} of mixtures olive oil with seed oils in all level percents showed similar criteria like refined olive oils except 1% colza(0.25) that is equal corresponding IOOC value 0.25 similar to virgin olive oil. Therefore other samples are not belonging to the class of virgin olive oils.

K parameter of all of the mixed samples, with the exception of level 1% colza (0.01), are more than 0.01 and are not placed in the class of virgin olive oils. Tables 2,3 and 4 are shown fatty acids of extra virgin olive oil for mixing and blend of olive oil with sunflower, colza and soybean oils, respectively.

In according to Tables 2,3 and 4 the most important changes are, increasing the values of C18:2t and C18:2c and decreasing the values of C18:1c and C16:0 by increasing the seed oils in the mixtures. As it is clear from the analyzing of fatty acids, we are not able the identification of adulterations less than 5 percent of added seed oils.

Therefore in addition to fatty acids we need the ECN (equivalent carbon number) which we used for calculation of ECN and R. In Tables 5,6 and 7 calculated values of ECN 42 (experimental) ECN42 (theoretical) ECN 44(experimental) ECN44 (theoretical), ECN 42, O (oleic acid) / L (linoleic acid) ratio and R related to all mixture samples and selected sample extra virgin olive oil for mixing are shown. The ECN oil 42 of Iranian extra virgin olive for mixing was 0.15 that is in accordance to codex criteria virgin olive oil and R value of it was in according to IOOC criteria that showed tested olive oil sample is genuine.

In according to the calculated chemical parameters, shown in Tables 5,6 and 7, by increasing of the amount of seed oils in olive oil, important chemical parameters such as ECN 42 (experimental), ECN42 (theoretical), ECN 44(experimental), ECN44(theoretical) and therefore the values of ECN 42 and R were increased with. The extreme limit of ECN 42 codex of virgin olive oil and refined olive oil are value ≤ 0.2 and ≤ 0.3 and R in according to IOOC method, for different kinds of olive oils should as follows:

$R \leq 0.95$, 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 ≥ 15

On the base of our findings, by increasing seed oils in olive oil, ECN 42 and R criteria were not in accordance with codex and IOOC, except 1 and 5% of colza that their ECN 42 are less than 0.2 but R values for 1 and 5% are 1.07 and 1.17 respectively that are greater than R value for genuine olive.

Table 1: Parameters of K_{232}, K_{270} and K of mixture olive oil with seed oils

Sample	K_{232}	K_{270}	K	No.
SSEVOO[1)]	1.77	0.17	0.00	N =1
1% sunflower	2.14	0.28	0.02	N =1
5% sunflower	2.23	0.43	0.03	N =1
5% crude sunflower	2.26	0.27	0.01	N =1
7% sunflower	2.24	0.54	0.05	N =1
10% sunflower	2.52	0.62	0.06	N =1
1%colza	2.1	0.25	0.01	N =1
5%colza	2.25	0.29	0.02	N =1
7% colza	2.30	0.34	0.02	N =1
10% colza	2.27	0.32	0.02	N =1
1%soya	2.67	0.30	0.02	N =1
5% soya	2.32	0.30	0.02	N =1
7% soya	2.30	0.38	0.02	N =1
10% soya	2.57	0.44	0.03	N =1
Codex, EC and IOOC regulation for virgin olive oil	≤2.6	≤0.25	≤0.01	

[1)] Selected sample extra virgin olive oil for mixing.

These findings may show that blends olive oil with seed oils such as sunflower oil and soybean that have high content of linoleic acid, ECN 42 and R parameters reveal the adulterations more easily than the contamination by colza.

Although ECN 42 is a critical parameter in recognition different types of olive oil such as virgin olive and refined olive oil but the our results showed the R parameter is a very important index in detection of adulterations. This parameter has different values for three kinds of mixtures of olive oil with seed oils, which shows the genuineness or non genuineness olive oils at all levels of added seed oils.

Therefore applying ECN 42 and specially R through the global method can detect the presence of small amounts of seed oils in olive oil. The extinction coefficients method may as a supplementary help us in the detection of the adulterations and classification olive oils. R values for mixings by 1% sunflower and soybean oil samples are 1.67 and 1.53 respectively which are greater than of the mentioned value of R for genuine olive oil.

Table 2: Fatty acid composition of mixture olive oil with sunflower oil at the 1% to 10%.

Fatty acid	SSEVOO	1%	5%	7%	10%	Codex, EC and IOOC regulation for virgin olive oil
C14:0	0.01	0.01	0.01	0.01	0.01	0.0 - 0.05
C15:1	0	0	0.01	0.01	0	-
C16:0	12.11	12.1	11.78	11.33	11.17	7.5 – 20.0
C16:1	0.92	0.89	0.83	0.77	0.73	0.3 - 3.5
C17:0	0.04	0.04	0.04	0.04	0.04	0.0 - 0.3
C17:1	0.08	0.07	0.07	0.07	0.06	0.0 - 0.3
C18:0	2.49	2.50	2.55	2.74	2.78	0.5 - 5.0
C18:1t	0.02	0.02	0.02	0.02	0.03	0.0 - 0.05
C18:1c	73.86	73.71	71.62	69.91		
	68	55.0 - 83.0				
C18:2t	0	0.01	0.05	0.08	0.08	0.0 - 0.05
C18:2c	8.95	9.22	11.54	13.47	15.38	
	3.5 – 21.0					
C20:0	0.4	0.39	0.39	0.39	0.47	0.0 - 0.6
C18:3alpha	0.56	0.53	0.5	0.40	0.47	-
C20:1	0.27	0.26	0.26	0.29	0.32	0.0 - 0.4
C22:0	0.1	0.1	0.13	0.23	0.27	0.0 - 0.2
C22:1	0	0	0	0	0.01	-
C24:0	0.04	0.05	0.07	0.1	0.18	-
Others	0.15	0.10	0.13	0.14		
0	0.0 - 0.2					
No.	1	1	1	1	1	

Table 3: Fatty acid composition of mixtures of olive oil with colza at the1% to 10%.

Fatty acid	SSEVOO	1%	5%	7%	10%
C14:0	0.01	0.01	0.01	0.01	0.02
C15:0	0	0.01	0.01	0	0.01
C15:1	0	0.01	0.01	0.01	0.01
C16:0	12.11	12.12	11.56	11.46	10.88
C16:1	0.92	0.9	0.84	0.82	0.77
C17:0	0.04	0.04	0.04	0.04	0.04
C17:1	0.08	0.07	0.07	0.07	0.07
C18:0	2.49	2.45	2.44	2.44	2.44
C18:1t	0.02	0.02	0.02	0.03	0.04
C18:1c	73.86	73.77	72.63	72.6	71.16
C18:2t	0	0.01	0.01	0.02	0.03
C18:2c	8.95	9.06	10.13	10.15	11.49
C20:0	0.4	0.39	0.45	0.47	0.52
C18:3alpha	0.56	0.6	1.07	1.1	1.65
C20:1	0.27	0.27	0.35	0.34	0.42
C22:0	0.1	0.01	0.12	0.12	0.14
C24:0	0.04	0.05	0.05	0.07	0.07
C24:1	0	0	0.01	0	0.02
Others	0.15	0.21	0.18	0.05	0.22
No.	1	1	1	1	1

Table 4: Fatty acid composition of mixtures of olive oil with soybean at the 1% to 10%.

Fatty acid	SSEVOO	1%	5%	7%	10%
C14:0	0.01	0.01	0.01	0.01	0.02
C15:0	0	0.01	0.01	0.01	0.01
C16:0	12.11	12.1	12.1	12.1	11.7
C16:1	0.92	0.9	0.8	0.80	0.76
C17:0	0.04	0.04	0.04	0.05	0.05
C17:1	0.08	0.07	0.07	0.07	0.07
C18:0	2.49	2.46	2.70	2.8	3.08
C18:1t	0.02	0.02	0.03	0.03	0.03
C18:1c	73.86	73.63	72.1	69.6	64.8
C18:2t	0	0	0.02	0.03	0.06
C18:2c	8.95	9.21	10.02	12.4	16.64
C18:3gamma	0	0	0.01	0.03	0.05
C20:0	0.4	0.38	0.37	0.37	0.32
C18:3alpha	0.56	0.58	0.76	1.14	1.69
C20:1	0.27	0.26	0.32	0.28	0.3
C21:1	0	0	0.01	0.02	0.02
C22:0	0.1	0.09	0.16	0.16	0.24
C23:0	0	0	0.01	0.02	0.04

Table 4: Continue.

C24:0	0.04	0.05	0.08	0.07	0.11
Others	0.15	0.21	0.07	0.06	0.01
No.	1	1	1	1	1

Table 5: Chemical parameters of mixtures of olive oil with sunflower oil at the 1% to 10%.

Sample	ECN42 (experimental)	ECN42 (theoretical)	ECN44 (experimental)	ECN44 (theoretical)	Δ ECN 42	O/L ratio	R	No.
SSEVOO	0.27	0.41	4.22	3.97	0.14	8.21	0.61	1
1%	0.68	0.37	4.22	3.85	0.31	8.01	1.67	1
5%	1.60	0.51	6.73	5.01	1.09	6.23	2.35	1
7%	2.48	0.83	8.07	7.42	1.66	4.40	2.77	1
10%	4.53	0.64	7.95	6.11	3.88	5.18	5.39	1

Table 6: Chemical parameters of mixings of olive oil with colza at the 1% to 10%.

Sample	ECN42 (experimental)	ECN42 (theoretical)	ECN44 (experimental)	ECN44 (theoretical)	Δ ECN 42	O/L ratio	R	No.
SSEVOO	0.2664	0.41	4.22	3.97	0.14	8.21	0.61	1
1%	0.470	0.42	4.24	4.01	0.06	8.11	1.07	1
5%	0.87	0.77	5.48	5.61	0.11	7.19	1.17	1
7%	1.82	1.25	6.68	7.46	0.57	6.19	1.63	1
10%	1.99	0.76	6.92	5.57	1.23	7.20	2.11	1

Table 7: Chemical parameters of mixings of olive oil with soybean at the levels from 1% to 10 %

Sample	ECN42 (experimental)	ECN42 (theoretical)	ECN44 (experimental)	ECN44 (theoretical)	Δ ECN 42	O/L ratio	R	No.
SSEVOO	0.27	0.41	4.22	3.98	0.14	8.21	0.61	1
1%	0.74	0.42	4.66	4.06	0.32	8.00	1.53	1
5%	1.12	0.58	5.42	4.84	0.55	7.21	1.74	1
7%	1.61	1.03	6.50	6.95	0.58	5.61	1.66	1
10%	3.97	1.93	9.60	10.33	2.03	3.90	2.12	1

REFERENCES

Aparicio R. and B. Aparicio-Ruíz, 2000. Authentication of vegetable oils by chromatographic techniques, review. *Journal of ChromatographyA* 881: 93-104.

Araghipour N *et al,* 2008. Geographical origin classification of olive oils by PTR-MS. *Food Chem* 108: 374-383.

Andrikopoulos N.K., 1986. Detection of olive oil adulteration with linoleic acid-rich oils by reversed-phase high-performance liquid chromatography. *Journal of ChromatographyA* 366: 311-320.

A Cert *et al*, 2000. Chromatographic analysis of minor constituents in vegetable oils, review. *J. Chromatogr* 881:131-148.

Ballabio D., A. Mauri, R. Odeschini and S. Buratti, 2006. Geographical classification of wine and olive oil by means of classification and influence matrix analysis (CAIMAN). *Analytica Chimica Acta* 570: 249-258

Bucci R., A.L. Magrı, D. Marini and F. Marini, 2002. Chemical authentication of extra virgin olive oil varieties by supervised chemometric procedures. *J. Agric. Food Chem*., 50:413-418.

Christopoulou E., M. Lazaraki, M. Komaitis and K. Kaselimis, 2004. Effectiveness of determinations of fatty acids and triglycerides for the detection of adulteration of olive oils with vegetable oils. *Food Chem*., 84: 463-474.

Codex standard for olive oils and olive pomace oils. *Codex Stan* 2003(2): 33-1981.

D'Imperio M., G. Dugo, M. Alfa, Mannina and L.A.L. Segre, 2007. Statistical analysis on Sicilian olive oils. *Food Chem*., 102: 956-965.

Di bella G., R. Maisano, La L. pera, V. Lo turco, F. Salvo and G. Dugo, 2007 Statistical Characterization of Sicilian Olive Oils from the Peloritana and Maghrebian Zones According to the fatty acid profile. *J. Agric. Food Chem*., 55: 6568-6574.

Firestone D., 2001. Assuring the integrity of olive oil products, Wiley award address. *J. AOAC Int*., 84: 176-180.

Flores, G., Ruiz del M.L. Castillo, M. Herraiz and G.P. Blanch, 2006. Study of the adulteration of olive oil with hazelnut oil by on-line coupled high performance liquid chromatographic and gas chromatographic analysis of filbertone. *Food Chem* 97: 742-749.

Galtier, O., N. Dupuy, Le Y. Dr´eau, D. Ollivier, C. Pinatel, J. Kister and J. Artaud, 2007. Geographic origins and compositions of virgin olive oils determinated by chemometric analysis of NIR spectra. *Analytica*

Chimica Acta.,595: 136-144.

Gerard, D., P. McIntyre, A.N. Davies, 2002. Detecting and quantifying sunflower oil adulteration in extra virgin olive oils from the eastern Mediterranean by visible and near-infrared spectroscopy *J. Agric. Food Chem*., 50: 5520-5525.

Hajimahmoodi, M., Y. Vander Heyden, N. Sadeghi, B. Jannat, M.R. Oveisi and S. Shahbazian, 2005. Gas-chromatographic fatty-acid fingerprints and partial least squares modeling as a basis for the simultaneous determination of edible oil mixtures. *Talanta*., 66: 1108-1116.

Haddada, F.M., H. Manaï, I. Oueslati, D. Daoud, J. Sánchez, E. Osorio and M. Zarrouk, 2007. Fatty acid, triacylglycerol, and phytosterol composition in six Tunisian olive varieties. *J. Agric. Food Chem*., 55: 10941-10946.

IOOC Trade standard applying to olive oil and olive pomace oil. COI/ T.15/NC no. 2/Rev. 10 2001.

IOOC 2006. Global method for the detection of extraneous oils in olive oils. COI/T.20/Doc. 25.

Kalua, C.M., M.S. Allen, J.r. DR. Bedgood, A.G. Bishop, P.D. Prenzler and K. Robards, 2007. Olive oil volatile compounds, flavour development and quality: a critical review. *Food Chem*., 100: 273-286.

Lapointe, A., C. Couillard and S. Lemieux, 2006. Effects of dietary factors on oxidation of low-density lipoprotein particles, reviews: current topics. *Journal of Nutritional Biochemistry*, 17: 645-658.

L´opez-Feria, S., S. C´ardenas, J. Garc´ıa-Mesa and M. Valc´arcel, 2008. Classification of extra virgin olive oils according to the protected designation of origin, olive variety and geographical origin. *Talanta*., 75: 937-943.

Moreno, J.J. and M.T. Mitjavila, 2003. The degree of unsaturation of dietary fatty acids and the development of atherosclerosis, reviews: current topics. *Journal of Nutritional Biochemistry*., 14: 182-195.

Marini, F., F. Balestrieri, R. Bucci, A.D. Magrı, A.L. Magrı and D. Marini, 2004. Supervised pattern recognition to authenticate Italian extra virgin olive oil varieties. *Chemometrics and Intelligent Laboratory Systems*., 73: 85-93.

Nagy, K., D. Bongiorno, G. Avellone, P. Agozzino, L. Ceraulo, K. V´ekey, 2005. High performance liquid chromatography–mass spectrometry based chemometric characterization of olive oils. *Journal of ChromatographyA* 1078: 90-97.

Vigli, F., A. Philippidis, A. Spyros and P. Dais, 2003. Classification of edible oils by employing 31P and 1H NMR Spectroscopy in combination with multivariate statistical Analysis. A proposal for the detection of seed oil adulteration in virgin olive oils. *J. Agric. Food Chem*., 51: 5715-572.