Grasas y Aceites
Vol. 51. Fasc. 3 (2000), 143-149

Algorithms for the detection of hazelnut oil in olive oil

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RESUMEN

Algoritmos para la detección de aceite de avellana en aceite de oliva.

La adición fraudulenta de aceite de avellana en aceite de oliva puede ser detectada sólo en altas proporciones (20-25%), usando el Δ7-estigmastenol y la diferencia entre los triglicéridos con número de carbono equivalente igual a 42, determinados experimentalmente por HPLC y teóricamente a partir de la composición de ácidos grasos (ΔECN42). Se propone un nuevo método que consiste en la comparación de los valores de varios algoritmos con una base de datos de valores experimentales obtenidos de aceites de oliva virgen genuinos. Estos algoritmos son: LLL_{exp} en función de %L; $(LLL/OLLn)_{exp}$ - $(LLL/OLLn)_{teor}$ en función de ΔECN44 y $(ECN44/LLL)_{exp}$ en función de %L; siendo: LLL_{exp}, $OLLn_{exp}$ y $ECN44_{exp}$ los porcentajes de los triglicéridos obtenidos por HPLC; LLL_{teor}, $OLLn_{teor}$ y $ECN44_{teor}$ los porcentajes de éstos calculados teóricamente a partir de la composición de ácidos grasos; ΔECN44 la diferencia entre el valor experimental y teórico del ECN44; y finalmente %L el porcentaje de ácido linoleico. La base de datos se ha recopilado considerando los valores obtenidos de aceites de oliva de diferentes composiciones de ácidos grasos y mezclas entre ellos. El método permite la detección de pequeños porcentajes (5%) de aceites de avellana en aceites de oliva.

PALABRAS-CLAVE: Aceite de avellana - Aceite de oliva - Ésteres de ácidos grasos - ECN42 - ECN44 - Triglicéridos.

SUMMARY

Algorithms for the detection of hazelnut oil in olive oil

The fraudulent addition of hazelnut oil to olive oil can be only detected in high proportions (20-25%) using the Δ7-stigmastenol and the difference between triacylglycerols of equivalent carbon number 42, determined experimentally by HPLC and calculated theoretically from the fatty acid composition (ΔECN42). A new method lies on a sequential comparison of the values of several algorithms with a database built with data obtained from genuine virgin olive oils. The algorithms are: LLL_{exp} *vs* %L; $(LLL/OLLn)_{exp}$ - $(LLL/OLLn)_{theor}$, *vs* ΔECN44 and $(ECN44/LLL)_{exp}$ *vs* %L; being: $LLLn_{exp}$ $OLLn_{exp}$ and $ECN44_{exp}$ the percentage of triacylglycerols determined by HPLC, LLL_{theor}, $OLLn_{theor}$ and $ECN44_{theor}$, the percentage of those calculated theoretically from the fatty acid composition; ΔECN44 the difference between the experimental and theoretical value of ECN44; and finally %L the percentage of linoleic acid. The database has been built considering the values obtained from olive oils of different fatty acid composition and from admixtures between them. The method allows the detection of low percentages of hazelnut oil in olive oil (5%).

KEY-WORDS: ECN42 - ECN44 - Fatty acid methyl esters - Hazelnut oil - Olive oil - Triacylglycerol.

1. INTRODUCTION

The fraudulent addition of hazelnut oil to olive oil had being produced in the last years as a consequence of the high market price reached by the olive oil compared to the price of the hazelnut oil in larger producer as Turkey. The analytical methods and maximum limits included in the European Union Commission regulations (EEC/2568/91) referred to the olive oil allow the detection of hazelnut oil only in high proportions, because the composition of hazelnut oil is similar to the olive one.

The hazelnut oil from different origins (Spain, Italy, Turkey and U.S.A.) has been widely researched in fatty acid composition and sterols (Parcerisa *et al*, 1995; Rugraff *et al*, 1982; Gargano *et al*, 1981; Colombini *et al*, 1979; García Olmedo *et al*, 1978 and Van Dijck *et al*, 1995). Although, the sterol composition is very similar between hazelnut and olive oil, the higher content in Δ7-stigmastenol present in hazelnut oil from Turkey allows their detection only when there is a high proportion of admixture, being it effectiveness limited (20-25%).

The triacylglycerol composition (Parcerisa *et al*, 1995; Gargano *et al*, 1981 and Casadei *et al*, 1987) shows differences respecting to the olive oil, in particular the increase of triacylglycerols containing linoleic acid and the decrease of those containing palmitic and linolenic acid. The higher trilinolein content allows detection of fraudulent admixtures in variable proportions (0-30%) depending on the varietal origin of the olive oil, being the limit of trilinolein 0.5% established in the EEC/2568/91 regulation. This parameter has been substituted in the EEC regulations by the differences between the percentage of triacylglycerols of equivalent carbon number 42 (ECN42) determined experimentally by HPLC and that theoretically calculated from the fatty acid composition (EEC/2472/97). This parameter improve slightly the detection level (20-25%), being not so dependent on the olive oil cultivar. Therefore, it is necessary to look for new parameters for the detection of this fraud.

In this paper, relations between different triacylglycerol, the ones showing larger differences in both oils, have been studied and compared to those obtained theoretically from the fatty acid

composition, in order to obtain algorithms to aid in the detection of low percentages of hazelnut oil in olive oil.

2. EXPERIMENTAL

2.1. Materials

All reagents were of analytical grade, except acetone and acetonitrile, which were of HPLC grade from Merck (Darmstad, Germany).

Silica gel cartridges of 1 g (6 ml) for solid phase extraction from Supelco (Bellefonte, PA, USA).

For the assays, virgin olive oils from different varietal origins were chosen in order to have a wide range of fatty acid composition. Virgin hazelnut oil from Turkey was used for the admixtures with olive oils.

2.2. Oil purification

The oil samples were purified by passing the oil through silica SPE. A silica SPE column was placed in a vacuum elution apparatus and washed under vacuum with 6 ml of hexane. The vacuum was released to prevent the dryness of the column and then a solution of the oil (0.12 g) in 0.5 ml of hexane was charged into the column. The solution was pulled through and then eluted with 10 ml of hexane diethylether (87:13 v/v) under vacuum. The combined elutes were homogenised and divided into two aliquots. Both solutions were evaporated to dryness in a rotary evaporator under reduced pressure at room temperature. The residues were dissolved, in 2 ml of acetone for triacylglycerol (TAG) analysis and in 2 ml of heptane for FAME analysis, respectively.

2.3. HPLC analysis of triacylglycerols

The analytical procedure is that described in the EEC Regulations (EEC/2568/91) using an HPLC column packed with particles of 0.4 μm instead of the 0.5 μm particles (León-Camacho and Cert, 1994).

A 10 μl aliquot of the purified oil solution in acetone (5%) was injected onto the HPLC system. The analyses were done on a Lichrosphere 100 RP-18 (4~m) column (25 cm x 4 mm I.D.) using an HP 1050 pumping unit (Hewlett Packard, Avondale, PA, U.S.A.) and refractive index detector (HP 1047). The mobile phase was acetone acetonitrile (1:1) at a flow rate to elute the trilinolein at 13 min (1.2 ml/min, approximately).

Special attention must be paid to the ECN42 (3 peaks) and ECN44 (4 peaks) as shown in Figure 1. LLL_{exp} and $OLLn_{exp}$ are the percentages of total area corresponding to the first and second peaks of the ECN42 group and the ECN44 is the percentage corresponding to the sum of the three peaks of the ECN44 group. In some cases the second peak of the ECN44 group splits in two peaks and then four peaks should be taken in account. For the calculation of the triacylglycerol percentage the peaks comprehended between ECN42 and ECN50 should be considered.

2.4. Determination of fatty acid composition

Fatty acid methyl esters (FAMES) were prepared by cold transmethylation with methanolic potash (IUPAC, 1987). To the solution of the purified oil in heptane (2 ml), 0.3 ml of 2N methanolic potash was added. The mixture was shaken vigorously for 30 s, and then, left to settle for 10 min. A 5 μl aliquot of the upper layer was injected onto the gas chromatograph.

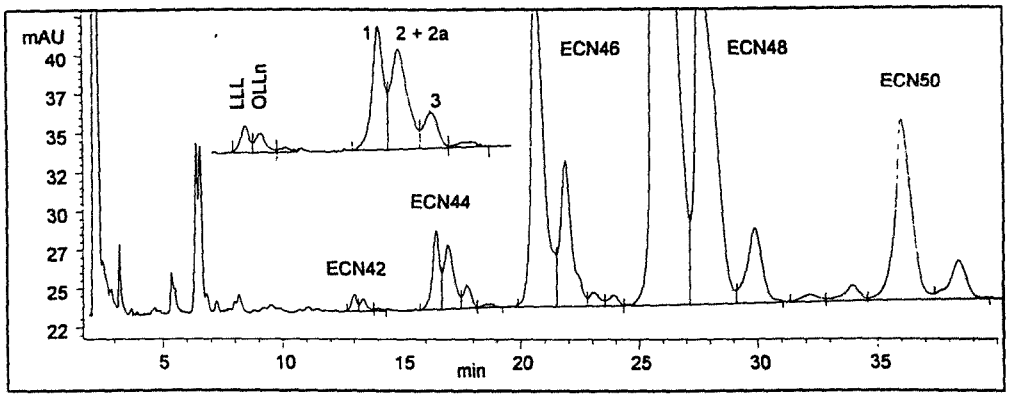

Figure 1
HPLC profile of an olive oil showing the peaks, which must be taken in account for the algorithm calculation. In the ECN44 peaks 1, 2 + 2^a and 3 should be taken in account

The gas chromatographic analysis of FAMES was carried out according to the method described for the determination of FAMES trans-isomers (EEC/1429/92). A fused silica capillary column (SP -2380, 60 m x 0.25 mm I.D., SUPELCO, Bellefonte, PA, U.S.A.) coated with cyanopropylsilicone (0.20 μm film thickness) and hydrogen as gas carrier were used. Operating conditions were: initial oven temperature 160°C for 13 min., then increased at 1.5°C/min until 190°C and maintained for 5 mm. Temperatures of injector and detector were 225° and 250°C respectively (León-Camacho and Cert, 1994). The oven temperature was adjusted to obtain the C18:3 peak eluting just before the C20:1 peak. For the calculation of the percentage of each fatty acid, the areas of the peaks corresponding to the double bond position isomers were summed.

2.5. Theoretical calculation of TAGs from FAME analysis

The calculation of the theoretical percentage of TAGs from FAME analysis was carried out taking in account only the major fatty acids palmitic P (C16:0), palmitoleic Po (C16:1), stearic 5 (C18:0), oleic O (C18:1), linoleic L (C18:2), and linolenic Ln (C18:3). The percentages of these six fatty acids were normalised up to 100%. According to the formula described in the EEC regulation (EEC/2472/97), a computer program was developed assuming an 1,3-random, 2-random distribution of the fatty acid in the glycerine molecule with the restriction in the percentages of saturated fatty acids in the 2-position (EEC/2472/97). To determine the TAG contribution to each HPLC peak, all the possible combinations among the 6 fatty acid isomers were determined. For each TAG the real equivalent carbon number was calculated according to the formula (Carelli and Cert, 1993).

$$ECN42 = CN - 2.52b_o - 2.43b_{Po} - 2.27b_L - 2.09b_{Ln}$$

The first peak of the ECN42 group includes the triacylglycerols LLL, and PoLL, the second one OLLn, PoPoPo, LPoPo, PoOLn. The three peaks of the ECN44 group comprehend OLL, PoOL, OOLn, PoPoO, PLL, PPoL, PPoPo, POLn, SLLn and SPoLn. The LnPP is not included for the calculation of the theoretical value of ECN44.

3. RESULTS AND DISCUSSION

The hazelnut oils show a fatty acid composition very homogeneous, while the olive oils have greater variations in the content of palmitic acid, oleic acid and mainly in linoleic acid. It can be seen from Table I that hazelnut oils have lower contents of palmitic, palmitoleic and linolenic acids. These differences are not enough significant to be used as an index for detecting the presence of hazelnut oil in olive oil. Similarly, the TAG composition of hazelnut oil is also very homogeneous as showed in Table II. On the other hand, olive oils show larger differences according to the variations in the fatty acid composition. Therefore, the detection of hazelnut oil in olive oil using the absolute values of triacylglycerol can not be used. A further step was looking for algorithms calculated from the experimental and theoretical values of TAGs that were quite different in both hazelnut and olive oils. The experimental trilinolein (LLL_{exp}), the difference between the experimental and theoretical ratios of the LLL, and OLLn (ΔR_1), and the ratio between the experimental value of the ECN44 and the TAG OLLn (R_{2exp}) were the algorithms that showed grater differences (Table II).

Table I
Fatty acid composition of different hazelnut and olive oil varieties

Oil	P	Po	S	O	L	Ln
Hazelnut Turkey I	5.0	0.2	2.2	82.1	10.4	0.1
Hazelnut Turkey II	4.9	0.2	2.2	79.8	12.7	0.1
Hazelnut Spain	4.9	0.2	2.6	81.7	10.5	0.1
Olive «Picual» I	12.6	1.1	2.4	79.5	3.5	0.8
Olive «Picual» II	10.2	0.7	3.3	81.6	3.6	0.5
Olive «Hojiblanca» I	10.5	0.6	2.8	79.4	6.0	0.7
Olive «Hojiblanca» III	8.0	0.5	3.0	80.3	7.5	0.7
Olive «Hojiblanca» IV	9.4	0.5	3.3	77.5	8.6	0.7
Olive «Hojiblanca» II	8.4	0.5	3.1	76.8	10.5	0.7
Olive «Picholine»	8.7	0.6	2.2	75.2	12.4	0.9
Olive «Blanqueta»	15.1	1.2	2.1	65.2	15.1	0.7
Olive «Chamlali» I	17.1	2.1	2.7	61.4	16.1	0.6
Olive «Chamlali» II	12.8	0.8	3.0	64.8	17.9	0.7

Table II

Triacylglycerol composition of different hazelnut and olive oil varieties

Oil	LLL	OLLn	PLLn	OLL	PLL+ OOLn	POLn	OOL+ PoOO	PLO+ SLL	OOO	POO	POP+ PLS	GOO	SOO	POS
Hazelnut Turkey I	1.34	—	—	4.68	0.61	—	16.23	2.49	58.56	10.37	0.37	0.41	4.64	0.33
Hazelnut Turkey II	1.80	0.07	—	5.49	0.96	—	17.70	3.29	52.44	11.29	0.76	0.55	4.57	0.50
Hazelnut Spain	1.16	—	—	4.54	0.46	—	17.09	2.42	59.30	9.94	0.23	0.39	4.42	0.44
Olive «Picual» I	0.01	0.17	—	0.46	1.60	0.70	7.73	3.45	47.23	25.51	3.79	0.78	5.12	1.51
Olive «Picual» II	0.01	0.13	—	0.44	1.31	0.49	7.37	2.97	50.61	22.96	2.81	0.87	7.07	1.44
Olive «Hojiblanca» I	0.03	0.15	—	1.02	1.73	0.68	10.79	4.07	46.63	22.10	2.66	0.82	5.49	1.12
Olive «Hojiblanca» III	0.05	0.21	0.02	1.46	1.68	0.44	13.25	4.10	49.48	18.11	1.85	0.83	5.49	0.98
Olive «Hojiblanca» IV	0.11	0.26	0.02	2.20	1.71	0.49	14.61	4.96	44.04	19.81	2.24	0.62	5.70	1.11
Olive «Hojiblanca» II	0.15	0.28	0.05	2.87	1.89	0.41	16.67	5.15	44.35	17.64	1.71	0.76	5.47	1.05
Olive «Picholine»	0.29	0.48	0.08	3.76	2.38	0.51	19.83	6.21	42.48	16.41	1.63	0.60	4.34	0.64
Olive «Blanqueta»	0.45	0.33	0.13	4.87	2.80	0.60	17.72	11.71	28.13	21.99	4.23	0.49	3.19	0.88
Olive «Chamlali» I	0.54	0.28	0.18	6.24	3.65	0.30	17.93	15.28	22.71	20.48	5.76	0.46	4.20	1.34
Olive «Chamlali» II	0.82	0.45	0.20	6.91	3.54	0.22	20.47	10.69	29.60	17.87	2.93	0.43	4.08	0.89

Table III

Values of the different parameters in hazelnut and olive oils

Oil	LLL theor. (%)	P (%)	L (%)	LLL exp (%)	ΔECN44	ΔR_1	R_{2exp}
Hazelnut Turkey I	0.22	4.9	12.7	1.80	0.97	14.5	3.0
Olive «Picual» I	0.01	12.6	3.5	0.01	0.14	0.01	276
Olive «Picual» II	0.01	10.2	3.6	0.01	0.44	0.01	224
Olive «Hojiblanca» I	0.02	10.5	6.0	0.03	0.57	0.06	114
Olive «Hojiblanca» III	0.05	8.0	7.5	0.05	0.23	0.06	69.1
Olive «Hojiblanca» IV	0.07	9.4	8.6	0.11	0.64	0.19	40.2
Olive «Hojiblanca» II	0.13	8.4	10.5	0.15	0.57	0.17	34.4
Olive «Picholine»	0.22	8.7	12.4	0.29	0.62	0.16	23.0
Olive «Blanqueta»	0.41	15.1	15.1	0.45	0.50	0.44	18.4
Olive «Chamlali» I	0.55	17.1	16.1	0.54	1.46	0.44	19.0
Olive «Chamlali» II	0.63	12.8	17.9	0.82	1.27	0.43	13.0

In olive oil, the LLL_{exp} has a direct relationship with the linoleic acid percentage while in hazelnut oil it has a higher value. Likely, the ΔR_1 and ΔECN44 parameters rise slightly with the linoleic acid percentage and a relationship between them can be established for the different levels of the theoretical trilinolein (LLL_{theo}). In hazelnut oil the ΔR_1 is high while ΔECN44 have values in the range of those of olive oil. In olive oil the R_{2exp} parameter shows a significant decrease with the increase of the linoleic acid percentage, and it is always higher than in hazelnut oils.

The effect of the addition of hazelnut oil to olive oils can be seen in Table IV. In olive oils with low percentage of linoleic acid, a great increase of LLL_{exp} occurs while a slight increase of linolenic acid is observed. In contrast, in olive oils with high percentages of linoleic acid, a slight increase of the LLL_{exp} when compared to the linolenic acid which remains constant or decreases. Therefore, the addition of hazelnut oil produce an increase of the LLL_{exp} value from the ones calculated in function of the linoleic acid percentage. Furthermore, it can be seen also from Table IV, that additions of hazelnut oil increase greatly the ΔR_1 parameter while ΔECN44

increase slightly. Finally, the R_{2exp} parameter diminishes significantly in olive oils with low and medium percentages of linoleic acid when hazelnut oil is added.

Table IV
Parameters changes for several olive oils adding different percentages of hazelnut oil

Oil	Hazelnut (%)	LLL theor. (%)	L (%)	P (%)	LLL exp (%)	ΔECN44	ΔR_1	R_{2exp}
Olive «Picual» I	0	0.01	3.50	12.50	0.01	0.14	0.01	276
	2.5	0.01	3.76	12.31	0.05	0.44	0.29	43.9
	5	0.01	3.99	12.12	0.10	0.52	0.57	26.3
	7.5	0.01	4.22	11.93	0.15	0.61	0.77	20.3
	10	0.01	4.45	11.74	0.19	0.68	1.02	15.3
Olive «Hojiblanca» II	0	0.13	10.30	8.30	0.15	0.57	0.17	34.4
	2.5	0.13	10.47	8.22	0.19	0.61	0.32	27.4
	5	0.13	10.53	8.13	0.23	0.65	0.46	22.8
	7.5	0.13	10.66	8.05	0.26	0.68	0.59	19.5
	10	0.13	10.78	7.97	0.30	0.71	0.75	15.5
Olive «Picholine»	0	0.22	12.32	8.66	0.29	0.62	0.16	23.0
	2.5	0.22	12.33	8.57	0.33	0.65	0.26	20.2
	5	0.22	12.35	8.35	0.37	0.68	0.35	18.0
	7.5	0.22	12.35	8.35	0.40	0.72	0.43	16.6
	10	0.22	12.36	8.28	0.44	0.75	0.53	15.1
Olive «Chamlali» II	0	0.63	17.80	12.70	0.74	1.27	0.15	13.6
	2.5	0.60	17.59	12.43	0.77	1.31	0.18	13.1
	5	0.59	17.47	12.24	0.79	1.34	0.27	12.6
	7.5	0.58	17.34	12.04	0.82	1.36	0.42	12.1
	10	0.56	17.21	11.85	0.85	1.38	0.56	11.5

The results indicate that these three algorithms (LLL_{exp} *vs* %L; ΔR_1 *vs* ΔECN44; R_{2exp} *vs* %L) allows the detection of hazelnut oil in olive oils. Nevertheless, the admixtures of genuine olive oils of different linoleic acid contents yield similar effects that the addition of hazelnut oils, although in lesser magnitude. The values of the different parameters for admixtures of genuine olive oils of extreme percentages of linoleic acid are shown in Table V.

The settlement of limits for the LLL_{exp}, ΔR_1 and R_{2exp} parameters were determined experimentally in genuine oils having a wide range of linoleic and palmitic acid content. The compositions of admixtures of different olive oils in several proportions were also determined. From the results, theoretical parameters were calculated and the results corrected to include the repeatability error, being increased the LLL_{exp} and ΔR_1 parameters by 0.03 and 0.15 respectively, and the R_{2exp} calculated by the formula:

$$R_{2exp} = \frac{ECN44_{exp} - 0.10}{LLL_{exp} + 0.03}$$

The genuine olive oils and the admixtures between them were classified in seven groups according to their theoretical trilinolein (LLL_{theor} < 0.5; 0.05 - 0.099; 0.10 - 0.199; 0.20 - 0.299; 0.30 - 0.399; 0.40 - 0.499 and> 0.50). Moreover, for each group, the LLL_{exp} *vs* %L were depicted for the maximum values of LLL_{exp} (Figure 2). Likely, the values of ΔR_1 *vss* ΔECN44 were plotted and curves for the maximum and minimum values were established (Figure 3). Under the lower curve lied the values of monovarietal oils and the ones lying between those curves correspond to mixtures between oils. For these mixtures, the R_{2exp} *vs* %L were calculated and a curve was traced for the minimum values of the parameter (Figure 4). For each group

Table V
Evolution of the parameters in admixtures of olive oils of different fatty acid composition

Oil	% Admixture	LLL theor. (%)	L (%)	P (%)	LLL exp (%)	ΔECN44	ΔR_1	R_{2exp}
Olive «Picual» II	—	0.01	3.63	10.22	0.01	0.44	0.01	224
Olive «Chamlali» II + «Picual» II	5:95	0.01	4.35	10.33	0.05	0.65	0.26	52.6
	10:90	0.02	5.07	10.46	0.08	0.83	0.36	37.8
	30:70	0.06	7.93	10.97	0.23	1.31	0.81	19.9
	50:50	0.15	10.76	11.47	0.38	1.45	0.91	16.1
	70:30	0.29	13.63	12.00	0.52	1.34	0.82	14.8
	90:10	0.50	16.47	12.51	0.67	0.96	0.67	13.8
Olive «Chamlali» II	—	0.63	17.86	12.76	0.79	1.27	0.43	13.0

of theoretical trilinolein, different curves were obtained.

From the above results, the method is applied in a sequential procedure starting with the calculation of the experimental triacylglycerol and the theoretical one from the fatty acid composition. The first step consists in the comparison of the value of the experimental LLL, if the value is lower than 0.05 the oil is genuine. Otherwise one proceeds to the second step, which consists in the comparison of experimental values in function of the % of linoleic acid. If the value is lower or equal to the limit, the oil is genuine, and if the value is above the limit, one proceeds to the third step, calculating the ΔR_1 in function of the ΔECN44. The value is bounded in two limits; if it is over the upper limit the oil is not genuine, if is below the lower limit the oil is genuine and if the value is comprehended between both, one must proceeds to compare the next parameter. The last one consist in the calculation of R_{2exp} parameter, again the parameter are bounded by a limit. In this case, if the value is above the limit the oil is genuine; but if is below the limit, the oil is not genuine. The flow diagram of the process is shown in the Figure 5.

To settle the detection level of the method, admixtures of each olive oil cultivar with several percentages (2.5, 5.0, 7.5 and 10%) of Turkish hazelnut oil were prepared. The method was applied concluding that all admixtures containing 7.5 and 10% of hazelnut oil and the majority of those containing 5.0% resulted in «no genuine oil». Besides, the majority of the admixtures containing

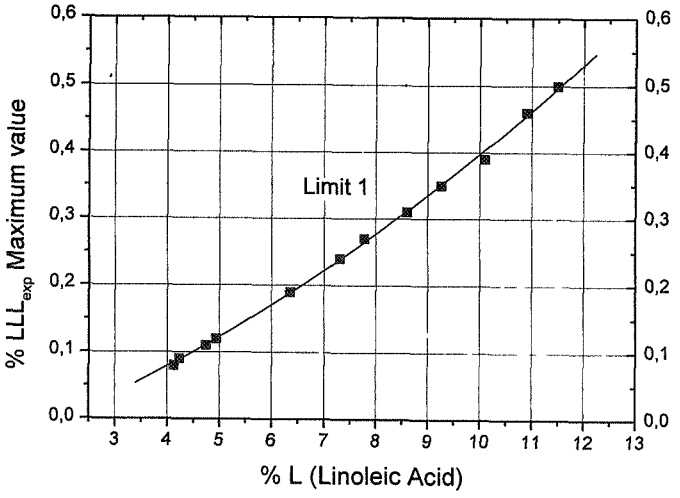

Figure 2
First limit for the classification of genuine and non-genuine olive oils (For $LLL_{theor.} < 0.2$)

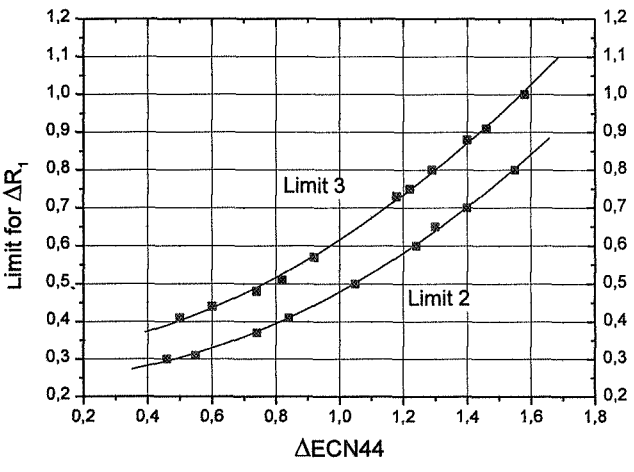

Figure 3
Second and third limits for the classification of genuine and non-genuine olive oils (For $LLL_{theor.} < 0.05$)

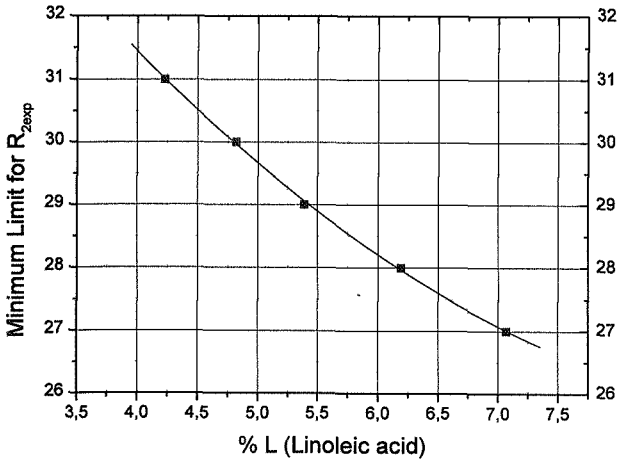

Figure 4

Fourth limit for the classification of genuine and non-genuine olive oils (For $LLL_{theor.} < 0.05$)

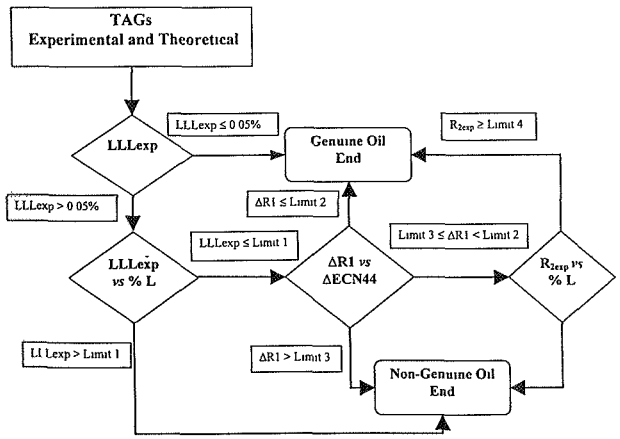

Figure 5
Flow diagram of the sequential procedure for the detection of hazelnut oil in olive oil

2.5% of hazelnut oil yielded «genuine oil» despite the parameter being very close to the limits. The results indicated that the method allows the detection of approximately 5.0% of hazelnut oil in olive oil.

Actually several laboratories are evaluating the method in order to establish the repeatability and reproducibility of the analytical procedure.

ACKNOWLEDGEMENTS

The authors wish to thank Manuel León-Camacho for the gas chromatographic and HPLC analysis of the oils. We also thank to Ministry of Agriculture for the financial support (INIA CA098-005).

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Recibido: Diciembre 1999
Aceptado: Febrero 2000