Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 86 (2004) 485–492
Food Chemistry
www.elsevier.com/locate/foodchem

Triglyceride, total and 2-position fatty acid composition of Cornicabra virgin olive oil: Comparison with other Spanish cultivars

F. Aranda, S. Gómez-Alonso, R.M. Rivera del Álamo, M.D. Salvador, G. Fregapane *

Departamento de Química Analítica y Tecnología de Alimentos, Facultad de Químicas, Universidad de Castilla – La Mancha, Avda. Camilo José Cela 10, 13071 Ciudad Real, Spain

Received 17 June 2003; received in revised form 11 September 2003; accepted 11 September 2003

Abstract

The main triglycerides (TG) found in the Cornicabra virgin olive oil variety samples analyzed ($n = 224$, from 1995/96 to 1999/2000 crop seasons) were OOO, SOL + POO, OLO + LnPP and OLA + SOO, as expected from the high oleic acid and low linoleic and linolenic acid contents observed for both the total and sn-2 position fatty acids (FA); these accounted for more than 85% of the total HPLC chromatogram peak area. Concentrations of most of the TGs and FAs presented highly significant statistical differences ($p \leqslant 0.001$) among the four Spanish varieties studied. Principal component analysis and discriminant analysis (PCA and DA) suggested that the TG variables are more suitable than total and 2-position FAs for optimum classification of the commercial samples analyzed. Finally, PCA and DA showed that there are several combinations of TGs and total FA variables (from 3 to 5) which can be selected for satisfactory classification of the four Spanish virgin olive oil varieties studied, guaranteeing 90%-plus correct classification.

Keywords: Triglycerides; Fatty acids; sn-2 Position; Virgin olive oil; Cornicabra

1. Introduction

The Cornicabra olive cultivar covers an area of 280,000 ha, mainly in the provinces of Ciudad Real and Toledo (Castilla-La Mancha, Spain), and accounts for more than 14% of the cultivated land under olive in Spain, the world's largest olive oil-producing country. The '*Montes de Toledo*' Protected Designation of Origin was recently created (EC 1187/2000) to certify the origin, authenticity and quality of the Cornicabra virgin olive oil produced in a specific geographic area and to promote the marketing of this oil variety locally and internationally. Cornicabra virgin olive oil is valued for its high stability and good sensory characteristics, which have been described as a dense sensation and a balanced aroma, sour and pungent (Salvador, Aranda, Gómez-Alonso, & Fregapane, 2001).

In many cases, chemometric and statistical procedures that employ series of chemical compounds and/or sensory descriptors are used to characterize or authenticate monovarietal virgin olive oils (Tsimidou & Karakostas, 1993; Aparicio & Luna, 2002; Bucci, Magri, Magri, Marini, & Marini, 2002; Mannina et al., 2003).

The chemical characteristics of this type of edible oil are defined in European Regulation EC 796/2002 (which amends EEC 2568/91), including the total, 2-position fatty acid (FA) and triglyceride (TG) composition of olive oils. Moreover, it has been proven that the ratio between saturated and unsaturated fatty acids can contribute to cultivar characterization, since it is known that the acidic profile of virgin olive oils is mainly affected by the fruit variety (Ranalli & Serraiocco, 1996; Gouveia, 1997; Stefanoudaki, Kotsifaki, & Koutsaftakis, 1997, 1999; Sanchez, De Miguel, & Marín, 1997; Cortesi, Fiorino, & Ponzetti, 2000). Nevertheless, other major factors, such as climatic conditions, cultivar irrigation and the stage of ripeness of the fruit, can affect the TG and FA composition (Ranalli, de Mattia, Ferrante, & Giansante, 1997; Aparicio & Luna, 2002).

* Corresponding author. Tel.: +34-902204100x3439; fax: +34-926295318.
E-mail address: giuseppe.fregapane@uclm.es (G. Fregapane).

0308-8146/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2003.09.021

Determination of the composition of the FAs located in position 2 of TGs has been widely used to detect the presence of synthetic TGs obtained by chemical esterification of glycerol with free fatty acids, since the regioselectivity of the enzymatic metabolic pathway is very specific and is not random, as is the chemical pathway (Wan, 1988). More importantly, however, this determination may also be useful for the characterization of specific virgin olive oil cultivars grown within a particular geographic region (Vlahov, Schiavone, & Simone, 1999).

The main objective of this study was to determine the TG, total and 2-position FA composition of the economically important Cornicabra virgin olive oil variety from several consecutive crop seasons (1995/96 to 1999/2000, $n = 224$). This is part of a wider project for characterization of this cultivar as grown in Castilla-La Mancha, in response to direct demand from the regional olive oil industry. In addition, we discuss the differences in the major components of Cornicabra olive oil with respect to other Spanish cultivars, with a view to a preliminary classification of varieties.

2. Materials and methods

2.1. Oil samples

Samples of commercial Cornicabra virgin olive oil ($n = 224$) were collected from industrial oil mills located in the provinces of Toledo and Ciudad Real (Castilla-La Mancha, Spain) during a series of crop seasons from 1995/1996 to 1999/2000. Most of these were collected from oil mills belonging to the *Montes de Toledo* Protected Designation of Origin. The rest were obtained from other oil mills located in Castilla-La Mancha, all exclusively processing the Cornicabra olive variety. All samples were filtered with anhydrous Na_2SO_4 and stored at 4 °C in darkness using amber glass bottles without headspace until analysis.

Samples of other Spanish monovarietal virgin olive oils, Arbequina ($n = 17$), Hojiblanca ($n = 14$) and Picual ($n = 13$), were obtained from specialized retailers soon after the crop seasons 1998/99 to 2000/01.

2.2. Analytical methods

2.2.1. Determination of triglyceride composition (Annex VIII of Regulation EC 2568/91)

A 5% solution of the samples to be analyzed was prepared by weighing 0.25 ± 0.001 g of the sample into a 5 ml graduated flask and making up to 5 ml with acetone. An HPLC system (HP 1100, Agilent Technology) equipped with a differential refractometer detector was employed, using a Spherisorb ODS-2 column (250×4.6 mm, 3 μm particle size; Teknocroma, Barcelona, Spain). Settings were: column oven, 30 °C; elution solvent: acetone-acetonitrile (60:40) at a rate of 1.0 ml/min and an injection volume of 10 μl of the sample prepared as indicated above. It was assumed that the sum of the areas of the peaks corresponding to the various TGs was equal to 100%, and the relative percentage of each TG was calculated.

2.2.2. Determination of fatty acids in the 2-position in the triglycerides (Annex VII of EEC 2568/91 and Annex XB of EC 796/2002)

This method entails several steps: purification through a column filled with alumina (15 g of activated alumina in 50 ml hexane) of a solution of 5 g of oil in 25 ml of hexane, selective hydrolysis of the 1,3-position of FAs in the TGs with pancreatic lipase, separation of the obtained monoglycerides by thin-layer chromatography, using silicagel 60 plates (Merck) and a developing solvent mixture of hexane, diethyl ether and formic acid in proportions 70/30/1 (v/v/v). Identification of the monoglyceride band (R_f about 0.035) under ultraviolet light. And finally, analysis of the monoglycerides by gas–liquid chromatography (HP 6890, Agilent Technologies) following conversion of the monoglycerides to methyl esters.

2.2.3. Determination of total fatty acids (Annex XA of EEC 2568/91 and XB of EC 796/2002)

For the determination of fatty acid composition, the methyl esters were prepared by vigorous shaking of a solution of oil in hexane (0.2 g in 3 ml) with 0.4 ml of 2 N methanolic potassium hydroxide and analyzed by a GC equipped with a FID detector (HP 6890, Agilent Technologies). A fused silica column (50 m length $\times$ 0.25 mm i.d.), coated with SGL-1000 phase (0.25 μm thickness; Sugerlabor, Spain), was used. The carrier gas was helium, with a flow through the column of 1 ml/min. The temperatures of the injector and detector were set at 250 °C and the oven temperature was 210 °C. The injection volume was 1 μl.

2.3. Statistical analysis

Statistical analysis was performed using the SPSS 10 statistical software (SPSS Inc., Chicago, USA). Descriptive analysis, one-way ANOVA, Duncan's comparison test, principal components and stepwise discriminant analyses were used.

3. Results and discussion

3.1. Triglyceride composition

The composition (%) of triglycerides (TGs), and of the TG fractions, expressed as the equivalent carbon number (ECN) found in virgin olive oil of the Cornic-

abra variety, studied ($n = 194$) in the crop seasons from 1995/96 to 1999/00, are shown in Table 1. The range and the 10, 25, 50, 75 and 90 percentiles are also reported for better description of the distribution of values.

The TG composition reported in this study may be considered as reference data for the Cornicabra virgin olive oil variety, since the previously published data on TGs for this economically important monovarietal olive oil were based on too few samples and crop seasons to be statistically reliable.

The main TG peaks in the Cornicabra virgin olive oil were OOO, SOL + POO, OLO + LnPP and OLA + SOO; these accounted for more than 85% of the total area of peaks in the chromatogram.

The level of triolein (OOO), the main TG in all olive oil varieties, was remarkably high, with a mean concentration (±SD) of $51.7 \pm 1.84\%$ and a range from 44.8% to 54.7%. Similar OOO content has been reported for the Picual variety: $48.4 \pm 2.1\%$ (Graciani, 1988), 50.9% (Osorio, Sanchez, Martinez Cano, & y Montaño, 2003) and $51.7 \pm 2.35\%$ (Gouveia, 1997), for green Manzanilla Cacereña olives (50.6%) and for ripped fruits of the Corniche variety (50.3%; Osorio et al., 2003). The OOO content of Cornicabra virgin olive oil is greater than that in other Spanish varieties such as Zorzaleña, Manzanilla, Verdial and Lechin, with average contents below 41% (Graciani, 1988), and the Carrasqueña, Cornezuelo, Morisca and Verdial varieties from Badajoz (Extremadura), with mean contents below 43% (Osorio et al., 2003).

The second peak in order of quantitative importance in the Cornicabra virgin olive oil corresponded to the SOL + POO TG mixture, with an average content of $20.8 \pm 1.33\%$ and a range from 18.1% to 25.6%. This peak is the second in importance for many other Spanish virgin olive oil varieties, such as Picual, with a reported mean content of $24.2 \pm 1.7\%$ according to Graciani (1988), and Manzanilla Cacereña and Verdial from Badajoz with mean contents of about 24.1–27.5% and 26.8–30.3%, depending on the ripening stage of the fruits (Osorio et al., 2003).

The next two TG fractions are OLO + LnPP and OLA + SOO, with mean contents of $7.79 \pm 0.91\%$ and $6.76 \pm 0.58\%$, and ranges from 5.26% to 10.7% and 5.50% to 8.61%, respectively. The OLO + LnPP content is similar to those of other Spanish monovarietal virgin

Table 1
Triglyceride composition (%) of Cornicabra virgin olive oil from 1995/96 to 1999/00 crops ($n = 194$)

Triglyceride	Mean ± SD	Range	Percentiles				
			10	25	50	75	90
LLL	0.06 ± 0.02	0.02–0.10	0.04	0.05	0.06	0.07	0.08
OLLn + PoLL	0.13 ± 0.03	0.05–0.24	0.10	0.11	0.12	0.14	0.16
OLL + PoOL	0.75 ± 0.16	0.34–1.35	0.56	0.65	0.73	0.84	0.94
OOLn + PLL + PoPoO	1.38 ± 0.15	1.09–1.97	1.20	1.29	1.36	1.47	1.59
POLn + PPoL + PPoPo	0.44 ± 0.08	0.31–0.71	0.35	0.38	0.42	0.48	0.54
OLO + LnPP	7.79 ± 0.91	5.26–10.7	6.68	7.21	7.84	8.35	8.87
PoOO	1.15 ± 0.20	0.72–1.78	0.88	1.00	1.13	1.25	1.44
POL + SLL	2.69 ± 0.32	2.10–4.19	2.39	2.49	2.62	2.81	3.05
PoOP	0.42 ± 0.10	0.22–0.74	0.31	0.34	0.40	0.48	0.57
SPoL + SOLn	0.20 ± 0.04	0.14–0.40	0.17	0.18	0.20	0.22	0.24
PPL	0.21 ± 0.04	0.16–0.26	0.18	0.19	0.21	0.22	0.23
OOO	51.7 ± 1.84	44.8–54.7	49.1	50.7	52.2	52.9	53.7
SOL + POO	20.8 ± 1.33	18.1–25.6	19.2	19.9	20.6	21.5	22.6
PSL + PPO	2.21 ± 0.29	1.50–2.99	1.87	2.00	2.14	2.39	2.66
MOO	0.14 ± 0.07	0.07–0.68	0.09	0.11	0.12	0.15	0.19
OOG	0.69 ± 0.12	0.47–1.02	0.55	0.58	0.67	0.76	0.85
OLA + SOO	6.76 ± 0.58	5.50–8.61	6.11	6.35	6.64	7.11	7.63
SOP + SLS	1.24 ± 0.14	0.71–1.78	1.08	1.14	1.22	1.32	1.4°
OOA	0.93 ± 0.06	0.60–1.12	0.86	0.90	0.93	0.97	1.01
SOS + POA	0.38 ± 0.07	0.12–0.63	0.31	0.33	0.36	0.42	0.47
ECN_{42}	0.18 ± 0.04	0.07–0.32	0.14	0.16	0.18	0.20	0.23
ECN_{44}	2.57 ± 0.30	1.84–3.82	2.23	2.37	2.52	2.71	2.94
ECN_{46}	12.5 ± 1.08	9.13–16.7	11.2	11.8	12.4	13.1	13.7
ECN_{48}	74.7 ± 1.62	69.0–79.2	72.8	73.7	74.6	75.8	76.4
ECN_{50}	8.68 ± 0.70	7.10– 11.09	7.89	8.19	8.52	9.04	9.71
ECN_{52}	1.31 ± 0.11	0.90– 1.60	1.19	1.24	1.30	1.38	1.46

SD, standard deviation; P, palmític; Po, palmitoleic; M, margaric; S, stearic; O, oleic; L, linoleic; Ln, linolenic; and A, arachidic acids. ECN_{42} = LLL + OLLn + PoLL. ECN_{44} = OLL + PoOL + OOLn + PLL + PoPoO + POLn + PPoL + PPoPo. ECN_{46} = OLO + LnPP + PoOO + POL + SLL + PoOP + SPoL + SOLn + PPL. ECN_{48} = OOO + SOL + POO + PSL + PPO. ECN_{50} = OOG + OLA + SOO + SOP + SLS. ECN_{52} = OOA + SOS + POA.

olive oils, such as Picual and Verdial, in which the reported mean content is 6.8 to 13.8% (Graciani, 1988), whereas mean contents of 8% and 18% have been found in the Cacereña and Verdial de Badajoz varieties (Osorio et al., 2003). The OLA + SOO content is relatively high such as compared to the average content in other virgin olive oil varieties, such as the Spanish Zorzaleña, Manzanilla, Lechin and Verdial (Graciani, 1988) with average contents below 5.5%; and Manzanilla Cacereña (3.8–4.1%), Verdial from Badajoz (4.6–5.2%), Morisca (4.5–4.9%) or Cornezuelo (4.8%) grown in Extremadura (Osorio et al., 2003). On the other hand, the content in the Cornicabra variety is similar to that found in Picual (5.3–7.2%), grown in southern Portugal (Gouveia, 1997) and Picual, grown in Andalusia ($7.4 \pm 0.5\%$ according to Graciani, 1988) and Corniche grown in Extremadura (with an average content above 7% according to Osorio et al. (2003)).

The mean contents of POL + SLL and PSL + PPO ($2.69 \pm 0.32\%$ and $2.21 \pm 0.29\%$, respectively) were very similar to those of Manzanilla Cacereña and Picual variety virgin olive oils. The concentrations of these TGs are much higher in virgin olive oil varieties with low OOO contents, such as Cornezuelo, Morisca and Verdial from Badajoz, with average contents in POL + SLL above 9% (Osorio et al., 2003), and Galega Vulgar (4.5%; Gouveia, 1997).

Finally, concentrations of trilinolein (LLL) and the ECN_{42} fraction (LLL, OLLn and PLLn) in the Cornicabra virgin olive oil were very low ($0.06 \pm 0.02\%$ and $0.18 \pm 0.04\%$, respectively).

3.2. Total fatty acid composition

The fatty acid (FA) composition (%) of Cornicabra virgin olive oil ($n = 224$) in the five crop seasons studied is depicted in Table 2. These analytical results are within EU Regulation limits for olive oils (myristic acid: $\leqslant 0.05\%$; linoleic: $\leqslant 0.9\%$; arachidic: $\leqslant 0.6\%$; gadoleic: $\leqslant 0.4\%$; behenic: $\leqslant 0.2\%$; and lignoceric: $\leqslant 0.2\%$).

In Cornicabra olive oil, oleic acid content is especially high ($80.4 \pm 0.96\%$) and linoleic acid content is especially low ($4.46 \pm 0.57\%$). Cornicabra and Picual from Andalusia are the two Spanish varieties with the highest oleic acid contents of relevant nutritional interest, and are the lowest in linoleic and linolenic acids (Alba et al., 1996), which explains their oxidative stability, as determined by the Rancimat method (Salvador et al., 2001). Moreover, the Cornicabra variety, like the Hojiblanca variety, also contains low levels of palmitic acid ($9.22 \pm 0.17\%$).

In addition to the cultivar, it should be considered that the other main known factors affecting total fatty acid composition, and especially oleic acid content, are latitude, climatic conditions, and the ripening stage of the fruit on harvesting (Ranalli et al., 1997; Aparicio & Luna, 2002).

3.3. Fatty acids in the 2-position of the triglycerides

The composition of the FAs in the sn-2 position of the TGs for the Cornicabra virgin olive oil samples studied ($n = 160$) is shown in Table 3.

As expected from the total FA profile of the Cornicabra variety, of the FAs in the 2-position of the TGs, oleic acid content was high ($93.4 \pm 0.65\%$, with a range from 91.5% to 95.1%) and linoleic ($4.96 \pm 0.68\%$, ranging from 3.27% to 6.60%) and linolenic acid ($0.34 \pm 0.06\%$, ranging from 0.23% to 0.57%) contents were low.

The composition of the FAs in the 2-position reported in this study can also be considered as reference data for the Cornicabra virgin olive oil variety, since the previously published data on these compounds did not represent enough samples and crop seasons to be statistically relevant.

Table 2
Total fatty acid composition (%) of Cornicabra virgin olive oil from 1995/96 to 1999/00 crops ($n = 224$)

Fatty acid	Mean ± SD	Range	Percentiles				
			10	25	50	75	90
Palmitic, $C_{16:0}$	9.22 ± 0.17	6.99–11.05	8.42	8.73	9.13	9.67	10.3
Palmitoleic, $C_{16:1}$	0.77 ± 0.11	0.49–1.11	0.65	0.68	0.76	0.85	0.93
Margaric, $C_{17:0}$	0.06 ± 0.01	0.04–0.07	0.05	0.05	0.06	0.06	0.07
Margaroleic, $C_{17:1}$	0.10 ± 0.01	0.08–0.11	0.09	0.09	0.10	0.10	0.11
Stearic, $C_{18:0}$	3.36 ± 0.29	2.61–4.43	3.05	3.15	3.29	3.56	3.77
Oleic, $C_{18:1}$	80.4 ± 0.96	76.5–82.5	79.1	79.9	80.6	81.0	81.5
Linoleic, $C_{18:2}$	4.46 ± 0.57	3.07–6.62	3.84	4.09	4.45	4.73	5.21
Linolenic, $C_{18:3}$	0.62 ± 0.08	0.48–0.95	0.53	0.56	0.60	0.66	0.72
Arachidic, $C_{20:0}$	0.51 ± 0.03	0.28–0.62	0.48	0.49	0.50	0.51	0.54
Gadoleic, $C_{20:1}$	0.34 ± 0.02	0.27–0.39	0.32	0.33	0.34	0.36	0.37
Behenic, $C_{22:0}$	0.14 ± 0.01	0.11–0.21	0.13	0.13	0.14	0.15	0.16
SFA	13.3 ± 0.67	11.40–15.13	12.5	12.9	13.2	13.7	14.3
MUFA	81.6 ± 0.90	78.0–83.6	80.5	81.3	81.8	82.2	82.6
PUFA	5.08 ± 0.58	3.67–7.22	4.46	4.66	5.03	5.37	5.79

Table 3
Composition (%) of the fatty acids in the sn-2 position of the triglyceride of Cornicabra virgin olive oil from 1995/96 to 1999/00 crops ($n = 160$)

Fatty acid	Mean ± SD	Range	Percentiles				
			10	25	50	75	90
$C_{16:0}$	0.57 ± 0.07	0.44–0.91	0.49	0.53	0.56	0.61	0.65
$C_{16:1}$	0.53 ± 0.09	0.32–0.81	0.43	0.46	0.51	0.58	0.65
$C_{17:1}$	0.10 ± 0.01	0.08–0.16	0.09	0.09	0.10	0.10	0.11
$C_{18:0}$	0.10 ± 0.01	0.07–0.16	0.09	0.09	0.10	0.11	0.12
$C_{18:1}$	93.4 ± 0.65	91.5–95.05	92.6	93.0	93.4	93.9	94.2
$C_{18:2}$	4.96 ± 0.68	3.27–6.60	4.06	4.46	5.00	5.35	5.92
$C_{18:3}$	0.34 ± 0.06	0.23–0.57	0.28	0.30	0.33	0.35	0.40
SFA	0.67 ± 0.07	0.54–1.07	0.59	0.62	0.66	0.71	0.75
MUFA	94.0 ± 0.67	92.3–95.72	93.1	93.6	94.0	94.5	94.9
PUFA	5.29 ± 0.69	3.58–6.93	4.37	4.79	5.33	5.68	6.27

3.4. Preliminary classification of the main Spanish virgin olive oil varieties on the basis of the TG and FA profiles

There were high statistically significant differences ($p \leqslant 0.001$ among the four Spanish varieties studied in terms of concentrations of most of the triglycerides, as reported in Table 4, for selected compounds, on the basis of their Anova-F ratio and content. The highest statistically significant differences among the cultivars, in terms of TGs, were found in the peaks corresponding to OLL + PoOL ($F = 404$), POL + SLL ($F = 858$), SPoL + SOLn ($F = 749$), PPL ($F = 513$) and OOO ($F = 386$) and the fraction ECN_{46} ($F = 674$).

Principal component analysis and stepwise discriminant analysis showed that one of the most reasonable combinations for the classification of the commercial virgin olive oil varieties studied comprised four variables: POL + SLL, OOA, ECN_{48} and OOLn + PLL + PoPoO. The first two discriminant functions of the statistical analysis explained 99.1% of the variance (90.2% and 8.9%, respectively), yielding a reasonable classification (86–99%) of each of the virgin olive oil varieties studied, as depicted in Fig. 1. Very similar results were obtained by replacing the OOLn + PLL + PoPoO peak with PPL.

Again, highly significant statistical differences ($p \leqslant 0.001$) were found between the four Spanish varieties studied in terms of total FA contents, as reported in Table 5 for selected compounds. The most significant peaks were those corresponding to $C_{17:1}$ ($F = 1014$), $C_{18:1}$ ($F = 461$), $C_{18:2}$ ($F = 482$), MUFA ($F = 457$) and PUFA ($F = 468$).

The Cornicabra and Picual varieties had the highest percentages of oleic acid (80.4 ± 0.96 and 78.9 ± 1.62, respectively) and MUFA (81.6 ± 0.90 and 80.2 ± 1.53

Table 4
Most relevant triglyceride content (mean ± SD, as %) of different Spanish virgin olive oil varieties

Triglyceride	Anova *F*- ratio	Variety			
		Cornicabra ($n = 194$)	Picual ($n = 13$)	Hojiblanca ($n = 14$)	Arbequina ($n = 17$)
OLL + PoOL	404	0.75 ± 0.16^{a}	0.68 ± 0.23^{a}	1.74 ± 0.44^{b}	2.42 ± 0.38^{c}
OOLn + PLL + PoPoO	148	1.38 ± 0.15^{a}	1.44 ± 0.12^{a}	1.76 ± 0.15^{b}	2.22 ± 0.31^{c}
OLO + LnPP	277	7.79 ± 0.91^{b}	7.16 ± 1.10^{a}	11.72 ± 1.60^{c}	13.93 ± 0.78^{d}
PoOO	32	1.15 ± 0.20^{b}	1.38 ± 0.26^{c}	0.77 ± 0.32^{a}	1.49 ± 0.31^{c}
POL + SLL	858	2.69 ± 0.32^{a}	2.86 ± 0.43^{a}	4.26 ± 0.66^{b}	7.72 ± 0.78^{c}
SPoL + SOLn	749	0.20 ± 0.04^{a}	0.26 ± 0.04^{b}	0.37 ± 0.10^{c}	1.01 ± 0.21^{d}
PPL	513	0.21 ± 0.04^{a}	0.24 ± 0.05^{b}	0.41 ± 0.06^{c}	0.40 ± 0.04^{c}
OOO	386	51.7 ± 1.84^{d}	48.5 ± 1.63^{c}	45.4 ± 2.94^{b}	35.5 ± 2.61^{a}
SOL + POO	28	20.8 ± 1.33^{a}	22.8 ± 1.37^{b}	20.1 ± 1.63^{a}	23.2 ± 0.89^{b}
PSL + PPO	151	2.21 ± 0.29^{a}	2.96 ± 0.38^{c}	2.54 ± 0.66^{b}	4.07 ± 0.61^{d}
OLA + SOO	181	6.76 ± 0.58^{c}	6.87 ± 0.70^{c}	6.29 ± 0.53^{b}	3.42 ± 0.38^{a}
OOA	225	0.93 ± 0.06^{c}	0.73 ± 0.06^{b}	0.74 ± 0.06^{b}	0.56 ± 0.08^{a}
ECN_{42}	209	0.18 ± 0.04^{a}	0.29 ± 0.07^{b}	0.37 ± 0.09^{c}	0.49 ± 0.14^{d}
ECN_{44}	358	2.57 ± 0.30^{a}	2.65 ± 0.32^{a}	4.03 ± 0.60^{b}	5.36 ± 0.72^{c}
ECN_{46}	674	12.5 ± 1.08^{a}	12.5 ± 1.33^{a}	17.9 ± 1.98^{b}	25.4 ± 1.45^{c}
ECN_{48}	300	74.7 ± 1.62^{c}	74.2 ± 2.09^{c}	68.0 ± 2.26^{b}	62.7 ± 2.06^{a}
ECN_{50}	154	$8.68 \pm 0.70^{b,c}$	8.92 ± 0.83^{c}	8.26 ± 0.78^{b}	4.95 ± 0.51^{a}
ECN_{52}	115	1.31 ± 0.11^{d}	1.20 ± 0.13^{c}	1.10 ± 0.10^{b}	0.81 ± 0.14^{a}

Mean values with different letters within the same row are statistically different ($p \leqslant 0.05$).

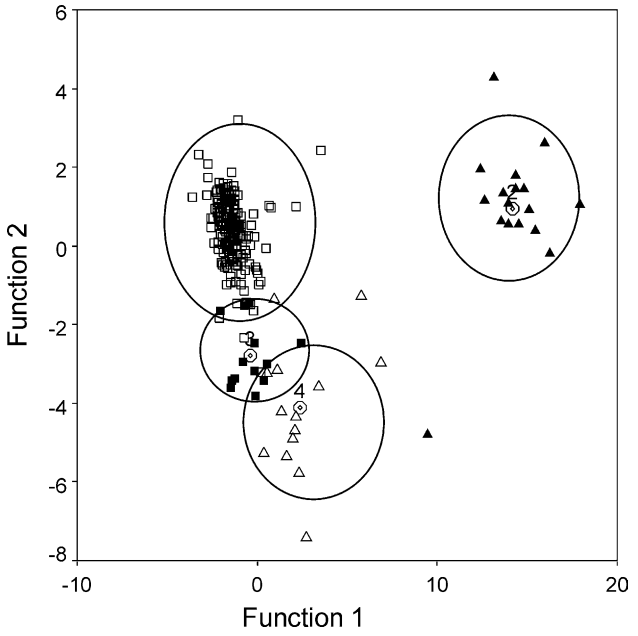

Fig. 1. Plot of discriminant functions using four variables to classify different commercial Spanish virgin olive oil varieties. *Variables*: POL + SLL, OOA, ECN48 and OOLn + PLL + PoPoO. (□) Cornicabra (1); (▲) Arbequina (2); (■) Picual (3); (△) Hojiblanca (4); (⊙) group centroid.

respectively), with a significant statistical difference for the first, which was higher in Cornicabra, but not for the second. These two varieties differed significantly in palmitic acid and SFA, which were lower in Cornicabra than in Picual. Arbequina had higher levels of palmitic ($13.7 \pm 0.99\%$), palmitoleic ($1.42 \pm 0.24\%$) and linoleic acid (10.3 ± 0.87) than any of the other varieties studied. Finally, Hojiblanca had statistically significantly higher linolenic content (0.75 ± 0.04) than the other monovarietal virgin olive oils analyzed (Table 5).

Principal component analysis and stepwise discriminant analysis showed that $C_{18:2}$, $C_{17:1}$, $C_{18:0}$, $C_{16:0}$, PUFA, MUFA and $C_{18:1}$ were the most relevant variables for classification of the commercial virgin olive oil varieties studied. Several combinations of from 3 to 5 of these variables produced similar results, providing a reasonable classification of the varieties studied (85–99%).

With regard to the FAs in the sn-2 position of the TGs, the compounds with the highest Anova *F*-ratio were practically the same as those with the highest total FAs. For this reason, when statistical analysis was applied to the ratio between the 2-position and total FAs, the Anova *F*-ratio values were much lower than those obtained with both independent variables (data not shown).

Cornicabra and Picual varieties had the highest concentrations of oleic aid and the lowest of linoleic and linolenic in the 2-position of the TGs. They differed in palmitoleic acid content, which was significantly higher in Picual ($0.67 \pm 0.12\%$) than in Cornicabra ($0.53 \pm 0.09\%$). Hojiblanca had the lowest concentrations of 2-position palmitic, palmitoleic acid and SFA. Arbequina had the highest concentrations of palmitic, palmitoleic

Table 5
Most relevant total and 2-position fatty acid contents (mean ± SD, as %) of different Spanish virgin olive oil varieties

Fatty acid	Anova *F*-ratio	Variety			
		Cornicabra ($n = 224$)	Picual ($n = 13$)	Hojiblanca ($n = 14$)	Arbequina ($n = 17$)
$C_{16:0}$	200	9.22 ± 0.17^{a}	10.6 ± 0.78^{b}	9.68 ± 1.00^{a}	13.7 ± 0.99^{c}
$C_{16:1}$	141	0.77 ± 0.11^{a}	0.91 ± 0.13^{b}	0.73 ± 0.15^{a}	1.42 ± 0.24^{c}
$C_{17:1}$	1013	0.10 ± 0.01^{a}	0.11 ± 0.03^{b}	0.23 ± 0.04^{c}	0.26 ± 0.02^{d}
$C_{18:0}$	110	3.36 ± 0.29^{b}	3.49 ± 0.47^{b}	3.48 ± 0.21^{b}	2.03 ± 0.19^{a}
$C_{18:1}$	461	80.4 ± 0.96^{d}	78.9 ± 1.62^{c}	76.6 ± 1.54^{b}	70.6 ± 1.70^{a}
$C_{18:2}$	482	4.46 ± 0.57^{a}	4.53 ± 1.14^{a}	7.51 ± 1.13^{b}	10.3 ± 0.87^{c}
$C_{18:3}$	16	0.62 ± 0.08^{a}	0.67 ± 0.05^{b}	0.75 ± 0.04^{c}	0.61 ± 0.05^{a}
SFA	116	13.3 ± 0.67^{a}	14.6 ± 0.70^{c}	13.9 ± 0.92^{b}	16.4 ± 0.97^{d}
MUFA	457	81.6 ± 0.90^{d}	80.2 ± 1.53^{c}	77.9 ± 1.44^{b}	72.6 ± 1.53^{a}
PUFA	468	5.08 ± 0.58^{a}	5.20 ± 1.14^{a}	8.26 ± 1.15^{b}	10.9 ± 0.90^{c}
sn-2 $C_{16:0}$	27	0.57 ± 0.07^{b}	0.53 ± 0.07^{b}	0.45 ± 0.07^{a}	0.73 ± 0.06^{c}
sn-2 $C_{16:1}$	106	0.53 ± 0.09^{a}	0.67 ± 0.12^{b}	0.42 ± 0.15^{a}	1.17 ± 0.29^{c}
sn-2 $C_{17:1}$	1251	0.10 ± 0.01^{a}	0.11 ± 0.02^{a}	0.25 ± 0.03^{b}	0.33 ± 0.02^{c}
sn-2 $C_{18:0}$	13	0.10 ± 0.01^{b}	0.10 ± 0.01^{b}	0.08 ± 0.01^{a}	0.08 ± 0.01^{a}
sn-2 $C_{18:1}$	518	93.4 ± 0.65^{c}	93.4 ± 0.86^{c}	89.6 ± 0.88^{b}	85.0 ± 0.81^{a}
sn-2 $C_{18:2}$	364	4.96 ± 0.68^{a}	4.82 ± 1.01^{a}	8.71 ± 0.87^{b}	12.1 ± 0.74^{c}
sn-2 $C_{18:3}$	53	0.34 ± 0.06^{a}	0.33 ± 0.01^{a}	0.51 ± 0.06^{b}	0.53 ± 0.09^{b}
sn-2 SFA	20	0.67 ± 0.07^{b}	0.64 ± 0.06^{b}	0.54 ± 0.07^{a}	0.80 ± 0.07^{c}
sn-2 MUFA	402	94.0 ± 0.67^{c}	94.2 ± 0.95^{c}	90.2 ± 0.88^{b}	86.5 ± 0.78^{a}
sn-2 PUFA	376	5.29 ± 0.69^{a}	5.15 ± 1.01^{a}	9.22 ± 0.91^{b}	12.7 ± 0.81^{c}

Mean values with different letters within the same row are statistically different ($p \leqslant 0.05$).

and linoleic acid, SFA and PUFA of the samples analyzed.

According to principal component analysis and discriminant analysis, the most useful sn-2 position FA variables for classification were $C_{17:1}$, $C_{16:1}$, $C_{18:3}$, $C_{20:0}$ and $C_{18:3}$. The final classification was somewhat lower with respect to total FAs, and, more importantly, the discrimination of Cornicabra and Picual samples is significantly lower. Therefore, although this parameter has been proposed for classification of virgin olive oil varieties (Vlahov et al., 1999), our results suggest that it is not worthwhile, since the analytical determination is more time-consuming without enhancing the discrimination power of discriminant analysis.

Finally, taking into account the three sets of analytical determinations studied, several combinations of some TG and total FA variables, from 3 to 5, could be selected by principal component analysis and discriminant analysis to satisfactorily classify the four virgin olive oil varieties studied with more than 90% certainty.

The statistical analysis suggested that the TG variables were more important than total and 2-position FAs for optimum classification of the commercial samples analyzed. However, TG and 2-position FA determination consume much more time and resources than simple, straightforward FA analysis, and so it is not really clear whether it is worth including these variables in the chemometric and statistical analyses used to classify virgin olive oil varieties.

In addition, this preliminary study can surely be improved by considering other minor chemical components of virgin olive oil, such as individual phenolic compounds (Gómez-Alonso, Salvador, & Fregapane, 2002) and sterols and alcohols (Rivera del Álamo, Fregapane, Aranda, Gómez-Alonso, & Salvador, 2003) which, as families of substances, have proved to be very important in the chemical classification of virgin olive oil varieties.

Acknowledgements

The authors express their sincere gratitude to the Spanish Comision Interministerial de Ciencia y Tecnología (CICYT) for supporting this research project (ALI95-0197-CO2-02 and 1FD97-0177).

References

Alba, J., Hidalgo, F., Ruíz, M. A., Martínez, F., Moyano, M. J., Cert, A., Pérez-Camino, M. C., & Ruíz, M. V. (1996). Características de los aceites de oliva de primera y segunda centrifugación. *Grasas y Aceites, 47*(3), 163–181.

Aparicio, R., & Luna, G. (2002). Characterization of monovarietal virgin olive oils. *European Journal of Lipid Science and Technology, 104*, 614–627.

Bucci, R., Magri, A. D., Magri, A. L., Marini, D., & Marini, F. (2002). Chemical authentication of extra virgin olive varieties by supervised chemometric procedures. *Journal of Agricultural and Food Chemistry, 50*(3), 413–418.

Cortesi, N., Fiorino, P., & Ponzetti, A. (2000). Influencia de los cultivares y sistemas de extracción en la composición del aceite de oliva. *Olivae, 81*, 36–39.

EEC 2568/91 (1991). Characteristics of olive and olive pomance oils and their analytical methods. *Official Journal of the European Communities, L248*, 1–82.

EC 1187/2000 (2000). Register of protected designations of origin and protected geographical indications. *Official Journal of the European Communities, L133*, 19–20.

EC 796/2002 (2002). Commission Regulation amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-pomace oil and on the relevant methods of analysis and the additional notes in the Annex to Council Regulation (EEC) No 2658/87 on the tariff and statistical nomenclature and on the Common Customs Tariff, L128, 8–28.

Graciani, E. (1988). Caracterización del aceite de oliva virgen español. III. Posibilidad de caracterización por variedades de aceituna o por zonas de producción de acuerdo con su contenido en triacilgliceroles. *Grasas y Aceites, 39*(2), 105–110.

Gómez-Alonso, S., Salvador, M. D., & Fregapane, G. (2002). Phenolic compounds profile of Cornicabra virgen olive oil. *Journal of Agricultural and Food Chemistry, 50*, 6812–6817.

Gouveia, J. M. B. (1997). Comparación de los Aceites de Oliva de los Cultivares Cobrançosa, Blanqueta, Azeiteira y Picual con los del cv. Galega Vulgar, Producidos en el Norte del Alentejo. I. Principales Características Químicasy Sensoriales. *Olivae, 66*, 34–45.

Mannina, L., Dugo, G., Salvo, F., Cicero, L., Ansanelli, G., Calcagni, C., & Segre, A. (2003). Study of the cultivar-composition relationship in Sicilian olive oils by GC, NMR, and statistical methods. *Journal of Agricultural and Food Chemistry, 51*(1), 120–127.

Osorio, E., Sanchez, J. J., Martinez Cano, M., & y Montaño, A. M. (2003). Estudio del contenido en triglicéridos de aceites monovarietales elaborados a partir de aceitunas producidas en la región extremeña. *Grasas y aceites, 54*(1), 1–6.

Ranalli, A., de Mattia, G., Ferrante, M. L., & Giansante, L. (1997). Incidence of olive cultivation area on the analytical characteristics of the oil. Note 1. *La Rivista Italiana delle Sostanze Grasse, 74*, 501–508.

Ranalli, A., & Serraiocco, A. (1996). Quantitative and qualitative effects of a pectolytic enzyme in olive oil production. *Grasas y Aceites, 47*(4), 227–236.

Rivera del Álamo, R. M., Fregapane, G., Aranda, F., Gómez-Alonso, S., & Salvador, M. D. (2003). Sterol and alcohol composition of cornicabra virgin olive oil: The campesterol content exceeds the upper limit of 4% established by EU regulations. *Food Chemistry* doi:10.1016/s0308-8146(03)00275-9.

Salvador, M. D., Aranda, F., Gómez-Alonso, S., & Fregapane, G. (2001). Cornicabra virgin olive oil: A study of five crop seasons. Composition, quality and oxidative stability. *Food Chemistry, 74*(3), 267–274.

Sanchez, J. J., De Miguel, C. y., & Marín, J. (1997). La calidad del aceite de oliva procedente de variedades cultivadas en Extremadura en relación con la composición y maduración de la aceituna. *Olivae, 75*, 31–36.

Stefanoudaki, E., Kotsifaki, F., & Koutsaftakis, A. (1997). The potential of HPLC triglyceride profiles for the classification of Cretan olive oils. *Food Chemistry, 60*, 425–432.

Stefanoudaki, E., Kotsifaki, F., & Koutsaftakis, A. (1999). Classification of virgin olive oils of the two major cretan cultivars based on

their fatty acid composition. *Journal of the American Oil Chemists' Society, 76*(5), 623–626.

Tsimidou, M., & Karakostas, K. X. (1993). Geographical classification of Greek virgen olive oil by non-parametric multivariate evaluation of fatty acid composition. *Journal of the Science of Food and Agriculture, 62*, 253–257.

Vlahov, G., Schiavone, C., & Simone, N. (1999). Triacylglycerols of the olive fruit (*Olea europaea* L.): Characterization of mesocarp and seed triacylglycerols in different cultivars by liquid chromatography and ^{13}C NMR spectroscopy. *Fett/Lipid, 101*(4), 146–150.

Wan, P. J. (Ed.). (1988). *Introduction to fats and oils technology*. Champaign, IL: American Oil Chemists' Society.