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Phenolic Compounds Profile of Cornicabra Virgin Olive Oil

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This study presents the phenolic compounds profile of commercial Cornicabra virgin olive oils from five successive crop seasons (1995/1996 to 1999/2000; $n = 97$), determined by solid phase extraction reversed phase high-performance liquid chromatography (SPE RP-HPLC), and its relationship with oxidative stability, processing conditions, and a preliminary study on variety classification. The median of total phenols content was 38 ppm (as syringic acid), although a wide range was observed, from 11 to 76 ppm. The main phenols found were the dialdehydic form of elenolic acid linked to tyrosol (*p*-HPEA-EDA; 9 ± 7 ppm, as median and interquartile range), oleuropein aglycon (8 ± 6 ppm), and the dialdehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA; 5 ± 8 ppm). In many cases the correlation with oxidative stability was higher when the sum of the dialdehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA) and oleuropein aglycon ($r^2 = 0.91-0.96$) or the sum of these two and hydroxytyrosol ($r^2 = 0.90-0.97$) was considered than was observed with HPLC total phenols ($r^2 = 0.91-0.95$) and especially with colorimetric determination of total polyphenols and *o*-diphenols ($r^2 = 0.77-0.95$ and 0.78−0.92, respectively). 3,4-DHPEA-EDA, p-HPEA-EDA, the aglycons of oleuropein and ligstroside, and HPLC total phenols content presented highly significant differences ($p = 0.001-0.010$) with respect to the dual- and triple-phase extraction systems used, whereas colorimetric total polyphenols content did not ($p = 0.348$) and *o*-diphenols showed a much lower significant difference ($p = 0.031$). The five variables that most satisfactorily classified the principal commercial Spanish virgin olive oil varieties were 1-acetoxypinoresinol, 4-(acetoxyethyl)-1,2-dihydroxybenzene (3,4-DHPEA-AC), ligstroside aglycon, p-HPEA-EDA, and RT 43.3 contents.

KEYWORDS: Phenols; Cornicabra; virgin olive oil; oxidative stability; extraction system; variety authenticity

INTRODUCTION

It is known that virgin olive oil contains phenolic substances which affect its stability and contribute to oil flavor and aroma (*1*, *2*). These compounds are different from those present in the olive fruit; during ripening and processing several chemical and enzymatic reactions may take place, yielding phenols of lower molecular weight (*3*, *4*).

Analysis of the phenolic compounds in virgin olive oil is currently performed by HPLC, yet this fraction is still a complex mixture and its chemical nature has not been completely elucidated (*5*−*10*). Also, although several studies on the relationship between the content of these compounds and the oxidative stability of the oil have been reported in the literature (*3*, *5*, *11*−*15*) and the influence of the extraction system on the phenolic content in the oil is being investigated (*3*, *16*−*18*), there is much still to be learned in this field. Moreover, no studies have been published that go deeply into the effect of the olive variety on the composition of the phenolic profile or the use of this variable as a tool to classify olive oil varieties, although it has been suggested that it be used as an analytical parameter (*8*, *10*, *17*).

This study presents the phenolic compounds profile of commercial Cornicabra virgin olive oils from five successive crop seasons (1995/1996 to 1999/2000; $n = 97$) as determined by SPE RP-HPLC and discusses the relationship of that profile with the quality parameter oxidative stability, processing conditions, and a preliminary study on variety classification.

The Cornicabra variety accounts for >14% of Spanish land under olive cultivation, and its virgin oil is valued for its high stability and good sensory characteristics. Of the Spanish varieties it is, along with Picual, one of the richest in phenolic compounds (*19*).

MATERIALS AND METHODS

Samples. Commercial Cornicabra virgin olive oil samples ($n = 97$) were collected from industrial oil mills located in the area of Toledo and Ciudad Real (Castilla-La Mancha) during the crop seasons from 1995/1996 to 1999/2000. Sixty oils were obtained by dual-phase centrifugation and 37 by triple-phase decanter. All samples were filtered

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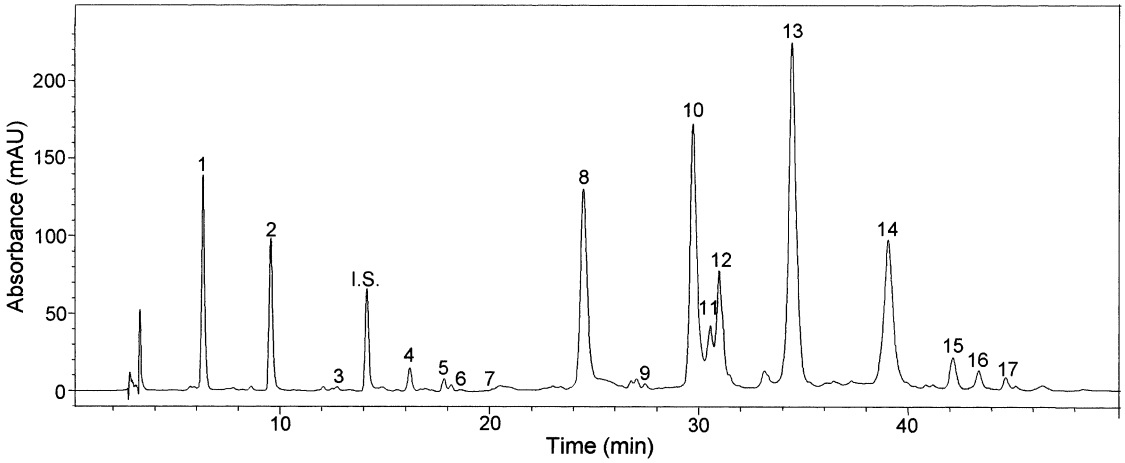

Figure 1. HPLC chromatogram of the phenolic extract of Cornicabra virgin olive oil. Signal was recorded at a wavelength of 280 nm. Peak assignments: 1, hydroxytyrosol (3,4-DHPEA); 2, tyrosol (p-HPEA); 3, vanillic acid; IS, syringic acid (internal standard); 4, vanillin; 5, *p*-coumaric acid; 6, 3,4-DHPEA-AC [hydroxytyrosol acetate, 4-(acetoxyethyl)-1,2-dihydroxybenzene]; 7, ferulic acid; 8, 3,4-DHPEA-EDA (dialdehydic form of elenoic acid linked to hydroxytyrosol); 9, p-HPEA-AC [tyrosol acetate, 4-(acetoxyethyl)-1-hydroxybenzene]; 10, p-HPEA-EDA (dialdehydic form of elenoic acid linked to tyrosol); 11, pinoresinol; 12, 1-acetoxypinoresinol + *trans*-cinnamic acid; 13, oleuropein aglycon (3,4-DHPEA-EA); 14, ligstroside aglycon (p-HPEA-EA); 15–17, RT 42.1, 43.3 and 44.3.

with anhydrous Na_2SO_4 and stored at 4 °C in darkness using amber glass bottles without headspace until analysis.

Cornicabra virgin olive oils ($n = 33$) with a higher concentration of phenolic compounds (*19*), for inclusion in the study of the relationship with oxidative stability, were obtained separately using the Abencor system (Comercial Abengoa, S.A., Sevilla, Spain). Samples of other Spanish monovarietal virgin olive oils, Arbequina ($n = 16$), Hojiblanca ($n = 14$), and Picual ($n = 12$), were obtained from specialized retailers at the beginning of their shelf life, soon after the crop seasons of 1998/1999 and 1999/2000.

Analytical Determinations. All reagents used were of analytical or HPLC grade.

Determination of Phenols by Solid Phase Extraction Reversed Phase High-Performance Liquid Chromatography (SPE RP-HPLC). A sample of filtered virgin olive oil was weighed (2.5 g), and 250 μL of a solution of the internal standard (15 ppm of syringic acid in methanol) was added. The solvent was evaporated in a rotary evaporator at 35 °C under vacuum, and then the oil was dissolved in 6 mL of hexane.

A diol-bonded phase cartridge (Supelco Co., Bellefonte, PA) was used to extract the phenolic fraction as described by Mateos et al. (*10*), and the residue was dissolved in 250 μL of methanol/water (1:1 v/v).

HPLC analysis was performed using an Agilent Technologies series 1100 system equipped with an automatic injector, a column oven, and a diode array UV detector. A Spherisorb S3 ODS2 column (250 × 4.6 i.d. mm, 5 μm particle size) (Waters Co., Milford, MA) was used, maintained at 30 °C, with an injection volume of 20 μL and a flow rate of 1.0 mL/min. Mobile phase was a mixture of water/acetic acid (95:5 v/v) (solvent A), methanol (B), and acetonitrile (C). The elution gradient was from 95% (A)−2.5% (B)−2.5% (C) to 34% (A)−33% (B)−33% (C) in 50 min, followed by 100% (B) for 15 min to clean the column. Chromatograms were taken at 240, 280, and 335 nm.

Phenolic compounds were identified using standard substances and their UV characteristic spectra, relative retentions times (*6*−*8*, *10*, *20*), and also alkaline hydrolysis of the aminopropyl-SPE extract (*9*, *10*). They were quantified at 280 nm using syringic acid as internal standard.

The following reference compounds were used: *p*-hydroxyphenylacetic, *p*-coumaric, *o*-coumaric, vanillic, *trans*-cinnamic, protocatechuic, caffeic, hydrocaffeic, syringic, chlorogenic, d-(−)-mandelic, gentisic, ferulic, gallic, and *p*-hydroxybenzoic acids; apigenin, luteolin, and vanillin from Sigma Chemical Co. (St. Louis, MO); and 2-(*p*-hydroxyphenyl)ethanol (tyrosol) from Aldrich Chemical (Gillingham, Dorset, U.K.).

Oxidative Stability. This was evaluated by the Rancimat method (*21*). Stability was expressed as the induction time (hours) measured with the Rancimat 679 apparatus (Metrohm Co., Basel, Switzerland), using an oil sample of 3.5 g, warmed to 100 °C, and an air flow of 10 L/h.

Colorimetric Determination of Total Polyphenols and o-Diphenol Compounds. The phenolic fraction was isolated by extraction of a solution of oil in hexane with a water/methanol mixture (60:40 v/v) three times (*22*). Total polyphenols were determined by adding the Folin−Ciocalteu reagent to a suitable aliquot of the combined extracts and measuring the absorbance at 725 nm 2 h later (*22*). Five milliliters of phenolic combined extracts was mixed with 1 mL of a 5% solution of sodium molybdate dihydrate in ethanol/water (1:1 v/v). The mixture was shaken vigorously, and 15 min later the absorbance at 370 nm was measured to determine the *o*-diphenol content (*23*). Total polyphenols and *o*-diphenols are given as milligrams of caffeic acid per kilogram of oil.

Statistical Analysis. Statistical analysis was performed using SPSS 10 statistical software (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Phenol Composition. As shown in the HPLC chromatogram depicted in **Figure 1**, 15 phenolic compounds from commercial Cornicabra virgin olive oil ($n = 97$) obtained from 1995/1996 to 1999/2000 crop seasons were identified and quantified by the SPE RP-HPLC method described under Materials and Methods. Three quantitatively significant compounds could not be identified and were designated RT 42.1, 43.3, and 44.3.

The results of statistical analysis of the phenolic composition of commercial Cornicabra virgin olive oil are reported in **Table 1**. The median of total phenol content, expressed as syringic acid (the internal standard used), was 38 mg/kg, although a wide range of concentrations was observed, from 11 to 76 mg/kg. These values correspond to a median of 314 mg/kg and a range from 37 to 680 mg/kg, expressed as the absolute concentration of phenols, calculated according to the response factors determined by Mateos et al. (*10*).

The dialdehydic form of elenolic acid linked to tyrosol (*p*-HPEA-EDA) was generally the main phenolic compound, with a median concentration of 9.4 ppm and an interquartile range (IQR, difference between the 75th and 25th percentiles) of 6.5 ppm. Similarly, the dialdehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA) was also found in high concentration: 5.0 ± 7.7 ppm (as median and IQR).

Table 1. Phenolic Composition of Commercial Cornicabra Virgin Olive Oil from 1995/1996 to 1999/2000 Crop Seasons ($n = 97$)

	concn[a] (mg/kg)							
compound	min	P 10	P 25	median	P 75	P 90	max	mean ± SD
hydroxytyrosol	0.00	0.26	0.48	0.93	1.67	3.18	5.52	1.30 ± 1.15
tyrosol	0.15	0.61	0.96	1.74	2.82	4.60	6.06	2.08 ± 1.39
vanillic acid	0.00	0.00	0.00	0.00	0.08	0.10	0.82	0.04 ± 0.09
vanillin	0.00	0.00	0.08	0.13	0.19	0.26	0.60	0.14 ± 0.11
p-coumaric acid	0.00	0.03	0.10	0.16	0.27	0.44	0.83	0.21 ± 0.17
3,4-DHPEA-AC[b]	0.00	0.00	0.00	0.00	0.00	0.06	0.21	0.01 ± 0.03
ferulic acid	0.00	0.00	0.00	0.00	0.00	0.06	0.16	0.01 ± 0.03
3,4-DHPEA-EDA[b]	0.00	0.66	2.01	5.00	9.66	14.87	25.73	6.65 ± 5.87
p-HPEA-AC[b]	0.00	0.00	0.00	0.00	0.07	0.08	0.14	0.03 ± 0.04
p-HPEA-EDA[b]	0.19	3.42	5.51	9.41	12.00	14.63	21.68	9.08 ± 4.32
pinoresinol	0.00	1.11	1.61	3.01	3.77	4.25	7.50	2.83 ± 1.35
1-acetoxypinoresinol + *trans*-cinnamic acid	0.00	0.46	0.80	1.22	2.17	3.40	4.68	1.57 ± 1.12
oleuropein aglycon	0.18	2.37	4.47	7.53	10.60	13.20	22.47	7.68 ± 4.35
ligstroside aglycon	0.50	1.76	3.01	3.98	5.39	6.09	8.28	4.09 ± 1.59
RT 42.1	0.10	0.39	0.51	0.95	1.96	3.99	6.63	1.51 ± 1.45
RT 43.3	0.00	0.10	0.19	0.38	0.86	1.66	2.62	0.63 ± 0.64
RT 44.3	0.03	0.14	0.21	0.37	0.59	1.18	1.89	0.48 ± 0.41
total as syringic acid	10.83	19.78	28.60	38.17	48.81	56.81	75.82	38.5 ± 14.1
total[c] as phenols	36.6	133.6	200.0	314.0	408.3	498.7	680.0	308 ± 139

[a] Concentration expressed as ppm of syringic acid, the internal standard used. [b] 3,4-DHPEA-AC, 4-(acetoxyethyl)-1,2-dihydroxybenzene; 3,4-DHPEA-EDA, dialdehydic form of elenolic acid linked to hydroxytyrosol; *p*-HPEA-AC, 4-(acetoxyethyl)-1-hydroxybenzene; p-HPEA-EDA, dialdehydic form of elenolic acid linked to tyrosol. [c] Concentration expressed as the absolute concentration of phenols, calculated according to the response factors determined by Mateos et al. (*10*).

p-HPEA-EDA was found in all samples analyzed, at concentrations ranging from 0.2 to 21.7 mg/kg, whereas 3,4-DHPEA-EDA, with a maximum value of 25.8 mg/kg, was not found in ~5% of the samples, probably because it degrades more readily due to its high oxidability.

Following in order of concentration were oleuropein and ligstroside aglycons, also derivatives of hydroxytyrosol and tyrosol, with contents of 7.5 ± 6.1 and 4.0 ± 2.4 ppm, respectively.

The main simple phenols found in the Cornicabra virgin olive oil were hydroxytyrosol and tyrosol, whereas the concentrations of other phenolic acids were very low. The concentration of tyrosol, at 1.7 ± 1.9 ppm, was generally higher than that of hydroxytyrosol (0.9 ± 1.2 ppm) and was found in all of the samples analyzed, at concentrations ranging from 0.2 to 6.1 ppm. These results are similar to those reported by several authors for other olive oil varieties (*12*, *14*, *16*, *24*), although the tyrosol/hydroxytyrosol ratio was lower in Cornicabra virgin olive oil than in some Italian varieties studied by Montedoro et al. (*5*). The reason for the wide content distribution observed for these two phenols may be that hydroxytyrosol and tyrosol can be produced by the partial hydrolysis of their derivatives (*6*, *25*). In addition, the high antioxidant activity of hydroxytyrosol, which is much higher than that observed for tyrosol (*11*), favors its degradation and disappearance.

In Cornicabra virgin olive oil, other phenolic acids were present in very low concentrations, in all cases <1 ppm, which is consistent with reports by other authors for other olive oil varieties (*5*, *12*, *14*, *26*). However, several phenolic compounds described by these authors, such as caffeic, *p*-hydroxybenzoic, *o*-coumaric, hydroxycaffeic, *p*-hydroxyphenylacetic, and syringic acids, were not detected in any of almost 100 samples of commercial Cornicabra olive oil analyzed.

Pinoresinol (3.0 ± 2.2 ppm) and 1-acetoxypinoresinol (1.2 ± 1.4 ppm, including small concentrations of *trans*-cinnamic acid) were only recently described in olive oil (*9*, *20*) and are relatively abundant in the Cornicabra virgin olive oil variety. According to Owen et al. (*9*) these compounds are the main components of the phenolic fraction of the olive seed and are practically absent from the pulp, leaves, and limbs, and therefore their presence in the oil must be due to breaking of the pits when the olives are crushed. These could be used as an index of the crushing conditions and of the fruit pulp/seed ratio during olive processing.

The presence of *trans*-cinnamic acid in Cornicabra virgin olive oil was verified, mainly by the amino-propyl SPE extraction, although it could not be independently quantified because it eluted along with 1-acetoxypinoresinol. This compound was described for the first time in olive oil by Montedoro et al. in 1992 (*5*) and Vacca et al. in 1993 (*27*) in Italian virgin olive oils. It was recently reported to be present in the Spanish varieties Picual (very abundant) and Arbequina (scarce) and to be absent in Manzanilla (*10*).

Hydroxytyrosol acetate (3,4-DHPEA-AC) has been described in Spanish varieties (*8*); however, the concentration in the Cornicabra variety was very low, and it was present in only 20% of the samples analyzed, with a maximum concentration of no more than 0.2 ppm. A similar pattern was observed for tyrosol acetate (p-HPEA-AC), which was only recently described in olive oil (*10*).

The SPE RP-HPLC method used, modified from that of Mateos et al. (*10*), allowed the separation and identification of a great number of phenolic compounds in the Cornicabra virgin olive oil, some of them not previously described in the literature; these included three compounds designated RT 42.1, 43.3, and 44.3, which have not yet been identified, although they are quantitatively significant in this monovarietal olive oil. Their median and IQR values were 1.0 ± 1.5, 0.4 ± 0.7, and 0.4 ± 0.4 ppm, respectively (**Table 1**).

With respect to luteolin and apigenin, the flavone compounds identified but not quantified in Cornicabra virgin olive oil were described by Vazquez-Roncero et al. in 1976 (*28*); however, HPLC quantification was not carried out until 1999, by Brenes et al. (*8*). They are formed by hydrolysis of the glucosides present in the olive pulp (*29*).

Correlation with Oxidative Stability. To enlarge the concentration range of the phenolic compounds, several Cornicabra virgin olive oils obtained by the Abencor system ($n = 33$) were included in the study of the relationship with the oxidative stability.

Table 2. Regression Coefficients[a] of the Correlation between Oxidative Stability and Phenolic Composition of Cornicabra Virgin Olive Oil from 1995/1996 to 1999/2000 Crop Seasons ($n = 130$)

	r^2				
compound	1996/1997	1997/1998	1998/1999	1999/2000	total
hydroxytyrosol [A]	0.209	0.340	0.285	0.197	0.061
3,4-DHPEA-EDA [B]	0.880	0.939	0.807	0.916	0.902
p-HPEA-EDA	0.883	0.828	0.515	0.833	0.786
oleuropein aglycon [C]	0.889	0.829	0.740	0.669	0.697
sum [B + C]	0.911	0.950	0.938	0.961	0.931
sum [A + B + C]	0.954	0.896	0.947	0.966	0.878
HPLC total phenols	0.921	0.940	0.914	0.951	0.932
total polyphenols[b]	0.774	0.928	0.862	0.949	0.894
o-diphenols	0.779	0.929	0.877	0.915	0.906

[a] Second-grade polynomial regression. [b] Linear regression.

As shown in **Table 2**, the phenolic substances that showed the highest correlation with oxidative stability by the Rancimat method through the five years studied were 3,4-HPEA-EDA ($r^2 = 0.81-0.94$), p-HPEA-EDA ($r^2 = 0.52-0.88$), and oleuropein aglycon ($r^2 = 0.74-0.89$). In all cases a second-degree polynomial relationship was found.

As reported earlier by this research group, the correlation in each of the crop seasons studied was generally higher than the total value, due to the seasonal effect on the composition and properties of virgin olive oil (*15*, *19*).

A higher correlation coefficient was generally obtained when the sum of 3,4-DHPEA-EDA and oleuropein aglycon ($r^2 = 0.91-0.96$) or the sum of these two compounds and hydroxytyrosol ($r^2 = 0.90-0.97$) was considered. As shown in **Table 2**, for each of the crop seasons studied, these values were often higher than the correlation coefficients obtained with total phenol content by HPLC ($r^2 = 0.91-0.95$) and more so with other traditional analytical parameters such as colorimetric determination of total polyphenols and *o*-diphenols ($r^2 = 0.77-0.95$ and 0.78−0.92, respectively), because these methods are less specific and measure compounds that do not possess antioxidant activity.

Despite its known antioxidant activity, hydroxytyrosol correlated very poorly with oxidative stability, as also reported by Baldioli et al. (*13*). This could be due to its highly variable content, because it can be formed by hydrolysis of its derivatives, as already mentioned. According to Papadopoulos and Boskou (*11*) and Baldioli et al. (*13*), tyrosol does not possess antioxidant activity, and therefore the high correlation found for p-HPEA-EDA could be due to its high correlation both with 3,4-HPEA-EDA ($r = 0.90$), the main antioxidant phenol compound contained in olive oil, and with the HPLC total phenol content ($r = 0.92$).

Effect of Extraction System Used: Dual- and Triple-Phase Centrifugation. Virgin olive oil in Castilla-La Mancha is almost exclusively extracted by centrifugation. As we know, there are two types of centrifugation systems, dual- and triple-phase decanters. The main difference is that in triple-phase centrifugation a significant amount of water is added to the olive paste, and therefore oil, residual water, and solid waste are obtained separately.

The chief variables that may affect the phenolic content in virgin olive oil are the olive variety (*8*, *16*), olive fruit quality before processing (*30*), the extraction system employed (*3*, *16*), and the oil storage conditions (*25*). In this study the centrifugation system is probably the most important of these factors because the virgin olive oil samples were obtained from oil mills certified by the Regulatory Board for the Protected Designation of Origin (PDO) of "Montes de Toledo" Virgin Olive Oil (*31*), which certifies that the olive oil is monovarietal and that good manufacturing practices and the HACCP system were used.

Table 3. Influence of the Extraction System on the Phenolic Composition of Cornicabra Virgin Olive Oil from 1995/1996 to 1999/2000 Crop Seasons ($n = 97$)

		concn[a] (mg/kg; mean ± SE)	
compound	*P*	dual phase ($n = 60$)	triple phase ($n = 37$)
hydroxytyrosol	0.120	1.57 ± 0.23	1.13 ± 0.18
tyrosol	0.765	2.05 ± 0.19	2.14 ± 0.20
3,4-DHPEA-EDA	0.001	8.09 ± 0.81	4.31 ± 0.70
p-HPEA-EDA	0.002	10.07 ± 0.57	7.47 ± 0.61
pinoresinol	0.231	2.97 ± 0.16	2.61 ± 0.25
1-acetoxypinoresinol + *trans*-cinnamic acid	0.624	1.53 ± 0.15	1.64 ± 0.25
oleuropein aglycon	0.002	8.70 ± 0.57	6.03 ± 0.62
ligstroside aglycon	0.010	4.41 ± 0.20	6.56 ± 0.26
RT 42.1	0.466	1.43 ± 0.19	1.65 ± 0.24
RT 43.3	0.289	0.57 ± 0.08	0.72 ± 0.12
RT 44.3	0.454	0.51 ± 0.05	0.44 ± 0.07
HPLC total phenols	0.001	42.45 ± 1.79	32.09 ± 2.01
total polyphenols[b]	0.348	169.79 ± 10.09	130.54 ± 9.01
o-diphenols[b]	0.034	9.07 ± 1.06	6.45 ± 0.63
oxidative stability (h)	0.002	76.23 ± 3.07	60.72 ± 3.57

[a] Concentration expressed as ppm of syringic acid. [b] Concentration expressed as ppm of caffeic acid.

The highest statistically significant difference ($p = 0.001$) observed in individual phenolic compounds with respect to the centrifugation system employed was recorded for 3,4-DHPEA-EDA (**Table 3**). Three other substances of the same family (secoiridoids) showed highly statistically significant differences: p-HPEA-EDA and the aglycons of oleuropein and ligstroside ($p = 0.002-0.010$). These four compounds are the main constituents of the phenolic fraction of the Cornicabra virgin olive oil, and therefore it is reasonable that the HPLC total phenols content showed as well significant difference ($p = 0.001$) between extraction systems. The observed results for secoiridoids are similar to those reported by De Stefano et al. (*16*) for other virgin olive oil varieties (Coratina and Oliarola from Italy), but not for hydroxytyrosol and tyrosol, which presented no statistically significant differences in the Cornicabra variety. A possible explanation might be, once again, that hydroxytyrosol and tyrosol can be produced by hydrolysis of the corresponding secoiridoid derivatives during storage of the oils, so that the actual concentration of these phenols did not reflect only the centrifugation operation.

There was no statistically significant difference ($p = 0.348$) in colorimetric total polyphenols content. Also, the difference in *o*-diphenols according to the extraction system employed was much less significant ($p = 0.031$) than in individual phenolic compounds. This explains why the effect of the extraction system on the phenols content in the oil as determined by these methods was not completely clear; although the mean total polyphenols content was generally higher in the oils extracted in dual-phase decanters, in many cases no statistically significant difference was found (*3*, *32*−*34*).

The addition of water during triple-phase decanter centrifugation produced olive oil with a significantly lower HPLC total phenolic compounds content than when the dual-phase decanter system was used. Concentrations were 32.1 and 42.5 ppm, respectively, and oxidative stability was lower (60.7 and 76.2

Table 4. Most Relevant Phenolic Components of Commercial Virgin Olive Oils from Different Spanish Varieties from 1998/1999 and 1999/2000 Crop Seasons ($n = 107$)

compound	ANOVA *F* ratio[b]	concn[a] (mg/kg; mean ± SE)			
		Cornicabra ($n = 65$)	Arbequina ($n = 16$)	Picual ($n = 12$)	Hojiblanca ($n = 14$)
hydroxytyrosol	12.7***	0.87 ± 0.09a	0.76 ± 0.22a	2.06 ± 0.55b	2.47 ± 0.38b
tyrosol	5.9***	1.51 ± 0.13a	0.72 ± 0.12a	2.87 ± 0.84b	1.67 ± 0.34a
3,4-DHPEA-AC	61.7***	0.01 ± 0.01a	1.50 ± 0.26c	0.80 ± 0.13b	1.03 ± 0.09b
ferulic acid	3.5**	0.02 ± 0.01a	0.05 ± 0.16ab	0.09 ± 0.05b	0.04 ± 0.15a
3,4-DHPEA-EDA	NS	7.56 ± 0.77	5.08 ± 1.11	5.50 ± 1.06	6.17 ± 0.95
p-HPEA-EDA	14.1***	9.49 ± 0.55b	3.39 ± 0.46a	5.61 ± 0.77a	5.45 ± 0.52a
pinoresinol	3.9**	2.70 ± 0.18b	2.23 ± 0.15ab	2.32 ± 0.36ab	1.44 ± 0.17a
1-acetoxypinoresinol + *trans*-cinnamic acid	70.3***	1.60 ± 0.14b	6.04 ± 0.40d	0.79 ± 0.15a	2.63 ± 0.30c
oleuropein aglycon	11.0***	8.68 ± 0.56b	2.60 ± 0.41a	10.93 ± 1.51b	8.29 ± 1.07b
ligstroside aglycon	37.5***	4.69 ± 0.18c	0.78 ± 1.65a	4.23 ± 3.12c	2.49 ± 0.36b
RT 42.1	NS	0.85 ± 0.11	0.75 ± 0.14	1.44 ± 0.60	0.48 ± 0.05
RT 43.3	12.7***	0.26 ± 0.02b	0.02 ± 0.07a	0.19 ± 0.05b	0.04 ± 0.02a
RT 44.3	19.6***	0.40 ± 0.03a	1.02 ± 0.01c	0.36 ± 0.10a	0.77 ± 0.07b
total	4.6**	39.2 ± 1.80b	25.5 ± 1.85a	37.9 ± 4.64b	33.6 ± 3.14ab

[a] Concentration expressed as ppm of syringic acid. Mean values with different letters are statistically different ($p \leq 0.05$). [b] NS, not significant at 95%; *, $p \leq 0.05$ (95%); **, $p \leq 0.01$ (99%); ***, $p \leq 0.001$ (99.9%).

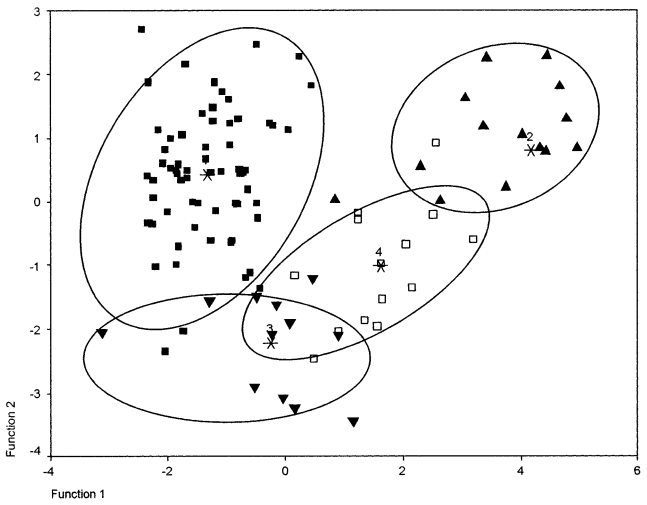

Figure 2. Plot of discriminant functions of five phenolic compounds used to classify different commercial Spanish virgin olive oil varieties: ■, Cornicabra (1); ▲, Arbequina (2); ▼, Picual (3); □, Hojiblanca (4); *, group centroids.

h, respectively) ($p = 0.002$). This means that, besides other economically and ecologically important advantages, olive oils produced by dual-phase centrifugation are of higher quality and are more stable to oxidation.

Influence of Olive Oil Variety. It has been suggested that the phenolic profile could be used to classify virgin olive oils according to their fruit variety (*8*, *10*, *17*, *35*); however, until now no statistically significant data have been published to confirm the feasibility of this approach.

As reported in **Table 4**, the concentrations of many phenolic compounds differed significantly ($p \leq 0.01$) among the main Spanish virgin olive oil varieties.

1-Acetoxypinoresinol (together with *trans*-cinnamic acid), 3,4-DHPEA-AC, and ligstroside aglycon presented the highest ANOVA *F* ratio values: 70.3, 61.7, and 37.5, respectively. The content distribution of these three components was different in almost every commercial virgin olive oil variety studied, which suggests the feasibility of variety classification by the HPLC phenolic compounds profile, in particular for Arbequina and Picual varieties, as qualitatively reported by García et al. in 2001 (*17*).

Principal component and stepwise discriminant analyses showed that the three compounds mentioned above, plus *p*-HPEA-EDA and RT 43.3, were the most useful variables for classification of the commercial virgin olive oil varieties studied. The first two discriminant functions of the statistical analysis explained 99.4% of the variance (81.3 and 18.1%, respectively), yielding a reasonable classification of the virgin olive oil varieties studied.

As the plot of discriminant functions shows (**Figure 2**), Cornicabra virgin olive oil (from Castilla-La Mancha in central Spain) was well separated from the Arbequina (from Cataluña in northeastern Spain) and Hojiblanca (from Andalucia in southern Spain) varieties. The phenolic profile of Picual virgin olive oil (from Andalucia) was midway between those of Cornicabra and Hojiblanca. In fact, Picual and Cornicabra are the two Spanish varieties richest in phenolic compounds.

The complete assessment of a validated predictive and classification model that maybe useful in variety identification and authenticity, of course, requires a much longer and focused study.

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