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Influence of extraction system, production year and area on Cornicabra virgin olive oil: a study of five crop seasons

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Abstract

This paper describes a study of the influence of the extraction system, crop season and production area on the chemical composition and quality of Cornicabra virgin olive oils ($n=152$) from five successive crop seasons (from 1994/1995 to 1998/1999). Analysis of the effect of the extraction system (dual-phase, triple-phase decanters and pressure) on the values of analytical determinations, revealed statistically significant differences ($P\leqslant 0.05$) in a few parameters only, mainly in antioxidant content (total phenols and α-tocopherol) and oxidative stability. All quality indices and major fatty acid and sterol compositions presented significant statistical differences ($P\leqslant 0.001$) with respect to the year of production, with the exception of total phenols. The crop season is therefore a critical variable, since the chemical composition of the olive oil may vary considerably from one year to the next. Many of the analytical parameters varied among the five production areas considered, in the provinces of Toledo and Ciudad Real. The results appear to confirm the general consensus on the quality of Cornicabra virgin olive oils from Castilla-La Mancha, namely that oils from mills located in the south and southeast of the province of Toledo are generally of higher quality.

Keywords: Cornicabra; Virgin olive oil; Extraction system; Crop season; Production area

1. Introduction

Virgin olive oil possesses a characteristic aroma, taste and colour that distinguishes it from other vegetable oils. Consumption is currently increasing thanks to its excellent organoleptic and nutritive qualities and to growing consumer preference for minimally processed foods.

The Cornicabra olive variety covers an area of 300,000 ha, located chiefly in central Spain in the provinces of Ciudad Real and Toledo, and accounts for more than 14% of total national production. The fruit is medium to large, characteristically elongated and asymmetrical in shape, with a fat yield of 22–24% of fresh weight. The oil is valued for its high stability and good sensory characteristics, such as a dense feel and a balanced aroma, sour and pungent (Barranco, Rallo, Uceda, & Hermoso, 1994; Salvador, Aranda, Gómez-Alonso, & Fregapane, 2001).

The main olive oil-producing countries have been particularly active in recent years in studying the chemical composition of olive oil varieties or oil produced in a specific area and how this relates to oil quality. In Spain, there have been exhaustive studies of some of the most important olive varieties, such as Picual, Hojiblanca and Arbequina from Andalucia and Cataluña (Aparicio, Ferreiro, Cert, & Lazón, 1990; Cert et al., 1999; Graell et al., 1993; Hidalgo et al., 1993; Motilva, Jaria, Bellart, & Romero, 1998).

Despite the economic importance of Cornicabra olive oil, this is the first study in which the effects of some production variables on chemical composition and quality have been examined, on the basis of a large enough number of successive crop seasons to be statistically relevant. The increasing interest in this virgin olive oil variety is reflected in the recent creation of the 'Montes de Toledo' Foundation (DOCM, 1998). The purpose of the Foundation is to certify the origin, genuineness and quality of the Cornicabra virgin olive oil

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produced in a specific geographical area in Castilla-La Mancha, and also to increase local and international awareness of the variety, of its added value and of its marketing.

This paper examines the influence of the extraction system, crop season and production area on the chemical composition of Cornicabra virgin olive oils ($n = 152$) from five successive crop seasons (from 1994/1995 to 1998/1999). Several analytical determinations were chosen: quality indices as defined by EU Regulations (free fatty acid content, peroxide value and spectrophotometric characteristics in the UV region); parameters of interest involved in oxidation processes (oxidative stability, total phenols, tocopherols and chlorophyll and carotenoids pigments); fatty acid and sterol composition.

2. Materials and methods

2.1. Oil samples

Samples of Cornicabra commercial virgin olive oil ($n = 152$) were collected from industrial oil mills located in the provinces of Toledo and Ciudad Real (Castilla-La Mancha), during a series of crop seasons from 1994/1995 to 1998/1999. Sixty-eight oils were extracted, using the dual-phase decanter, 63 with the triple-phase decanter and 12 with a combination of both systems, while nine were processed by the older pressure technique, which has now practically disappeared. The oil samples were classified according to production area in five groups: northern Toledo (TO-N), the least important growing area; two areas in the southeast of the province of Toledo (TO-SE1 and TO-SE2), the largest Cornicabra olive oil production area; and northern and southern Ciudad Real (CR-N and CR-S).

All samples were filtered through anhydrous Na_2SO_4 and stored at 4 °C in darkness using amber glass bottles without head space until analysis.

2.2. Analytical methods

Determination of free acidity, peroxide value, UV absorption characteristics, and fatty acid composition were carried out, following the analytical methods described in Regulations EEC/2568/91 and later modifications of the Commission of the European Union (EEC, 1991).

Free acidity, given as % of oleic acid, was determined by titration of a solution of oil dissolved in ethanol–ether (1:1) with ethanolic potash.

Peroxide value, expressed in milliequivalents of active oxygen per kilogram of oil (meq/kg), was determined as follows: a mixture of oil and chloroform–acetic acid was left to react with a solution of potassium iodide in darkness; the free iodine was then titrated with a sodium thiosulphate solution.

K_{232} and K_{270} extinction coefficients were calculated from absorption at 232 and 270 nm, respectively, with a UV spectrophotometer (Agilent Technologies, HP 8452A), using a 1% solution of oil in cyclohexane and a path length of 1 cm.

Oxidative stability was evaluated by the Rancimat method (Gutiérrez, 1989). Stability was expressed as the oxidation induction time (hours), measured with the Rancimat 679 apparatus (Metrohm), using an oil sample of 3.5 g warmed to 100 °C and an air flow of 10 l/h.

Total phenols and ortho-diphenol compounds were isolated by triple extraction of a solution of oil in hexane with a water–methanol mixture (60:40). To a suitable aliquot of the combined extracts, Folin–Ciocalteau reagent and sodium molybdate, 5% in ethanol, 50% (Merck) were added and the absorptions of the solution at 725 nm (total phenolic) and 370 nm (*o*-diphenolic components), respectively, were measured. Values are given as mg of caffeic acid per kilogramme of oil (Gutfinger, 1981; Vázquez, Janer, & Janer, 1973).

Tocopherols were evaluated following the AOCS Method Ce 8–89 (AOCS, 1989). A solution of oil in hexane was analysed by HPLC (HP series 1100) on a silica gel Lichrosorb Si-60 column (particle size 5 μm, 250×4.6 mm i.d.; Sugerlabor, Madrid, Spain) which was eluted with hexane–2-propanol (98.5:1.5) at a flow rate of 1 ml/min. A fluorescence detector (Waters 470), with excitation and emission wavelength set a 290 and 330 nm, was used.

Chlorophyll and carotenoid compounds (mg/kg) were determined at 472 and 670 nm in cyclohexane using the specific extinction values, by the method of Minguez-Mosquera, Rejano, Gandul, Sanchez, and Garrido (1991).

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 potash and analysed by GC with an Agilent Technologies (HP 6890) chromatograph equipped with a FID detector. A fused silica column (50 m length×0.25 mm i.d.), coated with SGL-1000 phase (0.25 μm thickness; Sugerlabor), was used. Helium was employed as carrier gas 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 (Regulation EEC 2568/91, corresponding to AOCS method Ch 2-91; EEC, 1991).

Sterols were determined according to the method described in the Annex V of the Regulation EEC 2568/91 (corresponding to AOCS method Ch 6-91; EEC, 1991). The olive oil, with added α-cholestanol as an

internal standard, was saponified with potassium hydroxide in ethanolic solution and the unsaponifiables were then extracted with ethyl ether. The sterol fraction was separated from the unsaponifiable extract by chromatography on a basic silica gel plate. The sterols recovered from the silica gel were transformed into trimethyl-silyl ethers and were analysed by an HP 6890 gas chromatographer with a capillary column (25 m length×0.25 mm i.d.), coated with SGL-5 (0.25 μm thickness; Sugerlabor). Working conditions were as follows: carrier gas, helium; flow through the column, 1.2 ml/min; injector temperature, 280 °C; detector temperature, 290 °C; oven temperature, 260 °C; injection volume 1 μl.

Analytical determinations were carried out at least in duplicate.

2.3. Overall quality index

The overall quality index (OQI) was introduced by the International Olive Oil Council (COI) in 1990 to express virgin olive oil quality numerically (COI, 1990). This is a scale from 0 to 10 that considers four quality parameters: the score for sensory evaluation (SE), free acidity (FA), K_{270}, and peroxide value (PV), according to the following equation: $OQI = 2.55 + 0.91SE - 0.78FA - 7.35K_{270} - 0.066PV$. Sensory analysis was performed only on about half of the olive oils studied (Salvador et al., 2001), and therefore a score of 6.5, the lower limit for the extra-virgin category, was adopted for the rest of the samples.

2.4. Sensory analysis

This was carried out by 12 selected and trained panellists from the panel of *D.O. Montes de Toledo* (Toledo; in collaboration with the University of Castilla-La Mancha) according to the method described in Annex XII of the European Union Commission (EEC 2568/91; EEC, 1991).

2.5. Statistical analysis

Statistical analysis was performed with the SPSS 10 statistical software (SPSS Inc., Chicago, USA), using mainly descriptive analysis, one-way ANOVA and least significant difference (LSD) comparison test.

3. Results and discussion

3.1. Extraction system

The quality indices of commercial Cornicabra virgin olive oil obtained by different extraction systems, dual phase (C2), triple-phase (C3) decanter centrifugation and pressure system (P), are listed in Table 1. Analysis of extraction system dependent differences, in the mean analytical values for the Cornicabra virgin olive oil studied, revealed statistically significant differences ($P \leqslant 0.05$) in a few quality parameters only, mainly antioxidant content (total phenols and α-tocopherol) and oxidative stability.

Table 1
Quality indices of commercial Cornicabra virgin olive oils from crop seasons 1994/1995 to 1998/1999 obtained by different extraction systems ($n = 140$)

Quality indices	ANOVA *F*-ratio	Extraction system		
		C2	C3	P
Number of samples[a]		68	63	9
Free fatty acid (% oleic)	1.8	0.58 a	0.58 a	0.86 a
Peroxide value (meq/kg)	0.7	10.2 a	9.4 a	11.1 a
K_{232}	0.1	1.619 a	1.616 a	1.653 a
K_{270}	1.0	0.139 a	0.132 a	0.140 a
Oxidative stability (h)	3.6*	65.8 b	57.2 a	46.3 a
Total phenols (mg/kg)	4.8**	160 b	142 b	100 a
ortho-diphenols (mg/kg)	3.1	9.2 a	6.9 a	–
α-Tocopherol (mg/kg)	8.3***	178 c	160 b	134 a
Chlorophylls (mg/kg)	5.7	11.4 b	8.6 a	11.4 a, b
Carotenoids (mg/kg)	4.1	7.6 b	6.5 a	6.8 a, b
Intensity of bitterness	6.5**	2.0 b	1.6 a	–
Overall quality index	1.7	6.4 a	6.5 a	6.1 a

Dual phase (C2), triple-phase (C3) decanter centrifugation and pressure system (P).

[a] By crop season. C2: 12 samples from crop 1994/1995; 9, 1995/1996; 9, 1996/1997; 17, 1997/1998; 21, 1998/1999. C3: 11, 1994/1995; 6, 1995/1996; 9, 1996/1997; 19, 1997/1998; 18, 1998/1999. P: 4, 1994/1995; 2, 1995/1996; 3, 1996/1997.

* $P \leqslant 0.05$ (95%).

** $P \leqslant 0.01$ (99%).

*** $P \leqslant 0.001$ (99.9%).

Values with different letters are statistically different ($P < 0.05$).

The α-tocopherol content decreased from the dual-phase to the triple-phase decanter and pressure systems, with mean concentrations of 178, 160 and 134 mg/kg respectively, and an ANOVA *F*-ratio value (*F*) of 8.3 ($P \leqslant 0.001$). Similarly, total phenol content was greater in the centrifuge-extracted oil than in the pressure extracted oil (*F*: 4.8, $P \leqslant 0.01$), and the mean concentration of total phenol was slightly lower in the oils extracted using the triple-phase decanter than those from the dual-phase decanter. The respective mean contents for the dual-phase, triple-phase centrifugal and pressure systems were 160, 142 and 100 mg/kg. These differences in natural antioxidant contents affected the observed Rancimat-method oxidative stability, with measured mean values of 66, 57 and 46 h, respectively. These results, which were for five consecutive crop seasons, confirm those reported by other authors (Alba et al., 1996; Angerosa & Di Giovacchino, 1996; Cert, Alba, León-Camacho, Moreda, & Pérez-Camino, 1996; Di Giovacchino, Solinas, & Miccoli, 1994).

The decreases in phenol compounds may be explained by their water-solubility. Higher water/paste ratios are used in triple-phase centrifugation, and therefore larger amounts of phenols are eliminated with water wastes. However, contradictory results have been reported in some studies with respect to differences in olive oil composition due to the extraction systems employed (Felice, Gomes, & Catalano, 1979; Negriz & Unal, 1991). This is because the amounts of these compounds in the oil differ widely, due to the many variables involved in the process of extraction, i.e. the olive crushing machinery, the temperatures applied, the duration of contact with the water and the total volume of water used, all of which may cause significant changes in the total phenol content (Boskow, 1996; Cert et al., 1999).

Phenolic substances do not only affect virgin olive oil stability; they also contribute to oil flavour and aroma, especially to the typical bitter taste of olive oil, which is a positive attribute in the sensory evaluation of virgin olive oils and a typical organoleptic characteristic of the Cornicabra variety. The intensity of bitterness, as defined by Gutierrez, Perdiguero, Gutierrez, and Olias (1992), was statistically higher ($P \leqslant 0.01$) in dual-phase than in triple-phase decanter extracted oils (with medium values of 2.0 and 1.6, respectively).

Mean contents of fatty acids and sterols differed slightly, depending on the extraction system employed, although the differences were statistically significant in some cases (Table 2). Palmitic, palmitoleic and linoleic acids (*F*: 3.3, 3.7 and 5.2, respectively) increased, whereas oleic acid content appeared to be lower with triple-phase and dual-phase centrifugation systems than with the pressure system. For sterol composition (%), it was found that the proportion of stigmasterol (*F*: 3.1) was higher in triple-phase decanter-extracted oils, whereas Δ5-avenasetrol, Δ5,24-stigmastadienol and apparent β-sitosterol (*F*: 3.8, 7.1 and 3.2, respectively)

Table 2
Major fatty acid and sterol composition (%) of Cornicabra virgin olive oils from crop seasons 1994/1995 to 1998/1999 obtained by different extraction systems ($n = 140$)

Fatty acid or sterol	ANOVA *F*-ratio	Extraction system		
		C2	C3	P
Palmitic acid, $C_{16:0}$	3.3*	9.23 b	8.99 a, b	8.62 a
Palmitoleic acid, $C_{16:1}$	3.7*	0.78 b	0.76 b	0.69 a
Stearic acid, $C_{18:0}$	1.3	3.38 a	3.46 a	3.45 a
Oleic acid, $C_{18:1}$	2.1	80.3 a	80.4 a, b	81.1 b
Linoleic acid, $C_{18:2}$	0.7	4.51 a	4.62 a	4.51 a
Linolenic acid, $C_{18:3}$	5.2**	0.62 b	0.60 b	0.55 a
Arachidic acid, $C_{20:0}$	1.1	0.51 a	0.51 a	0.49 a
SFA	2.6	13.3 b	13.2 a, b	12.8 a
MUFA	1.7	81.6 a	81.6 a	82.2 a
PUFA	0.5	5.13 a	5.22 a	5.06 a
Campesterol	0.4	4.19 a	4.20 a	4.25 a
Stigmasterol	3.1*	0.76 a, b	0.86 b	0.67 a
Clerosterol	0.1	0.90 a	0.90 a	0.91 a
β-Sitosterol	3.9*	85.4 b	84.9 a, b	84.1 a
Δ5-Avenasterol	3.8*	6.24 a	6.46 a	7.86 b
Δ5,24-Stigmastadienol	7.1***	0.55 a	0.48 a	0.79 b
Apparent β-sitosterol	3.2*	93.7 a	93.5 a	93.8 b
Total sterols (mg/kg)	1.2	1516 a	1470 a	1492 a

Dual phase (C2), triple-phase (C3) decanter centrifugation and pressure system (P).
* $P \leqslant 0.05$ (95%).
** $P \leqslant 0.01$ (99%).
*** $P \leqslant 0.001$ (99.9%)
Values with different letters are statistically different ($P < 0.05$).

were greater in the pressure system. Ranalli, De Mattia, Ferrante, and Giansante (1997) and Koutsaftakis, Kotsifaki, and Stefanoudaki (1999) reported that the campesterol/stigmasterol ratio was high in the centrifugation system for some Italian and Greek olive oil varieties. However, in this study on the Cornicabra oil variety, a higher ratio was found in the pressure system than in the dual-phase and triple-phase centrifugation, with mean values of 6.8, 5.8 and 5.4, respectively, although there was no statistically significant difference (F: 2.1).

Nowadays, the pressure system has fallen into disuse in Castilla-La Mancha and centrifugation is the most common procedure, since large amounts of olives have to be processed in a short time. In the dual-phase centrifugation system, no water is added, so that the system yields only oil and a plastic paste containing vegetable solids. The dual-phase decanter was introduced to Spain about 10 years ago, and is now widely used, mainly because it greatly reduces liquid waste, thus helping considerably to cut down environmental pollution. Moreover, the results of this study appear to indicate that these oils possess more natural antioxidants and are therefore more stable to oxidation.

3.2. Crop season

All quality indices of the Cornicabra olive oil showed a considerable statistically significant difference ($P \leqslant 0.001$) with respect to the year of production, with the exception of total phenols (Table 3).

Over the 5-year period considered, the free acidity decreased (F: 16.5) from the highest medium value of 1.01%, expressed as oleic acid, in the season 1994/1995 down to 0.34% in 1998/1999. The high acidity observed in 1994/1995 was probably the consequence of several years of drought followed by heavy rainfall shortly before harvesting in December 1995. A similar trend was observed for the peroxide value (F: 17.2), which decreased from a mean of 11.9 mg O_2/kg in 1994/1995 to 6.5 mg/kg in 1997/1998, although the value was much higher in the last crop season (13.0 mg/kg). This effect was caused by the low temperature registered in December 1998, when the olive fruit was frozen on the trees. The same pattern was observed for the UV absorption characteristics K_{232} and K_{270} (F: 57.3 and 26.4, respectively), with the lowest mean values in the crop season 1997/1998 and the highest values in 1994/1995 and 1995/1996. Oxidative stability (F: 8.3) increased from 1994/1995 to 1997/1998, with a maximum mean value of 74 h and decreased in 1998/1999; the same was observed for α-tocopherol (F: 10.4), with the exception of crop 1995/1996.

There was no clear chronological trend in major fatty acid and sterol composition (Table 4), although it is important to note that, in all cases, there were considerable statistical differences ($P \leqslant 0.001$) between crop seasons, as was also observed by the research group of Stefanoudaki, Kotsifaki, and Koutsaftakis (1999).

Some of these quality parameters—free fatty acids, K_{270} and peroxide value—affected the OQI, adopted by the COI in 1990. The highest OQI, with the limitations indicated in Section 2, was observed for the crop seasons 1996/1997 and 1997/1998 (mean value of 6.8), whereas the worst crop was 1994/1995 (5.8). Intermediate values were observed for the other years studied.

The results clearly indicate that, in studying the quality characteristics of virgin olive oil, the crop season is a critical variable, since its chemical composition may vary considerably from one crop season to the following. Unfortunately, most of the studies reported in the literature covered only one or at most two production years.

Table 3
Quality indices of commercial Cornicabra virgin olive oils from crop seasons 1994/1995 to 1998/1999 ($n = 152$)

Quality indices	ANOVA F-ratio	Crop season				
		1994/1995	1995/1996	1996/1997	1997/1998	1998/1999
Number of samples[a]		27	17	21	41	46
Free fatty acid (% oleic)	16.5***	1.01 c	0.76 c	0.51 a, b	0.53 b	0.34 a
Peroxide value (meq/kg)	17.2***	11.9 b	7.5 a	7.1 a	6.5 a	13.0 b
K_{232}	57.3***	1.916 c	1.709 c	1.518 a	1.474 a	1.556 b
K_{270}	26.4***	0.158 b	0.171 b	0.118 a	0.118 a	0.126 a
Oxidative stability (h)	8.3***	42.1 a	57.5 b	62.6 b, c	73.9 c	61.0 b
Total phenols (mg /kg)	1.0	158 a	145 a	130 a	155 a	141 a
ortho-diphenols (mg/kg)	3.6	–	–	–	9.0 a	6.8 a
α-Tocopherol (mg/kg)	10.4***	138 a	179 b, c	163 b	188 c	166 b
Chlorophylls (mg/kg)	11.1***	10.7 b	7.7 a	7.2 a	8.4 a	13.2 b
Carotenoids (mg/kg)	12.9***	6.7 b	5.5 a	5.5 a	7.0 b	8.5 c
Intensity of bitterness	0.1	–	–	–	1.8 a	1.8 a
Overall quality index	20.3***	5.8 a	6.2 b	6.8 c	6.8 c	6.4 b

[a] By extraction system. 1994/1995: 12 samples by C2; 11, C3; 4, P. 1995/1996: 9, C2; 6, C3; 2, P. 1996/1997: 9, C2; 9, C3; 3, P. 1997/1998: 17, C2; 19, C3; 5, C2/C3 blend. 1998/1999: 21, C2; 18, C3; 7, C2/C3.

*** $P \leqslant 0.001$ (99.9%).

Values with different letters are statistically different ($P < 0.05$).

3.3. Production area

Quality indices and major fatty acid and sterol composition of Cornicabra virgin olive oil from different production areas located in the north and southeast of the province of Toledo (TO-N, TO-SE1 and TO-SE2) and in northern and southern Ciudad Real (CR-N and CR-S), the main growing areas of this olive variety (see Section 2 for more details), are listed in Tables 5 and 6.

Table 4
Major fatty acid and sterol composition (%) of Cornicabra virgin olive oils from crop seasons 1994/1995 to 1998/1999 ($n = 152$)

Fatty acid or sterol	ANOVA *F*-ratio	Crop season				
		1994/1995	1995/1996	1996/1997	1997/1998	1998/1999
Palmitic acid, $C_{16:0}$	18.5***	8.36 a	8.95 b	8.75 b	9.42 c	9.41 c
Palmitoleic acid, $C_{16:1}$	23.6***	0.65 a	0.75 b	0.75 b	0.84 c	0.77 b
Stearic acid, $C_{18:0}$	16.6***	3.49 c	3.55 c	3.34 b	3.19 a	3.60 c
Oleic acid, $C_{18:1}$	18.7***	81.46 c	79.79 a	80.89 b	80.20 a	80.02 a
Linoleic acid, $C_{18:2}$	8.4***	4.37 a	5.19 b	4.62 a	4.48 a	4.47 a
Linolenic acid, $C_{18:3}$	32.6***	0.55 a	0.63 c	0.55 a	0.68 d	0.59 b
Arachidic acid, $C_{20:0}$	13.5***	0.50 b	0.49 a, b	0.48 a	0.53 c	0.51 b
SFA	25.5***	12.5 a	13.2 b	12.8 a	13.4 b	13.7 c
MUFA	17.0***	82.5 c	81.0 a	82.1 c	81.5 b	81.2a, b
PUFA	8.9***	4.92 a	5.82 b	5.17 a	5.16 a	5.05 a
Campesterol	5.3***	4.36 c	4.18 a, b	4.28 b, c	4.16 a	4.19 a
Stigmasterol	14.4***	0.53 a	0.75 b	0.62 a	0.95 c	0.80 b
Clerosterol	29.1***	0.98 c	0.86 a	0.90 b	0.86 a	0.97 c
β-Sitosterol	23.8***	85.3 b	83.0 a	85.2 b	85.7 b	85.5 b
Δ5-Avenasterol	76.3***	6.79 b	9.24 c	7.12 b	5.50 a	5.42 a
Δ5,24-Stigmastadienol	145***	0.72 c	0.83 d	0.73 c	0.31 a	0.47 b
Apparent β-Sitosterol	31.8***	93.9 c	94.0 c	94.1 c	93.2 a	93.4 b
Total sterols (mg/kg)	19.7***	1551 c, d	1638 d	1464 b	1507 b, c	1347 a

*** $P \leqslant 0.001$ (99.9%).
Values with different letters are statistically different ($P < 0.05$).

Table 5
Quality indices of commercial Cornicabra virgin olive oils from crop seasons 1994/1995 to 1998/1999 obtained from different production areas ($n = 152$)*

Quality indices	ANOVA *F*-ratio	Toledo (TO)			Ciudad Real (CR)	
		N	SE 1	SE 2	N	S
Number of samples[a]		18	32	38	43	21
Free fatty acid (% oleic)	5.8***	0.95 c	0.43 a	0.40 a	0.65 b	0.57 a, b
Peroxide value (meq/kg)	2.3	12.9 b	8.5 a, b	7.8 a	10.5 b	9.2 a, b
K_{232}	6.0***	1.622 b,c	1.548 a,b	1.495 a	1.689 c	1.644 b,c
K_{270}	10.7***	0.127 a,b	0.128 b	0.111 a	0.147 c	0.147 c
Oxidative stability (h)	3.4**	33.4 a	69.9 b	64.2 b	59.6 b	54.9 a, b
Total phenols (mg /kg)	5.0***	91 a	171 b	123 a	161 b	142 a, b
ortho-Diphenols (mg/kg)	3.7**	1.48 a	10.4 b	6.6 a	10.4 b	6.1 a, b
α-Tocopherol (mg/kg)	6.0***	128 a	152 a, b	174 c, d	167 b, c	195 d
Chlorophylls (mg/kg)	2.1	7.2 a	8.4 a	10.1 a, b	10.1 a, b	12.9 b
Carotenoids (mg/kg)	2.2	5.7 a	6.4 a	7.3 a, b	6.8 a, b	8.2 b
Intensity of bitterness	3.4**	0.8 a	2.1 c	1.6 a, b	2.0 b, c	2.2 b, c
Overall quality index	10.8***	6.0 a	6.7 b, c	6.9 c	6.2 a	6.4 a, b

[a] By extraction system. TO-N: 8 samples by C2; 10, C3. TO-SE1: 14, C2; 12, C3; 2, P; 4, C2/C3 blend. TO-SE2: 14, C2; 16, C3; 3, P; 5, C2/C3. CR-N: 20, C2; 16, C3; 4, P; 3, C2/C3. CR-S: 12, C2; 9, C3. By crop season. TO-N: three samples from crop 1994/1995; 2, 1995/1996; 3, 1996/1997; 5, 1997/1998; 5, 1998/1999. TO-SE1: 5, 1994/1995; 4, 1995/1996; 5, 1996/1997; 8, 1997/1998; 10, 1998/1999. TO-SE2: 7, 1994/1995; 3, 1995/1996; 5, 1996/1997; 12, 1997/1998; 11, 1998/1999. CR-N: 9, 1994/1995; 6, 1995/1996; 6, 1996/1997; 9, 1997/1998; 13, 1998/1999. CR-S: 3, 1994/1995; 2, 1995/1996; 2, 1996/1997; 7, 1997/1998; 7, 1998/1999.

* $P \leqslant 0.05$ (95%).
** $P \leqslant 0.01$ (99%).
*** $P \leqslant 0.001$ (99.9%).
Values with different letters are statistically different ($P < 0.05$).

Table 6
Major fatty acid and sterol composition (%) of Cornicabra virgin olive oils from crop seasons 1994/1995 to 1998/1999 obtained from different production areas ($n=152$)

Fatty acid or sterol	ANOVA F-ratio	Toledo (TO)			Ciudad Real (CR)	
		N	SE 1	SE 2	N	S
Palmitic acid, $C_{16:0}$	4.5**	9.33 b, c	8.61 a	8.85 a, b	9.13 b, c	9.34 c
Palmitoleic acid, $C_{16:1}$	5.1***	0.84 b	0.71 a	0.73 a	0.78 b	0.78 a, b
Stearic acid, $C_{18:0}$	3.8**	3.29 a	3.60 c	3.47 a,b,c	3.35 b	3.56 b, c
Oleic acid, $C_{18:1}$	2.6*	80.0 a	80.8 b	80.7 b	80.6 a, b	80.1 a
Linoleic acid, $C_{18:2}$	2.3	4.81 b	4.63 b	4.49 a, b	4.36 a	4.50 a, b
Linolenic acid, $C_{18:3}$	8.8***	0.64 c	0.54 a	0.58 b	0.62 c	0.62 b, c
Arachidic acid, $C_{20:0}$	3.7**	0.50 a, b	0.49 a	0.51 b	0.51 b	0.50 a, b
SFA	2.9*	13.3 a,b,c	12.9 a	13.0 a, b	13.2 b, c	13.6 c
MUFA	2.5*	81.2 a, b	81.9 c	81.9 c	81.8 b, c	81.3 b
PUFA	1.8	5.4 b	5.2 a, b	5.1 a, b	5.0 a	5.1 a, b
Campesterol	4.5**	4.32 b	4.27 b	4.26 b	4.23 b	4.06 a
Stigmasterol	12.3***	1.67 b	0.76 a	0.80 a	0.70 a	0.77 a
Clerosterol	2.2	0.81 a	0.90 a, b	0.90 a, b	0.91 b	0.94 b
β-Sitosterol	5.4***	83.5 a	84.9 a,b	85.3 b,c	85.8 c	84.1 a
Δ5-Avenasterol	1.9	6.8 a, b	6.57 a, b	5.94 a	6.01 a	7.5 b
Δ5,24-Stigmastadienol	3.1*	0.34 a	0.56 a, b	0.43 a, b	0.56 a, b	0.70 b
Apparent β-sitosterol	8.2***	92.3 a	93.6 b,c	93.4 b	93.8 c	93.8 c
Total sterols (mg/kg)	10.8***	1451 a, b	1375 a	1422 a	1563 b	1573 b

* $P \leqslant 0.05$ (95%).
** $P \leqslant 0.01$ (99%).
*** $P \leqslant 0.001$ (99.9%).
Values with different letters are statistically different ($P < 0.05$).

Many of these parameters presented statistically significant differences ($P \leqslant 0.05$). For instance, the cultivar area affected the concentration of natural antioxidants, phenols and tocopherols, and therefore the oxidative stability of the Cornicabra virgin olive oil, as reported by Ranalli et al. (1997) for Italian varieties (such as Frantoio and Leccino). Although some authors have used the main fatty acid composition to differentiate olive oils according to the geographical area of production for oils from Greece (Tsimidou & Karakostas, 1993), Italy (Lanza, Russo, & Tomaselli, 1998) and Spain (Motilva, Ramo, & Romero, 2001), in this study only 50% of the samples could be correctly classified on the basis of discriminant analysis.

The Cornicabra virgin olive oil obtained from the south-east of Toledo (TO-SE 2) had the highest Overall Quality Index (with a medium value of 6.9), reflecting the measured lowest values for free fatty acids, peroxide and UV characteristics (0.40%, 7.8 mg/kg, 1.495 and 0.111, respectively). Moreover, these oil samples presented high values of α-tocopherol and oxidative stability, and a medium value for total phenols. With respect to fatty acids and sterols, these samples presented the highest MUFA, the lowest SFA and low PUFA content, and also medium content for apparent β-sitosterol and total sterols. The chemical compositions of the Cornicabra oils produced in the TO-SE 1 area were similar.

On the other hand, the olive oils obtained from cultivars from northern Toledo (TO-N) presented the lowest OQI (6.0), reflecting mainly high free acidity and peroxide values (0.95% and 12.9 mg/kg, respectively). Furthermore, they possessed the lowest natural antioxidant content and oxidative stability, as well as the lowest MUFA and the highest PUFA (which may also have affected the oxidative stability), and the lowest apparent β-sitosterol content.

The virgin olive oil, processed in the province of Ciudad Real (CR-N and CR-S), presented medium values for most of the quality indices.

The results appear to confirm the general judgement as to the quality of Cornicabra virgin olive oils from Castilla-La Mancha, expressed mainly by the industrial sector itself, namely that the oils produced by the mills located in the south and southeast of the province of Toledo are generally of higher quality. A more detailed study is required to establish whether the observed differences in the chemical composition and quality of the olive oils are mainly due to agronomical and climatologic variables or, on the contrary, to the processing practice employed by the oil-mills. It is also important to note that a large number (about 30%) of the virgin olive oils evaluated failed the minimum sensory requirements for the extra-virgin category (Salvador et al., 2001). It is therefore strongly recommended that the industry work to improve the organoleptic quality of the virgin olive oils produced.

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References

Alba, J., Hidalgo, F., Ruiz, M. A., Martinez, F., Moyano, M. J., Cert, A., Perez, M. C., & Ruiz, M. V. (1996). Características de los aceites de oliva de primera y segunda centrifugación. *Grasas y Aceites*, *47*, 163–181.

Angerosa, F., & Di Giovacchino, L. (1996). Natural antioxidants of virgin oil obtained by two and tri-phases centrifugal decanters. *Grasas y Aceites*, *47*, 247–254.

AOCS. (1989). *Determination of tocopherols and tocotrienols in vegetable oils and fats by HPLC. Official Method, Ce 8–89*. Champaign, IL (USA): AOCS Press.

Aparicio, R., Ferreiro, L., Cert, A., & Lazón, A. (1990). Caracterización de aceites de oliva vírgenes andaluces. *Grasas y Aceites*, *41*, 23–39.

Barranco, D., Rallo, L., Uceda, M., & Hermoso, M. (1994). *Fichas varietales del olivo, Cornicabra (1, no. 3)*. Jaén (Spain): Editorial Unaproliva.

Boskow, D. (1996). *Olive oil: chemistry and technology*. Champaign, IL (USA): AOCS Press.

Cert, A., Alba, J., Camino, M. C., Ruiz, A., Hidalgo, F., Moreda, W., Moyano, M. J., Martinez, F., Tubaileh, R., & Olias, J. M. (1999). Influencia de los sistemas de extracción sobre las características y los componentes menores del aceite de oliva virgen extra. *Olivae*, *79*, 41–50.

Cert, A., Alba, J., León-Camacho, M., Moreda, W., & Pérez-Camino, M. C. (1996). Effects of talc addition and operating mode on the quality and oxidative stability of virgin oils obtained by centrifugation. *Journal of Agricultural and Food Chemistry*, *44*, 3930–3934.

COI. (1999). Activities of cooperation technique. *Olivae*, *38*, 12.

Di Giovacchino, L., Solinas, M., & Miccoli, M. (1994). Effect of extraction systems on the quality of virgin olive oil. *Journal of the American Oil Chemists' Society*, *71*, 1189–1194.

DOCM. (1998). *Diario oficial Castilla La Mancha*, 18 Septiembre, pp. 7460–7461.

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

Felice, M., Gomes, T., & Catalano, M. (1979). Oil extraction from olives by continuous industrial processing "Three years of research". *Rivista Italiana delle Sostanze Grasse*, *56*, 361–379.

Graell, J., Arbos, J., Duro, V., Clota, J., Torrelles, R., Ciria, R., Puig, J., & Munté, M. (1993). Calidad del aceite de oliva virgen D.O. Borjas Blancas. *Alimentación, Equipos y Tecnología*, *10*, 97–127.

Gutfinger, T. (1981). Polyphenols in olive virgin oils. *Journal of the American Oil Chemists' Society*, *58*, 966–968.

Gutiérrez, F. (1989). Determinación de la estabilidad oxidativa de aceites de oliva vírgenes: comparación entre el método A.O.M. y el método Rancimat. *Grasas y Aceites*, *40*, 1–5.

Gutierrez, F., Perdiguero, S., Gutierrez, R., & Olias, J. M. (1992). Evaluation of the bitter taste in virgin olive oil. *Journal of the American Oil Chemists' Society*, *69*, 394–395.

Hidalgo, F., Navas, M. A., Guinda, A., Ruiz, A., León, M., Lazón, A., Maestro, R., Janer, M. L., Pérez, M. C., Cert, A., Alba, J., Gutierrez, F., Dobarganes, M. C., & Graciani, E. (1993). La calidad del aceite de oliva virgen: posibles nuevos criterios para su evaluación. *Grasas y Aceites*, *44*, 10–17.

Koutsaftakis, A., Kotsifaki, F., & Stefanoudaki, E. (1999). Effect of extraction system, stage of ripeness and keading temperature on the sterol composition of virgin olive oils. *Journal of the American Oil Chemists' Society*, *76*, 1477–1481.

Lanza, C. M., Russo, C., & Tomaselli, F. (1998). Relationship between geographical origin and fatty acid composition of extra-virgin olive oils produced in three areas of eastern Sicily. *Italian Journal of Food Science*, *4*, 359–366.

Mínguez-Mosquera, M. I., Rejano, L., Gandul, A., Sanchez, B..H., & Garrido, J. (1991). Color-pigment correlation in virgin olive oil. *Journal of the American Oil Chemists' Society*, *68*, 322–337.

Motilva, M. J., Jaria, I., Bellart, I., & Romero, M. P. (1998). Estudio de la calidad del aceite de oliva virgen de la Denominación de Origen "Les Garrigues" Lleida durante la campaña 1995/1996. *Grasas y Aceites*, *49*, 425–433.

Motilva, M. J., Ramo, T., & Romero, M. P. (2001). Caracterización geográfica de los aceites de oliva virgenes de la denominación de origen protegida 'Les Garrigues' por su pérfil de ácidos grasos. *Grasas y Aceites*, *52*, 26–32.

Negriz, C., & Unal, K. (1991). The effect of extraction systems on triterpene alcohols and squalene content of Virgin Olive Oil. *Journal of the Science of Food and Agriculture*, *56*, 79.

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. *Rivista Italiana delle Sostanze Grasse*, *74*, 501–508.

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*, 274–276.

Stefanoudaki, E., Kotsifaki, F., & Koutsaftakis, A. (1999). Classification of virgin olive oil of the two mayor Cretan cultivars based on their fatty acids composition. *Journal of the American Oil Chemists' Society*, *76*, 623–626.

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

Vázquez, A., Janer, C., & Janer, M. L. (1973). Determination of total polyphenols in olive oils. *Grasas y Aceites*, *24*, 350–357.