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Sterol and alcohol composition of Cornicabra virgin olive oil: the campesterol content exceeds the upper limit of 4% established by EU regulations

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Abstract

The sterol and alcohol composition of Cornicabra virgin olive oil during the crop seasons from 1997/1998 to 2001/2002 ($n = 334$) are reported. The median value of campesterol was 4.0% and ranged from 3.4 to 4.5% in the five crop seasons studied, indicating that high natural content is a peculiar characteristic of the Cornicabra virgin olive oil. Consequently, a large proportion (between 25 and 85%) of the annual production of this commercial monovarietal virgin olive oil exceeds the upper limit established by the current EU legislation. Also, sterols and alcohols are identified as highly useful compounds for chemical authentication of the main Spanish virgin olive oil varieties, in which campesterol presented the highest ANOVA *F*-ratio.

Keywords: Sterols; Alcohols; Campesterol; Cornicabra; Virgin olive oil

1. Introduction

The Cornicabra olive variety covers an area of 280,000 Ha, mainly in the provinces of Ciudad Real and Toledo (Castilla-La Mancha) and accounts for more than 14% of cultivated land under olive in Spain, the world's largest olive oil producing country. The fruit is medium to large with a characteristically elongated and asymmetric shape. The fat yield is 22–24% of fresh weight and the oil is valued for its high stability and good sensory characteristics, such as a dense sensation and a balanced sour and pungent aroma (Salvador, Aranda, Gómez-Alonso, & Fregapane, 2001a).

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 this oil variety locally and internationally.

Previous analytical determinations of sterols—which are important constituents of olive oil in that they relate to the quality and are widely used to check authenticity of the oil—showed that many samples of Cornicabra virgin oil exceeded in the campesterol content the upper limit of 4% of total sterols established by the current legislation (EEC 2568/91 and later amendments). Consequently, many manufacturers are forced to mix Cornicabra virgin olive oil with other olive oil varieties to avoid exceeding the legal campesterol limit, with the paradoxical result that the authenticity of this prized product is lost.

Sterol and alcohol profiles are used to characterize virgin olive oils and especially to detect the adulteration of olive oil with hazelnut oil (e.g., Mariani, Bellan, Morchio, & Pellegrino, 1999; Vichi, Pizzale, Toffano, Bortolomeazzi, & Conte, 2001). Recently, it has also been proposed that these profiles be used to classify virgin olive oils according to their fruit variety (Aparicio, Morales, & Alonso, 1997; Bucci, Magri, Magri, Marini, & Marini, 2002; Ranalli et al., 2002). Also, phytosterols apparently help to reduce the total plasma and LDL-cholesterol, and as a result these compounds are being considered as ingredients of functional foods (e.g., Ostlund, 2002).

The main objective of this study, in direct response to demand from the olive oil sector, was therefore to

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determine the composition of Cornicabra virgin olive oil as regards sterols and triterpene and higher aliphatic alcohols, and particularly the real campesterol content.

2. Materials and methods

2.1. Oil samples

Samples of commercial Cornicabra virgin olive oil ($n = 334$) 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 1997/1998 to 2001/2002. Most of these were collected from oil mills belonging to the *Montes de Toledo* Protected Origin Designation and the rest were obtained from other oil mills located in Castilla-La Mancha that exclusively process the Cornicabra olive variety, in order to ensure chemical characterization of oils of a single 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 from 1998/1999 to 2000/2001.

2.2. Analytical methods

2.2.1. Determination of sterols, erythrodiol and uvaol content (EC 2568/91, Annexes V and VI)

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 bands corresponding to the sterol and triterpene alcohol fractions were separated from the extract by thin-layer chromatography on a basic silica gel plate. The sterols and the erythrodiol and uvaol recovered from the plate were transformed into trimethylsilyl ethers and the mixture was analysed by gas chromatography using an HP 6890 (Agilent Technologies) chromatographer and a capillary column (25 m length×0.25 mm i.d.) coated with SGL-5 (0.25 μm thickness; Sugerlabor).

2.2.2. Determination of aliphatic alcohol content (EC 796/2002, Annex XIX)

The fatty substance, with 1-eicosanol added as internal standard, was saponified with ethanolic potassium hydroxide, then the unsaponifiable matter was extracted with ethyl ether. The alcoholic fraction was separated from the unsaponifiable matter by chromatography on a basic silica gel plate; the alcohols recovered from the silica gel were transformed into trimethylsilyl ethers and analysed by capillary gas chromatography.

2.2.3. Statistical analysis

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

3. Results and discussion

3.1. Sterol composition

The sterol composition (%) of commercial Cornicabra virgin olive oil for the crop seasons from 1997/1998 to 2001/2002 ($n = 334$) is reported in Table 1. As expected for virgin olive oil, the main sterols found were β-sitosterol, Δ5-avenasterol and campesterol, with respective contents (mean ±S.D.) of 84.4±2.4, 6.9±2.2 and 4.0±0.2%.

The campesterol content was remarkably high, with a median value of 4.0, an interquartile range of 0.4, and a global range from 3.4 to 4.5% in the five crop seasons studied (from 1997/98 to 2001/02). More than half of the samples analysed exceeded the upper limit of the 4% established by the EU (Regulation 2568/91/EEC and later amendments).

As regards other authenticity indices established by the current legislation, the apparent β-sitosterol content was lower than the legal minimum of 93% in about 15–20% of the samples analysed. Between 10 and 15% of the samples also exceeded the upper limits for cholesterol, brassicasterol and Δ7-stigmastenol, although these are minor components in the sterol fraction.

All of the Cornicabra olive oil samples analysed contained more than 1000 mg/kg of total sterols, the minimum value established by EU Regulation for olive oil, with a median value of 1489 mg/kg, and a range from 1125 to 1906 mg/kg.

3.2. Campesterol content

Table 2 shows that the natural high campesterol content in Cornicabra virgin olive oil is independent of the seasonal effect in the sense that, every year, between 25 and 85% of the commercial oil analysed in the five crop seasons studied exceeds the legal upper limit.

The slight decrease in campesterol content in the crop seasons 1999/2000 and 2001/2002, when 75% of analysed samples presented less than 4% campesterol, may partially be explained by adverse climatic conditions during these seasons, when the olive fruit was frozen on the trees.

In previous papers of this research group the campesterol content of Cornicabra virgin olive oil was also evaluated in relation to other factors affecting the composition and quality of this product, such as the

Table 1
Sterol composition (%) of commercial Cornicabra virgin olive oils from 1997/1998 to 2001/2002 crop seasons ($n = 334$)

Sterol	Mean ± S.D.	Range	Percentiles				
			10	25	50	75	90
Cholesterol[a]	0.35 ± 0.22	0.06–1.30	0.17	0.20	0.28	0.43	0.66
Brassicasterol[a]	0.08 ± 0.12	0.00–0.56	0.00	0.00	0.03	0.08	0.28
24-Metilencolesterol	0.24 ± 0.09	0.09–0.51	0.14	0.17	0.21	0.30	0.38
Campesterol[a]	4.01 ± 0.21	3.42–4.50	3.73	3.83	4.03	4.17	4.25
Campestanol	0.34 ± 0.06	0.03–0.64	0.29	0.31	0.33	0.36	0.39
Stigmasterol[a]	0.68 ± 0.23	0.28–1.75	0.45	0.51	0.63	0.80	0.99
Δ7-Campesterol	0.14 ± 0.12	0.00–0.87	0.05	0.07	0.11	0.18	0.26
Δ5.23-Stigmastadienol	0.03 ± 0.07	0.00–0.30	0.00	0.00	0.00	0.01	0.11
Clerosterol	0.93 ± 0.08	0.68–1.16	0.83	0.87	0.93	0.99	1.02
β-Sitosterol	84.4 ± 2.41	74.8–87.9	81.0	82.7	85.3	86.2	86.8
Sitostanol	0.70 ± 0.26	0.20–1.87	0.37	0.50	0.70	0.85	0.99
Δ5-Avenasterol	6.94 ± 2.24	4.18–14.60	4.88	5.17	6.10	8.62	10.18
Δ5.24-Stigmastadienol	0.46 ± 0.16	0.23–1.56	0.30	0.35	0.44	0.52	0.61
Δ7-Stigmastenol[a]	0.37 ± 0.24	0.13–1.68	0.17	0.24	0.32	0.40	0.58
Δ7-Avenasterol	0.33 ± 0.09	0.13–0.65	0.23	0.25	0.32	0.39	0.46
Apparent β-Sitosterol[a]	93.5 ± 0.5	91.9–95.0	92.8	93.2	93.5	93.8	94.1
Total sterols[a] (mg/kg)	1489 ± 126	1125–1906	1312	1413	1488	1567	1639

[a] Limits established by the current European Legislation: cholesterol, ⩽0.5; brassicasterol, ⩽0.1; campesterol, ⩽4.0; stigmasterol, <campesterol; Δ7-Stigmastenol, ⩽0.5; apparent β-Sitosterol, ⩾93; Total sterols, ⩾1000 mg/kg.

Table 2
Campesterol content (%) of Cornicabra virgin olive oil from 1997/1998 to 2001/2002 crop seasons ($n = 334$)

	Crop season				
	1997/1998	1998/1999	1999/2000	2000/2001	2001/2002
Number of samples	41	46	42	110	95
Mean	4.16	4.19	3.85	4.07	3.84
Standard deviation	0.15	0.15	0.14	0.17	0.15
Minimum	3.82	3.84	3.45	3.42	3.49
Percentile 10	3.90	3.97	3.65	3.82	3.67
Percentile 25	4.09	4.07	3.79	3.98	3.73
Median	4.16	4.20	3.86	4.12	3.84
Percentile 75	4.27	4.26	3.97	4.19	3.98
Percentile 90	4.33	4.39	4.05	4.24	4.07
Maximum	4.44	4.50	4.11	4.33	4.19

geographical growing area, the extraction system and the ripening stage of the fruit employed in the manufacturing of olive oil. Practically no statistically significant differences were observed in the campesterol content in relation to the geographical area (with the exception of a slight decrease in the Cornicabra olive oil produced in southern Ciudad Real) or with respect to the extraction system used (centrifugation dual- and triple-phase decanter, or pressure) (Salvador, Aranda, Gómez-Alonso, & Fregapane, 2003). Also, there was no clearly observable trend with respect to the relationship between campesterol content and the ripening stage of the Cornicabra olives used to produce virgin olive oil (Salvador, Aranda, & Fregapane, 2001b).

3.3. Alcohol composition

The concentrations (mg/100 g) of triterpenic and higher aliphatic alcohols in Cornicabra virgin olive oil from the 2000/01 and 2001/02 crop seasons ($n = 205$) are shown in Table 3.

The sum of erythrodiol and uvaol, another authenticity index established by law, was below the upper legal limit of 4.5% in all cases, with a median value of 2.8% and a range from 0.3 to 4.1%.

The main aliphatic alcohol components found in commercial Cornicabra virgin olive oil were tetracosanol (C24), hexacosanol (C26) and dicosanol (C22), with respective median values of 3.8, 3.2 and 3.0 mg/100

Table 3
Alcohols composition (mg/100 g) of Cornicabra virgin olive oils from 2000/2001 and 2001/2002 crop seasons ($n=205$)

Alcohol	Mean ± S.D.	Range	Percentiles				
			10	25	50	75	90
Erythrodiol	3.56 ± 1.18	0.37–7.79	1.75	3.08	3.77	4.18	4.69
Uvaol	0.64 ± 0.21	0.14–1.25	0.39	0.54	0.67	0.76	0.90
Erythrodiol + Uvaol[a] (%)	2.68 ± 0.69	0.32–4.09	1.59	2.48	2.78	3.09	3.38
Dicosanol (C22)	3.09 ± 1.15	0.82–7.25	1.66	2.24	3.03	3.91	4.50
Tricosanol	0.24 ± 0.09	0.08–0.56	0.14	0.18	0.23	0.29	0.37
Tetracosanol (C24)	4.11 ± 1.84	1.13–12.0	2.17	2.88	3.75	4.93	6.62
Pentacosanol	0.30 ± 0.10	0.11–0.59	0.16	0.22	0.29	0.36	0.45
Hexacosanol (C26)	3.53 ± 1.27	1.32–7.03	2.00	2.71	3.24	4.39	5.42
Heptacosanol	0.23 ± 0.09	0.08–0.47	0.13	0.17	0.21	0.29	0.36
Octacosanol (C28)	1.58 ± 0.65	0.55–3.66	0.84	1.05	1.45	1.99	2.51
Total aliphatic alcohols	13.1 ± 3.80	4.42–29.1	8.98	10.8	12.4	14.7	18.1

[a] Limit established by the current European legislation: ⩽4.5%.

g, and concentration ranges of 1.1–12.0, 1.3–7.0 and 0.8–7.3 mg/100 g, respectively. As expected, the concentrations of odd carbon number moiety alcohols were much lower, with median values under 0.3 mg/100 g.

Since the published data on these compounds for this economically important monovarietal oil did not represent enough samples and crop seasons to be statistically relevant, the aliphatic alcohol composition values reported in this study can be considered as reference data for the Cornicabra virgin olive oil variety.

3.4. Influence of olive oil variety

It is well known that the sterol composition can be used to determine adulteration of olive oil and it has recently been suggested that it be used to classify virgin olive oils according to their fruit variety (Aparicio et al., 1997; Ranalli et al., 2002).

The concentration of many sterol and alcohol compounds differed significantly ($P \leqslant 0.01$) among the main Spanish virgin olive oil varieties, as shown in Table 4 for selected sterols, on the basis of their content and the ANOVA-F ratio. Campesterol was the sterol component with the highest ANOVA F-ratio (160) of the main Spanish olive oil varieties. Moreover, the average erythrodiol content was also the highest of the studied varieties, as also reported by Aparicio et al. (1997).

In this preliminary study, principal component and stepwise discriminant analyses showed that four variables, two sterols, campesterol and Δ5-avenasterol, and two alcohols, hexacosanol and octacosanol (or erythrodiol in which case the results are very similar), were the most useful parameters for classification of the commercial virgin olive oil varieties studied. The first two discriminant functions of the statistical analysis explained 95.0% of the variance (69.9 and 25.1%, respectively), yielding a reasonable classification (79-99%) of each of the virgin olive

Table 4
Most relevant sterol and alcohol contents of commercial virgin olive oils of different Spanish varieties

Compound	Anova F-ratio	Content (mean ± S.E.)			
		Cornicabra ($n=205$)	Arbequina ($n=17$)	Picual ($n=13$)	Hojiblanca ($n=14$)
Campesterol (%)	160.0***	3.97 ± 0.02[d]	3.48 ± 0.05[c]	3.32 ± 0.07[b]	2.96 ± 0.04[a]
β-Sitosterol (%)	36.5***	83.4 ± 0.21[b]	76.7 ± 0.91[a]	83.90 ± 0.43[b]	84.0 ± 0.37[b]
Δ5-Avenasterol (%)	39.8***	7.86 ± 0.20[a]	14.4 ± 0.79[b]	7.23 ± 0.38[a]	7.85 ± 0.27[a]
Δ5.24-Stigmastadienol (%)	26.0***	0.51 ± 0.01[a,b]	0.85 ± 0.02[c]	0.45 ± 0.01[a]	0.57 ± 0.04[b]
Δ7-Avenasterol (%)	19.9***	0.38 ± 0.01[a]	0.45 ± 0.03[b]	0.45 ± 0.03[b]	0.56 ± 0.02[c]
Apparent β-Sitosterol (%)	11.2***	93.4 ± 0.05[a]	93.6 ± 0.10[a]	93.4 ± 0.14[a]	94.3 ± 0.10[b]
Erythrodiol (mg/100 g)	21.0***	3.56 ± 0.10[c]	2.66 ± 0.14[b]	1.59 ± 0.07[a]	2.22 ± 0.09[a,b]
Dicosanol (mg/100 g)	6.6***	3.10 ± 0,09[b]	3.23 ± 0.33[b]	2.22 ± 0.18[a]	1.98 ± 0.16[a]
Tetracosanol (mg/100 g)	2.8*	4.11 ± 0.15[a,b]	5.04 ± 0.40[b]	3.76 ± 0.34[a]	3.31 ± 0.27[a]
Hexacosanol (mg/100 g)	48.6***	3.53 ± 0.10[a]	7.17 ± 0.45[c]	6.46 ± 0.56[c]	4.81 ± 0.41[b]
Octacosanol (mg/100 g)	25.1***	1.58 ± 0.05[a]	3.07 ± 0.24[b]	1.65 ± 0.16[a]	1.70 ± 0.11[a]

* $P \leqslant 0.05$ (95%). *** $P \leqslant 0.001$ (99.9%).
[a–d] Mean values with different letters are statistically different ($P \leqslant 0.05$).

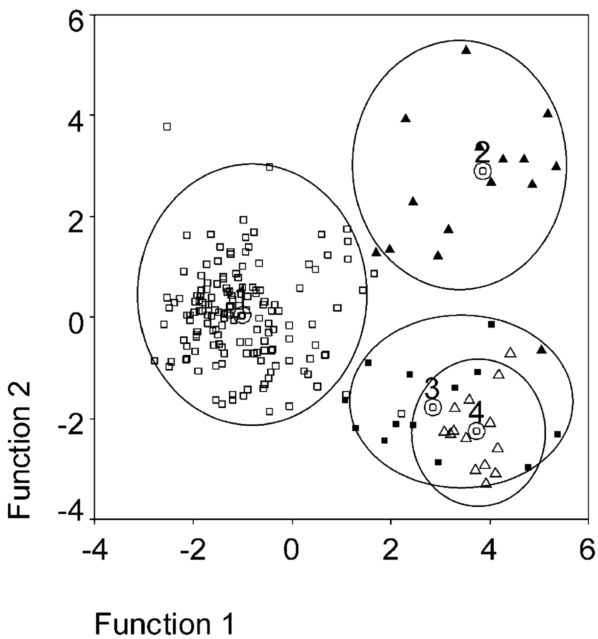

Fig. 1. Plot of discriminant functions using four variables to classify different commercial Spanish Virgin Olive Oil varieties. Variables: campesterol, Δ5-avenasterol, hexacosanol and octacosanol. □, Cornicabra (1); ▲, Arbequina (2); ■, Picual (3); △, Hojiblanca (4); ○, group centroid.

oil varieties studied (Fig. 1). This result can certainly be improved enough to achieve satisfactory chemical authentication of the Spanish virgin olive oil varieties by considering other olive oil components such as fatty acid and phenol composition (Gómez-Alonso, Salvador, & Fregapane, 2002).

The experimental results suggest that high campesterol content is a peculiar characteristic of Cornicabra virgin olive oil. Consequently, a major proportion of this commercial monovarietal virgin olive oil naturally exceeds the upper limit currently established by law. It is also concluded that sterols and alcohols are both very important families of compounds for the chemical authentication of virgin olive oil varieties.

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