Relation of Acidity and Sensory Quality with Sterol Content of Olive Oil from Stored Fruit

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Composition of the sterol fraction, fatty acid, acidity, and the sensorial evaluation of virgin olive oils were studied in two eastern Spanish varieties grown and processed under the same conditions. Fruits were stored at 5 °C and ambient temperature for different times. During fruit storage, there was no significant variation ($P = 0.05$) in fatty acid composition. However, the sterol composition of the oil varied markedly (in particular, there was an increase in stigmasterol), acidity increased, and there was a very significant decrease in sensorial quality. The stigmasterol content presented a high correlation with the acidity and sensory evaluation ($P < 10^{-6}$). The total sterol content increased gradually with olive storage time. Oils with stigmasterol greater than campesterol are graded to a low level (lampant). It is of interest that sensorial quality is revealed by stigmasterol content, a fact unknown until now.

Keywords: *Virgin olive oil; storage; sterols; quality*

INTRODUCTION

The sterol composition of oily fruits is currently the analytical method of choice to determine the plant species from which the oil has been extracted. Changes in the unsaponifiable sterol fraction of virgin olive oil have been reported during fruit ripening (Malta et al., 1972; Amelotti et al., 1973; Camera et al., 1975; and Tiscornia et al., 1978). In general, the sterol fraction did not vary substantially, except for a significant increase in Δ-5-avenasterol that coincided with the highest oil content in the fruit. Sitosterol declined during the same period. Gutiérrez et al. (1999) have shown that the percentage increase in Δ-5-avenasterol was inversely related to the decrease in sitosterol. During the phase of oil formation, there was no significant variation in the total sterol content. The oil obtained from fruit picked in the first stage of ripeness contained a greater amount of sterols than that obtained in the last stage. To our knowledge, only two reports deal with the effect of olive storage (from picking to milling at ambient temperature) on sterol composition. Camera et al. (1987) showed that the oil obtained from olives stored at ambient temperature for different intervals contained higher titratable acidity, stigmasterol (with values equal to or higher than that of campesterol), and total sterol content. Marianni et al. (1991) studied the variation in minor free and esterified components of the oil with ripeness and storage at ambient temperature. They found that total sterol content varied more in the oils obtained from olives stored for different periods than in those obtained at different ripeness stages.

The aim of the present study is to quantify the major components of the sterol fraction of oils obtained from two olive varieties grown in eastern Spain that were harvested at the same time and extracted at laboratory scale after different periods of storage at 5 °C and ambient temperature. Storage at 5 °C has never been studied before, nor has any correlation been found between two such important parameters of quality as acidity and sensorial evaluation.

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EXPERIMENTAL PROCEDURES

Materials. Olive fruits (*Olea europaea* cvs. Blanqueta and Villalonga) were harvested (130 000 kg each variety) from the groves of a cooperative society in Valle de Albaida (Valencia, Spain) and distributed randomly in plastic containers having a capacity of 14 kg of olives. Two different storage conditions were tested: under refrigeration at 5 ± 1 °C with a relative humidity of 95%, and under ambient conditions (12 ± 5 °C, RH 85 ± 5%). Storage times were 0, 7, 15, 30, and 45 days for the Blanqueta variety, and 0, 15, 30, and 45 days for the Villalonga variety. Samples of 14 kg of olives were taken from three containers chosen at random for each sampling date and condition. Oil was extracted using an Abencor analyzer (Comercial Abengoa, S. A. Seville, Spain). The analyzer consisted of three basic elements: a mill, a thermobeater, and a pulp centrifuge (Martínez et al., 1975), and yielded two subsamples per sample.

Analytical Determinations. *Sterols.* The qualitative and quantitative sterol content of the samples was determined according to the European Official Methods Analysis described in Regulations EEC/2568/91 and EEC/1429/92 of the European Union Commission. The determination gave sterols expressed in ppm total sterols only and % individual sterols, as follows:

(a) Extraction of the unsaponifiable matter. As an internal standard, exactly 0.5 mL of α-cholestanol 0.2% (w/v) in chloroform was placed in a 250 mL flask and evaporated to dryness in a rotary evaporator at 30 °C under reduced pressure. Next, 5 g of oil was placed in the same flask. The oil was saponified for 60 min with 50 mL of 2 N ethanolic potassium hydroxide. The mixture was transferred to a 500 mL decanting funnel with 100 mL of distilled water and 80 mL of ethyl ether. The mixture was shaken vigorously and two phases were allowed to separate. The operation was repeated twice more with the ether extraction phase. The ether fractions were combined in a funnel and washed with distilled water until the washings gave no reaction with 1% ethanolic phenolphthalein. The solution was dried with anhydrous sodium sulfate and filtered into a 250 mL flask. The filter was

10.1021/jf9907337 CCC: $19.00
Published on Web 03/14/2000

washed with small amounts of ethyl ether. The ether was evaporated under nitrogen, and then dried in an oven at 100 °C for approximately 15 min. The residue was allowed to cool, and then it was weighed.

(b) Separation of the sterol fraction. Silicagel plates (0.25 mm thick) were submerged in 0.2 N ethanolic potassium hydroxide for 10 s, oven dried at 100 °C for 1 h, and cooled in a desiccator. A 5% solution of the unsaponifiable matter in chloroform was prepared. Using a 100 μL microsyringe, 0.3 mL of this solution was deposited on the activated plate. The plate was developed in a tank containing hexane−ether 65:35 (v/v), dried, and sprayed with a solution of 2,7-dichlofluorescein to visualize the sterol band. The band was scraped off and washed once with 10 mL of warm chloroform and twice with 7 mL of ethyl ether. The solvent was evaporated to dryness under nitrogen to obtain the sterol fraction. Sterols were silanized with a mixture of anhydrous pyridine−hexamethyldisilazane−trimethylchlorosilazane 9:3:1 (v/v/v) at 50 μL per mg of sterol.

Gas Chromatography. Hydrogen was used as the carrier gas at a flow rate of 49 cm/s. The temperatures were: oven 265 °C, injector 280 °C, and detector 300 °C. The column was 30 m × 0.25 mm (J&W Scientific DS-5MS) and the ratio split was 1/80. The chromatograph was a HP 6890 equipped with a HP 6890 integrator.

Fatty Acids. Fatty acids were determined following the analytical methods described in Regulations EEC/2568/91 and EEC/1429/92 of the European Union Commission.

Acidity value, given as % of oleic acid, was determined by titration of a solution of oil in ethanol−ether 1:1 (v/v) with ethanolic potassium hydroxide according to European Official Methods of Analysis EEC/2568/91 and EEC/1492/92.

Sensory Analysis. Sensory analysis was carried out by an analytical panel of the Instituto de la Grasa, comprising 12 selected and trained tasters, according to European Official Methods of Analysis EEC/2568/91. Outliers were separated using the method of Albi and Gutiérrez (1991). Each oil was graded on a scale from 1 to 9, where 1 is very poor quality. The results from duplicated samples were subjected to statistical analysis.

Statistical Analysis. The assays were carried out in quadruplicate. The results are shown as graphs (drawn using Sigma Plot, version 1.2 (Jandel Scientific Corp.)) or tables of mean values. The same program (Sigma Plot) was used in the correlation study.

RESULTS AND DISCUSSION

Table 1 gives the initial composition in % of fatty acids for the two varieties studied. It can be seen that the Blanqueta variety has a lower content in oleic acid and a higher one in linoleic than the Villalonga variety, the former thus being more susceptible to oxidation (Villafranca et al., in revision). The mean values and coefficients of variation for acidity, total sterol content (ppm), percentage of the major sterols campesterol, stigmasterol, sitosterol, and Δ-5-avenasterol, and overall evaluation of quality for the two storage temperatures studied are shown in Tables 2 and 3 for the Blanqueta variety, and Tables 4 and 5 for the Villalonga variety. It can be seen that the acidity of the oil increased significantly throughout storage of the fruit, as a result of hydrolysis caused by lipolytic activity of pathogenic lipases. This increase was greater in the oils from olives stored ambient temperature. The results are in agreement with those obtained by García et al. (1996). The limit of 1% for "extra" oils (value established in the European Community for Virgin Olive Oil Regulations) was exceeded in the Blanqueta variety at 7 and 15 days for ambient temperature and 5 °C respectively, and in the Villalonga variety between 15 and 30 days for both conditions. This can be explained by the higher initial acidity content of the Blanqueta variety (50% higher).

Table 1. Initial Fatty Acid Composition (Percent) of the Virgin Olive Oil from the Blanqueta and Villalonga Varieties

fatty acid	Blanqueta	Villalonga
16:0	14.18[a]	11.67
	0.14[b]	0.15
16:1	1.07	0.77
	0.02	0.01
17:0	0.15	0.19
	0.01	0.01
17:1	0.28	0.33
	0.01	0.0
18:0	2.39	2.57
	0.01	0.02
18:1	65.18	71.39
	0.13	0.10
18:2	15.27	11.24
	0.04	0.03
18:3	0.62	0.87
	0.01	0.02
20:0	0.43	0.43
	0.01	0.01
20:1	0.28	0.32
	0	0
22:0	0.14	0.12
	0.01	0.01

[a] Mean. [b] Standard deviation.

The initial sterol content in total sterols of the two varieties was significantly different ($P = 0.05$), and much higher in the Blanqueta. Also, significant differences ($P = 0.05$) were found for the sitosterol and Δ-5-avenasterol; the former was higher in the Villalonga variety and the latter more important in Blanqueta (5-fold). This indicates that the biosynthesis of sterols is different in the two varieties, although this has no effect on the aim of our study. Total sterol content increased throughout storage, at the same rate as a decrease in dry matter per kg of olives. These results are in agreement with those obtained by Camera et al. (1987) and Marianni et al. (1991). The increase was greater in both varieties for the samples stored at ambient temperature, in which there was a larger decrease in dry matter.

Storage time and temperature influenced the percentage composition of the sterol fraction. The variation in composition was significant ($P = 0.05$), with a sharper increase at stigmasterol in the oils from fruits stored at ambient temperature. In the Blanqueta variety (Tables 2 and 3), the campesterol content was practically constant throughout storage and remained within the established limit (<4%); stigmasterol increased 2.79% at ambient temperature and 1.78% at 5 °C. After 30 days of storage at ambient temperature, the stigmasterol value was higher than that of campesterol, contrary to regulations for virgin olive oils. At 5 °C, the stigmasterol value remained lower than that of campesterol, which complied with the regulation value. In the Villalonga variety (Tables 4 and 5), the behavior was similar. The stigmasterol content increased by 3.65% at ambient temperature and by 2.49% at 5 °C. These results are in agreement with those obtained by (Camera et al. (1987) when studying the storage of olives (1 kg) at ambient temperature in layers on a trellis and in plastic bags with holes with other varieties.

The results suggest that the influence of storage temperature on sterol composition is more important

Table 2. Acidity, Sterol Composition, and Overall Sensorial Evaluation of the Oil of the Blanqueta Olive Fruit Stored at Ambient Temperature[a]

	time (days)									
	0		7		15		30		45	
	mean	RSD%[b]	mean	RSD	mean	RSD	mean	RSD	mean	RSD
acidity	0.57	0.40	1.19	0.38	1.94	0.36	5.28	0.36	6.12	0.67
TE	1544	3.20	1947	2.60	1852	3.10	1873	3.73	2085	3.61
CA	3.38	0.83	3.84	0.42	3.62	0.40	3.54	0.73	3.39	0.88
ST	1.15	0.23	1.97	0.42	2.62	0.94	3.84	0.46	3.94	0.47
BS	73.83	0.92	77.66	0.50	80.50	0.95	77.13	0.51	77.22	0.83
Δ5	21.64	0.38	16.52	0.30	13.55	0.31	15.85	0.23	15.97	0.37
OE[c]	8.2		7.8		5.3		3.4		1.7	

[a] Abbreviations: TE, total sterols (ppm); CA, campesterol (%); ST, stigmasterol (%); BS, sitosterol (%); Δ5, Δ-5-avenasterol (%); OE, overall evaluation. [b] RSD% = (SD/mean) × 100, $n = 4$. [c] Limits of confidence ±0.5.

Table 3. Acidity, Sterol Composition, and Overall Sensorial Evaluation of the Oil of the Blanqueta Olive Fruit Stored at a Temperature of 5 °C[a]

	time (days)									
	0		7		15		30		45	
	mean	RSD%[b]	mean	RSD	mean	RSD	mean	RSD	mean	RSD
acidity	0.57	0.40	0.66	0.38	1.29	0.38	2.33	0.36	3.74	0.57
TE	1544	3.20	1636	3.41	1724	1.58	1722	1.73	1800	2.11
CA	3.38	0.83	3.42	0.62	3.39	0.70	3.49	1.39	3.62	0.88
ST	1.15	0.23	1.62	0.44	1.96	0.84	2.64	0.46	2.93	0.57
BS	73.83	0.92	75.56	0.50	74.60	0.65	75.94	0.84	77.45	0.83
Δ5	21.64	0.38	19.42	0.36	19.97	0.81	17.79	0.83	16.42	0.37
OE[c]	8.2		7.9		7.3		4.1		3.7	

[a] Abbreviations: TE, total sterols (ppm); CA, campesterol (%); ST, stigmasterol (%); BS, sitosterol (%); Δ5, Δ-5-avenasterol (%); OE, overall evaluation. [b] RSD% = (SD/mean) × 100, $n = 4$. [c] Limits of confidence ±0.5.

Table 4. Acidity, Sterol Composition, and Overall Sensorial Evaluation of the Oil of the Villalonga Olive Fruit Stored at Ambient Temperature[a]

	time (days)							
	0		15		30		45	
	mean	RSD%[b]	mean	RSD	mean	RSD	mean	RSD
acidity	0.28	0.38	0.60	0.48	4.45	0.36	6.43	0.57
ET	829	2.90	1130	1.78	1201	3.00	1477	1.29
CA	3.64	0.93	3.69	0.76	3.59	1.59	3.68	0.88
ST	1.66	0.27	1.99	0.89	3.34	0.66	3.991	0.75
BS	90.64	0.29	89.69	0.51	88.53	0.28	87.61	0.37
Δ5	4.06	0.34	4.54	0.91	4.67	0.93	4.62	0.37
OE[c]	8.1		7.0		3.5		1.8	

[a] Abbreviations: TE, total sterols (ppm); CA, campesterol (%); ST, stigmasterol (%); BS, sitosterol (%); Δ5, Δ-5-avenasterol (%); OE, overall evaluation. [b] RSD% = (SD/mean) × 100, $n = 4$. [c] Limits of confidence ±0.5.

Table 5. Acidity, Sterol Composition, and Overall Sensorial Evaluation of the Oil of the Villalonga Olive Fruit Stored at a Temperature of 5 °C[a]

	time (days)							
	0		15		30		45	
	mean	RSD%[b]	mean	RSD	mean	RSD	mean	RSD
acidity	0.28	0.38	0.48	0.28	2.36	0.66	4.97	0.78
TE	829	2.90	1005	0.98	1094	4.09	1138	1.39
CA	3.64	0.93	3.69	0.76	3.59	1.59	3.62	0.88
ST	1.66	0.27	1.77	0.89	2.14	0.66	3.15	0.75
BS	90.64	0.29	89.18	0.55	89.00	0.92	87.59	0.62
Δ5	4.06	0.34	5.34	0.91	5.57	0.93	5.63	0.37
OE[c]	8.1		7.9		6.3		3.5	

[a] Abbreviations: TE, total sterols (ppm); CA, campesterol (%); ST, stigmasterol (%); BS, sitosterol (%); Δ5, Δ-5-avenasterol (%); OE, overall evaluation. [b] RSD% = (SD/mean) × 100, $n = 4$. [c] Limits of confidence ±0.5.

than the influence of storage time. In this sense, the greater increase in stigmasterol content observed in oils stored at ambient temperature could be the consequence of a higher hydrolysis of sitosterol than at 5 °C.

The concentration of sitosterol (the major sterol of virgin olive oil) behaved differently in the two varieties. In Blanqueta, it increased significantly throughout storage at 5 °C and ambient temperature whereas in Villalonga it tended to decline. The Δ-5-avenasterol also behaved differently in the two varieties, decreasing in Blanqueta and increasing slightly in Villalonga. The enzyme desaturase, which transforms sitosterol into Δ-5-avenasterol, acts throughout storage in the Villalonga variety, possibly due to the higher initial acidity. The results found for these two sterols in the Blanqueta variety are contrary to those reported by Camera et al.

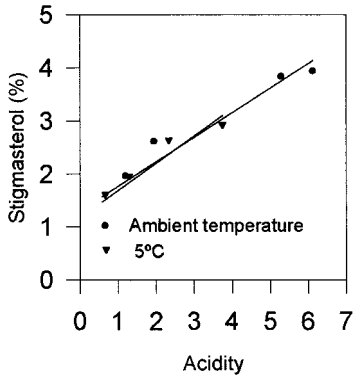

Figure 1. Relation between percent of stigmasterol and acidity in the virgin olive oil exctracted from the Blanqueta olive variety at different times.

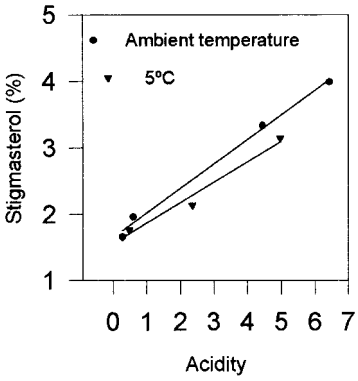

Figure 2. Relation between percent of stigmasterol and acidity in the virgin olive oil exctracted from the Villalonga olive variety at different times.

(1975) and Gutiérrez et al. (1999) for changes during the olive ripening cycle. This discrepancy may be due to differences in ripening during storage compared with ripening on the tree.

The sensorial evaluation determined by overall score (Tables 2−5) declined throughout storage. The decrease was much sharper in the oils from olives stored at ambient temperature, which became lampant (score less than 3.5) after 30 days of storage for the Blanqueta variety and 45 days for Villalonga. Both oils were extra quality (score greater than 6.5) at the beginning of storage. In the samples stored in the cold (5 °C), the oil of neither variety had become lampant at the end of storage. These results are in accord with those obtained by Garcia et al. (1996).

The aspect of interest in this work is the good regressions found between the percent of stigmasterol and both the acidity (Figures 1 and 2) for both varieties under the two storage conditions (in which can be observed the effect of temperature), and the sensorial evaluation (Figures 3 and 4), with correlation coefficients with $P < 10-6$. For this relationship, which at present we cannot explain, no reference has been found in the literature. We consider that this work provides information of great practical interest, namely, the possibility of determining the category of an oil by means of its stigmasterol content without the need for an analytical panel to test sensory quality.

New studies on shelf life in our laboratories have confirmed the results of the correlations with sensorial evaluation.

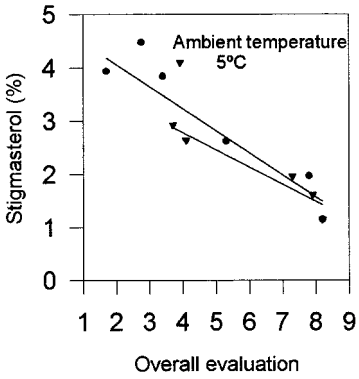

Figure 3. Relation between percent of stigmasterol and overall evaluation in the virgin olive oil exctracted from the Blanqueta olive variety at different times.

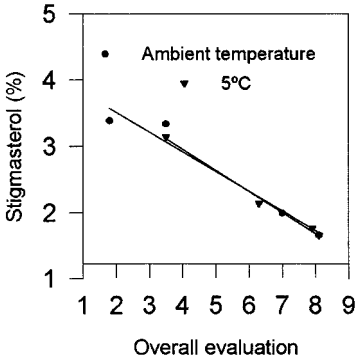

Figure 4. Relation between percent of stigmasterol and overall evaluation in the virgin olive oil exctracted from the Villalonga olive variety at different times.

Table 6. Ordinates at the Origin, Slopes, Correlation Coefficients, and Probabilities (*r*) of the Two Olive Varieties Studied

	a	b	r	P
Blanqueta ambient	1.31	0.46	0.9639	2.1×10^{-7}
Blanqueta 5 °C	1.18	0.51	0.9552	3.2×10^{-7}
Villalonga ambient	0.97	0.52	0.9720	2.7×10^{-8}
Villalonga 5 °C	0.91	0.46	0.9592	1.9×10^{-7}
Blanqueta ambient	4.89	−0.41	0.9640	2.0×10^{-7}
Blanqueta 5 °C	4.08	−0.32	0.9686	1.4×10^{-7}
Villalonga ambient	5.33	−0.55	0.9920	1.6×10^{-9}
Villalonga 5 °C	4.90	−0.47	0.9617	2.3×10^{-7}

Table 6 shows the ordinates at the origin, the slopes, the correlation coefficients, and the probabilities (*r*) of Figures 1, 2, 3, and 4 for the Blanqueta and Villalonga varieties.

ACKNOWLEDGMENT

The authors wish to thank Ms. M. A. Viera for her assistance.

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Received for review July 6, 1999. Revised manuscript received December 23, 1999. Accepted December 30, 1999. This study was supported by CICYT (ALI 95- 0197-CO2-01).

JF9907337