Effect of Extraction System, Stage of Ripeness, and Kneading Temperature on the Sterol Composition of Virgin Olive Oils

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ABSTRACT: Comparative extraction trials were carried out among a classical pressing, a dual-, and a three-phase centrifugation system using olive crops of Koroneiki variety. Two different kneading temperatures, 30 and 45°C, were tested at three stages of ripeness for two consecutive years of harvest, 1995–1996 and 1996–1997. Composition of the sterol fraction was determined in the resulting olive oil samples ($n = 72$). Stigmasterol was found to be affected by the extraction system; it was obtained in the highest amount in the pressing system. The ratio campesterol/stigmasterol was significantly higher in oils extracted by dual- and three-phase centrifugation. Sterols were significantly affected by the ripening stage of the fruit. During December, the ratio campesterol/stigmasterol reached the maximal and β-sitosterol the minimal values; this appears to be the optimal period for harvesting the olives. Comparison of the different kneading temperatures showed that at 30°C, Δ^5-avenasterol and campesterol/stigmasterol ratio reached higher values than at 45°C.

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KEY WORDS: Extraction technology, kneading temperature, maturation, sterols, virgin olive oil.

Pressing, the oldest method of extracting oil from olives, has several advantages, including the low pomace moisture content and the limited amount of vegetation water produced. However, it has some drawbacks, including high labor requirements, the cost of the filtering diaphragms, and the discontinuous manner of processing the olives. Consequently, technological progress has lead to increasing use of continuous centrifugation systems (1). These systems require addition of warm water to the olive paste to separate the oil from the other phases, resulting in the production of a considerable amount of vegetation water and the decrease in the oil of phenols, which are important in oil stability and organoleptic quality (2). The most recent innovation in oil extraction technology is dual-phase decanters, which are able to centrifuge the oily paste without addition of warm water.

Extraction technology and kneading temperature are known to affect oil yields and the properties of the oils and oil by-products (3–6). Yield, pomace moisture content, and pomace oil content from comparative extraction trials at 30 and 45°C for different ripening stages have been reported under industrial conditions (7).

The sterol fraction is an important determinant of the genuineness of an olive oil, and the ratio of campesterol/stigmasterol has been reported as a quality index of an oil (8). Several researchers have studied the evolution of sterols during maturation (9,10).

In this report, we present the sterol composition of oils extracted from the Koroneiki variety during a comparative study between a pressing extraction system, a dual-, and a three-phase decanter at two different kneading temperatures and at three different stages of maturity.

MATERIALS AND METHODS

Olives of Koroneiki variety were selected at three stages of maturity (unripe, normal, and overripe) from November to January for two consecutive years of harvest, 1995–1996 and 1996–1997. Olive oil was extracted using a classical pressing system, a continuous dual-phase centrifugation system, and a three-phase centrifugation system. For extraction, 2300 kg of olives of a certain stage of maturity were sufficiently mixed in order to be as homogeneous as possible and were separated into three lots. The quantity of olives for each extraction trial was 400 kg for the centrifugation systems and 350 kg for the classical one. Two trials were carried out for each extraction system.

For the classical system the following steps were carried out: (i) leaf removal from olive lots; (ii) washing; (iii) crushing by cylindrical triturators; (iv) grinding by round granite mill wheels; (v) kneading of the paste for 30 min at 30 and 45°C; (vi) load formation with four paste-filled mats (one empty filter disk) and one empty between two metal disks; (vii) pressing at 400 atm for 55 min; (viii) separation of the oil by means of an automated vertical discharge centrifuge.

For the dual- and three-phase decanters, the following procedure was performed: (i) leaf removal from olive lots; (ii) washing; (iii) milling of drupes by a hammer crusher with 6-mm diameter holes operating at 3000 rpm; (v) kneading of the paste for 30 min at 30 and 45°C; (vi) centrifugation using a Pieralisi M1 dual-phase decanter (Jesi, Italy) or fluidifica-

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TABLE 1
Oil Content Koroneiki Olives of During Maturation over Two Consecutive Harvest Years

	Oil content (wt%, dry basis)					
	1995–1996			1996–1997		
	November	December	January	November	December	January
Mean[a]	42.8	44.8	46.8	39.5	44.7	44.1
Std. dev.	1.09	0,.67	0.91	0.95	1.27	1.32
Min.	39.8	43.7	45.5	38.1	43.1	41.5
Max.	44.2	46.0	48.1	40.6	46.3	45.1

[a]Mean values of six determinations.

tion of the paste with 40% water and centrifugation using an Alfa-Laval (Monza, Italy) type 314 three-phase decanter; (vii) separation of the oil by means of a manual and an automated vertical discharge centrifuge.

The percentage of oil content on both a wet and dry weight basis (Table 1) was determined in the crop prior to extraction using a Soxhlet apparatus. The sterol composition of the oils was determined according the European Community (EC) official method 2568/91 (11). The sterol fraction was analyzed by a Hewlett-Packard 6890 gas chromatograph (Hewlett-Packard GmbH, Waldbronn, Germany) with a split injector and a flame-ionization detector. The analytical column was an HP-5 5% phenyl methyl siloxane stationary phase (30 m × 0.32 mm × 0.25 μm). The gas chromatographic conditions were as follows: Inlet temperature: 285°C; oven temperature: 265°C; detector temperature: 300°C; split ratio: 30:1; amount injected: 2 μL. Helium was used as a carrier gas at a flow rate of 1.2 mL min^{-1}. Sterols were quantified using cholestanol as internal standard.

Multivariate statistical analysis was applied to the data set using the statistical package SPSS, version 8.0 (12), in order to study the effect of the extraction system, the stage of maturation, and the kneading temperature on the sterol composition of the corresponding virgin olive oils. The experimental design included two extraction trials for each of the extraction systems at three stages of ripeness for two harvesting years. Each oil sample was analyzed in duplicate ($n = 72$). The model used was full factorial including the foregoing mentioned factors.

RESULTS AND DISCUSSION

Effect of extraction system on sterol concentration. Campesterol, stigmasterol, β-sitosterol, Δ^5-avenasterol, and Δ^7-avenasterol are significantly ($P < 0.001$) affected by the system of extraction Table 2. Among them stigmasterol is the one most affected (F value = 74.0). The campesterol/stigmasterol ratio, which has been reported as an index of quality of an oil, is high in the centrifugation systems. This result agrees with other researchers (6,8). Oils from the classical pressing system had significantly higher values for β-sitosterol and stigmasterol than the oils from centrifugation systems (13).

TABLE 2
Sterol and Triterpene Dialcohol Concentrations[a] (values as % total sterols) of Oils Extracted by a Classical Pressing, Dual-, or Three-Phase Decanter

	Classic	Dual-phase	Three-phase	Std. error	F[b]	Significance
Cholesterol	0.3[a]	0.3[a]	0.3[a]	0.006	3.090	0.058
24-Methylene cholesterol	0.4[a]	0.4[a]	0.4[a]	0.005	4.991	0.012
Campesterol	4.2[b]	4.3[a]	4.3[a]	0.009	12.484	0.000
Campestanol	0.4[a]	0.4[a]	0.4[a]	0.007	2.080	0.140
Stigmasterol	0.9[a]	0.8[b]	0.8[b]	0.006	73.970	0.000
Campesterol/stigmasterol	4.5[b]	5.0[a]	5.1[a]	0.041	33.161	0.000
Clerosterol	0.9[a]	0.9[a]	0.9[a]	0.009	1.413	0.257
β-Sitosterol	76.0[a]	75.7[b]	75.5[c]	0.062	16.955	0.000
Sitostanol	0.3[a]	0.3[a]	0.3[a]	0.006	0.167	0.847
Δ^5-Avenasterol	15.2[b]	15.3[b]	15.5[a]	0.051	10.385	0.000
Δ^5-24-Stigmastadienol	0.7[a]	0.7[a]	0.7[a]	0.016	2.001	0.150
β-Sitosterol sum[c]	93.1[a]	93.0[a]	92.9[a]	0.483	2.769	0.076
Δ^7-Stigmasterol	0.3[a]	0.3[a]	0.3[a]	0.011	4.207	0.023
Δ^7-Avenasterol	0.4[a]	0.4[a]	0.4[a]	0.008	17.581	0.000
Erythrodiol	1.4[a]	1.5[a]	1.4[a]	0.087	0.429	0.654
Uvaol	0.1[a]	0.1[a]	0.1[a]	0.010	4.085	0.025

[a]Mean sample size = 24. Means followed by the same superscript roman letter within each row do not present significant differences (Duncan's multiple range test α = 0.05).
[b]F tests the effect of the extraction system.
[c]β-Sitosterol sum = clerosterol + β-sitosterol + sitostanol + Δ^5-avenasterol + Δ^5-24-stigmastadienol.

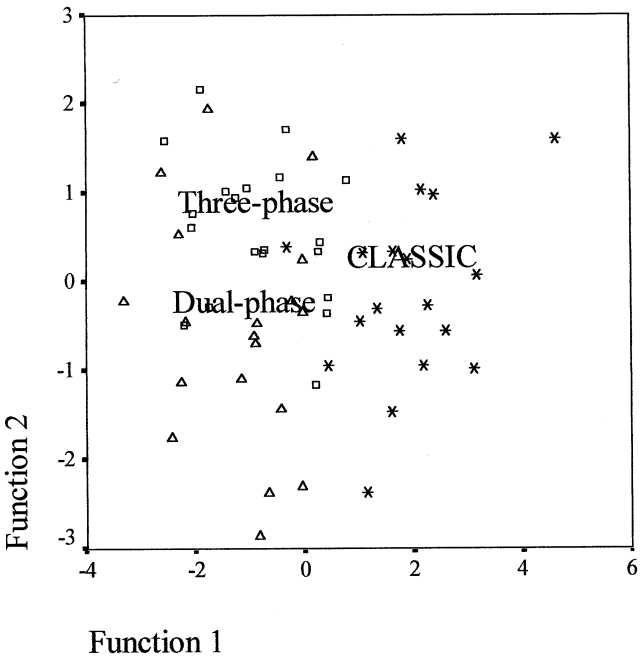

FIG. 1. Classification of olive oil samples extracted by (∗) a classical pressure system; a dual-phase (△) and a three-phase (□) decanter system on the basis of their sterol compositions, using canonical discriminant analysis.

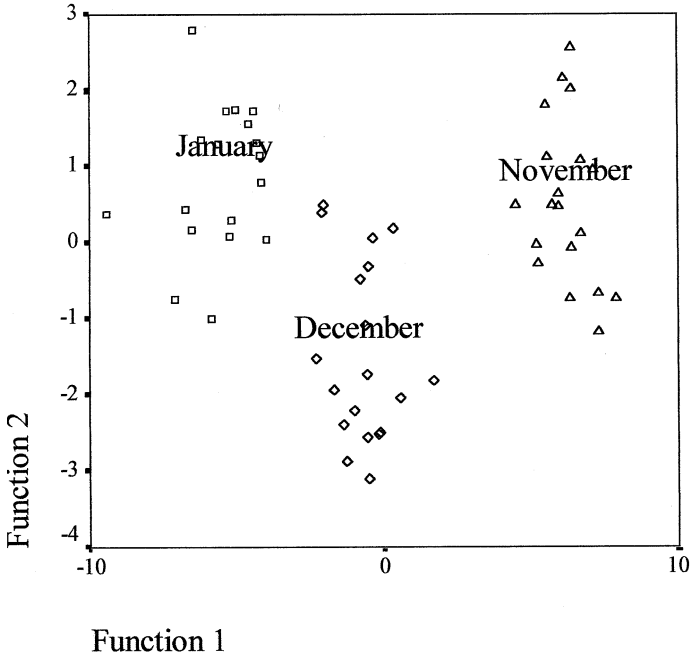

FIG. 2. Classification of olive oil samples harvested at three different ripening stages (November, △; December, ◇; January, □) on the basis of their sterol compositions, using canonical discriminant analysis.

Further multivariate analysis based on canonical discriminant analysis of the oils' sterol composition revealed sufficient potential for the sterol fraction composition to act as a means to group the different extraction systems (Fig. 1). The oils of the classical system (Table 3) were correctly classified 91.7% of the time. Only two samples of this system were misclassified into other groups. The oils extracted by dual- or three-phase centrifugation systems had more nearly similar sterol profiles, and the percentages of correct classification for the corresponding samples were 75% .

Effect of maturation stage on sterol concentration. The evolution of sterols and triterpene dialcohols during maturation is presented in Table 4. Most of these compounds are significantly affected by the maturation stage of the fruit, particularly campesterol, β-sitosterol, β-sitosterol sum, and Δ^5-avenasterol (highest *F* values). Campesterol and β-sitosterol decrease during ripening while β-sitosterol sum and Δ^5-avenasterol significantly increase. It is noteworthy that the campesterol/stigmasterol ratio—as quality index—reaches its highest value in December, which reveals that this is the optimal stage of maturation for harvesting the olives of the Koroneiki variety. Other researchers (9) reported that β-sitosterol is minimal and Δ^5-avenasterol is maximal when olives are harvested at their optimum. This is in accordance with our results for the month of December. Another important point is that at early stages of maturation campesterol content is very close to 4.5%, which is above the upper limit set by the EC norm, and it decreases afterward.

TABLE 3
Classification Results of Oils Obtained from Different Extraction Systems on the Basis of Their Sterol Composition[a]

		Predicted group membership			
	Extraction system	Pressing	Dual-phase	Three-phase	Total
Count	Pressing	22	1	1	24
	Dual-phase	0	18	6	24
	Three-phase	2	4	18	24
Percentage	Pressing	91.7	4.2	4.2	100.0
	Dual-phase	0.0	75.0	25.0	100.0
	Three-phase	8.3	16.7	75.0	100.0

[a]Of the original grouped cases, 80.6% were correctly classified.

The strong correlation between sterol composition and ripening is also evident from the canonical discriminant plot of the samples (Fig. 2). On the basis of sterol compositional data, the oils are classified into three separate groups, each corresponding to a certain stage of maturity.

Effect of kneading temperature on sterol concentration. The paste temperature at the malaxation stage is an important parameter among the rules of Good Manufacturing Practice (GMP). The sterols most significantly affected by the temperature applied (Table 5) are stigmasterol (*F* value = 44.188) and Δ^5-avenasterol (*F* value = 41.931). Stigmasterol is higher in the oils kneaded at 45°C whereas Δ^5-avenasterol is significantly lower. The campesterol/stigmasterol ratio is higher when the kneading temperature is 30°C, which leads to the conclusion that higher-quality oils are extracted at lower tem-

TABLE 4
Evolution of Sterols and Triterpene Dialcohols (values as % total sterols) During Fruit Maturation in Olive Oils from Koroneiki Variety[a]

	November	December	January	Std. error	F^b	Significance
Cholesterol	0.3[a]	0.3[a]	0.2[b]	0.006	84.580	0.000
24-Methylene cholesterol	0.4[b]	0.5[a]	0.4[b]	0.005	74.284	0.000
Campesterol	4.5[a]	4.2[b]	4.0[c]	0.009	771.782	0.000
Campestanol	0.5[a]	0.5[a]	0.4[b]	0.007	97.480	0.000
Stigmasterol	0.9[a]	0.8[b]	0.8[b]	0.006	134.287	0.000
Campesterol/stigmasterol	4.9[a]	5.0[a]	4.7[b]	0.041	54.012	0.001
Clerosterol	1.0[a]	0.9[b]	0.9[b]	0.009	12.399	0.000
β-Sitosterol	77.9[a]	74.4[c]	74.9[b]	0.062	940.962	0.000
Sitostanol	0.4[a]	0.3[b]	0.2[c]	0.006	195.440	0.000
Δ^5-Avenasterol	12.5[c]	16.6[b]	16.8[a]	0.051	2,302.683	0.000
Δ^5-24-Stigmastadienol	0.7[a]	0.7[a]	0.6[b]	0.016	3.413	0.044
β-Sitosterol sum	92.5[c]	93.0[b]	93.5[a]	0.041	170.547	0.000
Δ^7-Stigmasterol	0.4[a]	0.3[b]	0.3[b]	0.011	28.615	0.000
Δ^7-Avenasterol	0.4[a]	0.4[a]	0.3[b]	0.008	55.304	0.000
Erythrodiol	1.4[b]	1.2[b]	1.7[a]	0.087	9.826	0.000
Uvaol	0.1[a]	0.0[b]	0.1[a]	0.010	9.258	0.001

[a]Mean sample size = 24. Means followed by the same superscript roman letter within each row do not present significant differences (Duncan's multiple range test $\alpha = 0.05$).
[b]*F* tests the effect of maturity stage. For abbreviation see Table 2.

TABLE 5
Sterol and Triterpene Dialcohol Concentration (values as % total sterols) of Koroneiki Virgin Olive Oils at Two Different Malaxation Temperatures[a]

	30°C	45°C	Std. error	F^b	Significance
Cholesterol	0.3	0.3	0.005	13.190	0.001
24-Methylene cholesterol	0.4	0.4	0.004	1.581	0.217
Campesterol	4.2	4.2	0.007	7.100	0.011
Campestanol	0.4	0.4	0.006	0.851	0.362
Stigmasterol	0.8	0.9	0.005	44.188	0.000
Campesterol/stigmasterol	5.0	4.8	0.045	8.795	0.005
Clerosterol	0.9	0.9	0.008	0.665	0.420
β-Sitosterol	75.6	75.9	0.051	18.967	0.000
Sitostanol	0.3	0.3	0.005	0.024	0.877
Δ^5-Avenasterol	15.5	15.1	0.042	41.931	0.000
Δ^5-24-Stigmastadienol	0.7	0.7	0.013	0.282	0.599
β-Sitosterol sum	93.0	92.9	0.033	2.050	0.161
Δ^7-Stigmasterol	0.3	0.3	0.009	1.846	0.183
Δ^7-Avenasterol	0.4	0.4	0.007	6.052	0.019
Erythrodiol	1.5	1.4	0.071	0.009	0.923
Uvaol	0.1	0.0	0.008	34.430	0.000

[a]Mean sample size = 36.
[b]*F* tests the effect of malaxation temperature. For abbreviation see Table 2.

peratures. This is in accordance with the rules of GMP that suggest paste temperature never exceed 35°C (14).

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