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Sterol and erythrodiol + uvaol content of virgin olive oils from cultivars of Extremadura (Spain)

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Abstract

Sterol profiles were determined and the levels of erythrodiol + uvaol in 63 samples of oil elaborated in seven varieties of olives that predominate in the Extremadura Community Cornezuelo, Corniche, Cacereña, Carrasqueña, Morisca, Verdial de Badajoz and Picual at three ripening stages green, spotted, and ripe. The data were compared statistically, for differences by variety and ripeness. The data of 42 samples were subjected to a discriminant analysis with 'variety' as the grouping variable. This was found to explain 77% of the variance, with 100% success in classifying all the varieties. The model was validated with the data of the 21 remaining samples, with a mean of more than 90% of the samples correctly classified.

Keywords: Olive; Oil; Virgin; Phytosterols; Triterpene alcohols

1. Introduction

Plant sterols, or phytosterols, belong to the group of the desmethylsterols steroid alcohols present in all living organisms except bacteria. The contents of these sterols in different olive oils are limited by regulations established by the European Union, the International Olive Oil Council, and the Codex Alimentarius of the FAO/WHO, to control against fraud.

Erythrodiol and uvaol, triterpene alcohols that are chromatographed together with the sterols, are generally located in the exocarp of the olive (Christopoulou, Lazaraki, Alexiou, Synouri, & Frangiscos, 1996). During ripening, they not only increase in concentration but the effect is enhanced since the exocarp becomes more fragile, and fragments better during milling.

Several workers have described the use of an olive oil's sterol profile to detect possible fraudulent admixtures with lower value fats. The presence of olive–pomace oil in virgin oil can be detected from the levels of erythrodiol + uvaol (Reina, White, & Jahngen, 1997). Furthermore, these two parameters have been proposed as applicable to the characterization of olive oils, since the sterol profile differs from one variety to another, some sterol concentrations surpassing the regulatory maxima (Albi, Lanzón, Cert, & Aparicio, 1970). Even so, these characteristics are known to be affected by many factors agronomic (climate, soil, water) (El Antari, Hital, Boulouha, & El Moudni, 2000; Stefanoudaki, Chartzoulakis, Koutsaftakis, & Kotsifaki, 2001), geographic (altitude, longitude) (Christopoulou et al., 1996; Duarte & Martins, 1976; Harwood & Aparicio, 2000), harvesting (cultivar, ripeness) (De la Torre, López, & Coleil, 1985; Fiorino & Nizzi, 1991; Gutiérrez, Jimenez, Ruíz, & Albi, 1999; Hajana, El-Antari, & Hafifi, 1998; Koutsaftakis, Kotsifaki, & Stefanoudaki, 1999; Koutsaftakis, Kotsifaki, Stefanoudaki, & Cert, 2000; Mariani, Fedeli, Grob, & Artho, 1991), technological (conservation of the fruit or of the oil, extraction systems) (Gracia, 2001; Gutierrez, Varona, & Albi, 2000; Koutsaftakis et al., 1999), and processing (refining, solvent extraction) (Christopoulou et al., 1996; De Blas & del Valle, 1996; Määtta et al., 1999; Pasqualone & Catalano, 2000; Piironen, Lindsay, Miettinen, Toivo, & Lampi, 2000; Reina et al., 1997).

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The diversity of these factors and their interrelationships make it very difficult to completely characterize the sterol profile and erythrodiol + uvaol content of a given product. The aim of the present work was therefore, first to determine the differences in composition of the main single-variety virgin olive oils produced in Extremadura (Spain), and then to make an initial study of the use of this information as a tool to characterize and differentiate the varieties of olive involved.

2. Materials and methods

2.1. Oil samples

The samples used came from fruit of seven varieties – Carrasqueña, Cacereña, Cornezuelo, Corniche, Morisca, Picual, and Verdial de Badajoz – at three ripening stages green, spotted, and ripe- of the 2000–2001 harvest. For each variety, the samples were collected from three different plots located in the corresponding characteristic production zone of that variety. In 5 kg samples of each variety, ripeness index was determined (Hermoso et al., 1991), and the oil was immediately extracted by means of the Abencor extraction system (Martinez Suarez, Muños Aranda, Alba, & Lanzaón, 1975). The samples were then kept in opaque containers, under cold storage, until assay. Analyses were performed in duplicate.

2.2. Analytical method

The qualitative and quantitative sterol contents of the samples were determined according to the European Official Methods Analysis described in Annexes V and VI of Regulation EEC/2568/91 of the European Union Commission. The oil sample was saponified with ethanolic potassium hydroxide solution. The unsaponifiable fraction was removed with ethyl ether. The unsaponifiable sterol fraction was separated by Silicagel plate chromatography. Separation and quantification of the silanized sterol fraction was carried out by capillary column gas chromatography, on a Hewlett Packard 6890 chromatograph equipped with a 30 m TRB-5 column of 0.32 mm internal diameter and 0.25 μm film thickness. The working conditions of the chromatograph were: injector 300 °C, isothermal column 260 °C, and detector 325 °C. The injected quantity was 0.2 μl at a flow rate 1.1 ml/min, using helium as carrier gas. Analyses were performed in duplicate. Fig. 1 shows an illustrative chromatogram obtained for one of the samples. Sterols peak identification was carried out according to the reference method. Quantification was achieved by addition of an internal standard (*o*-cholestanol).

2.3. Evaluation of the data and statistical analysis

With the data obtained for the different sterols in the total of samples (63), the fulfillment of the limits established for the different types of olive oil (EC Regulation 2568, 1991) were checked as well as any, relationship between the sterols that might contribute to the quality of the oil. Also undertaken was an analysis of variance using the statistical package SPSS Base 10, with variety and ripeness as the sources of variation, in order to detect any possible differences with respect to these factors. Finally, we performed a discriminant analysis using the same statistical package, variety being the grouping variable. Furthermore, on remaining samples at random, a validation analysis of this discriminant functions was performed.

3. Results and discussion

3.1. Study of sterols and erythrodiol + uvaol

Table 1 lists the sterol levels obtained for the different oils. One observes that in general they lie within the established regulatory limits (EC Regulation 2568, 1991). The exception was the variety Corniche whose

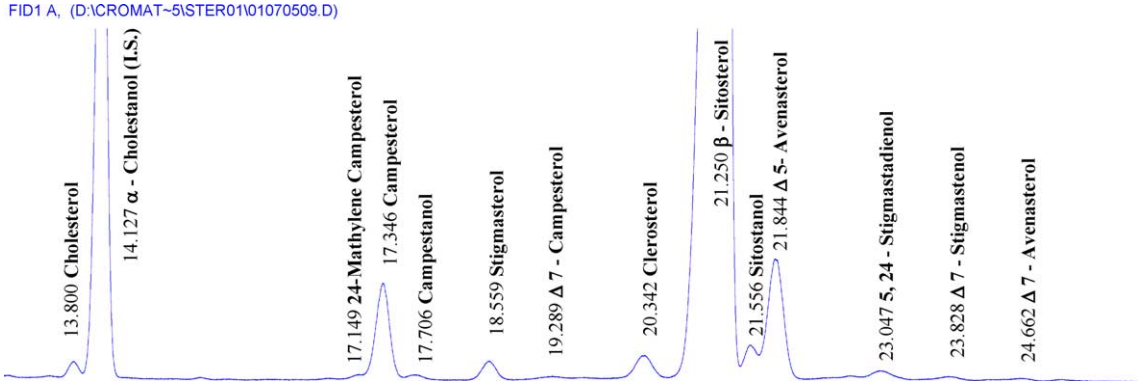

Fig. 1. Chromatogram of sterols on a oil olive sample.

Table 1
Mean values of the contents of sterols and erytrodiol + uvaol in the olive varieties and ripeness index studied (mg kg^{-1})

Variety	N	Cholesterol	24-Methylene cholesterol	Campesterol	Campestanol	Stigmasterol	Δ-7-Campesterol	Clerosterol	β-Sitosterol	Sitostanol	Δ-5-Avenasterol	5,24-Stigmastadienol	Δ-7-Stigmastenol	Δ-7-Avenasterol	Total sterols	Erythrodiol + uvaol
Carrasqueña	9	0.39 ab	0.13 a	2.58 ab	0.23 a	1.05 c	0.11 abc	1.11 ab	86.8 b	1.01 b	5.67 ab	0.67 ab	0.13 a	0.15 a	1426 bc	2.61 bc
Cacereña	9	0.43 ab	0.19 ab	2.63 b	0.15 a	0.80 abc	0.03 a	1.01 a	81.0 a	0.47 a	11.94 c	0.55 a	0.26 bc	0.67 d	1109 a	1.54 a
Cornezuelo	9	0.51 b	0.31 c	2.64 b	0.18 a	0.68 ab	0.22 c	1.26 b	79.4 a	0.82 ab	12.48 c	0.70 ab	0.32 c	0.47 bcd	1571 cd	2.42 b
Corniche	9	0.50 b	0.16 a	4.01 d	0.17 a	0.67 ab	0.16 bc	1.12 ab	87.3 b	0.71 ab	4.22 a	0.55 a	0.20 ab	0.23 ab	2030 e	3.28 c
Morisca	9	0.35 a	0.27 bc	2.31 a	0.24 a	0.69 ab	0.13 abc	1.00 a	80.5 a	0.85 ab	12.33 c	0.74 b	0.21 ab	0.38 abcd	1658 d	2.41 b
Picual	9	0.39 ab	0.20 ab	3.42 c	0.16 a	0.55 a	0.04 ab	0.95 a	86.4 b	0.82 ab	6.11 ab	0.55 a	0.19 ab	0.33 abc	1263 ab	1.30 a
VerdialBadajoz	9	0.51 b	0.20 ab	3.18 c	0.18 a	0.95 bc	0.02 a	0.97 a	84.9 b	0.98 b	7.43 b	0.66 ab	0.19 ab	0.48 cd	1423 bc	3.03 bc
Index ripeness																
Green	21	0.43 a	0.16 a	2.84 a	0.17 a	0.66 a	0.10 a	1.09 a	85.4 b	0.90 a	7.14 a	0.63 a	0.20 a	0.37 a	1564 b	2.12 a
Spotted	21	0.43 a	0.21 b	2.96 ab	0.20 a	0.84 b	0.08 a	1.08 a	83.6 a	0.78 a	8.89 b	0.64 a	0.21 a	0.35 a	1495 ab	2.50 a
Ripe	21	0.46 a	0.26 c	3.11 b	0.19 a	0.81 ab	0.12 a	1.01 a	82.2 a	0.76 a	9.76 b	0.63 a	0.23 a	0.42 a	1432 a	2.48 a

Means in a column with different letters are significantly different ($p < 0.05$).

campesterol content was close to these limits, and slightly over in some cases. With respect to brassicasterol, it was possible to quantify this sterol because it presented areas of very low, trace level, peaks in the chromatogram, together with the appearance of impurities with the same retention times that impeded good reproducibility in the results (Moreda, Pérez Camino, & Cert, 1995). Nonetheless, it has been thoroughly proven (Gutiérrez et al., 1999; Koutsaftakis et al., 2000) that brassicasterol has a negligible presence in these virgin oils.

Table 1 also shows the multivariate analysis with the factors of variation being the different varieties and the three ripening stages green, spotted, and ripe.

In general, there were significant differences between factors according to the specific sterol under consideration. Particularly notable was the case of 24-methylenecholesterol. This is an immediate metabolite in the synthesis of campesterol and is characteristic of the oil in the pulp of the olive, but is not found in the oil of the stones (Christopoulou et al., 1996). It was the only sterol to present clearly significant differences between the three stages of ripening as well as between some varieties.

Campesterol is present at higher levels in the varieties Corniche, Picual, and Verdial de Badajoz than in the varieties Carrasquesimña, Cacereña, Cornezuelo, and Morisca. This sterol could have major differentiating power as it is insensitive to variations in such factors as hydric stress (Stefanoudaki et al., 2001), geographical location (Christopoulou et al., 1996; Duarte & Martins, 1976; Gracia, 2001), and conservation (Gutierrez et al., 2000; Harwood & Aparicio, 2000).

Stigmasterol is related to various parameters of the quality of virgin olive oil. High levels correlate with high acidity and low organoleptic quality (Gracia, 2001; Gutiérrez et al., 1999). The samples presented low levels of this sterol, which is indicative that the oil came from healthy fruit not obtained by systems of forcing (Koutsaftakis et al., 1999). There were differences between varieties but not between ripening stages.

The highest sterol levels found corresponded to sitosterol, followed by Δ-5-avenasterol, like 24-methylenecholesterol characteristic of the oil in the pulp of the olive (Christopoulou et al., 1996). These two majority sterols were strongly negatively correlated, and there was a clear differentiation into two groups of varieties on the one hand Cacereña, Morisca, and Cornezuelo, and on the other Carrasqueña, Corniche, Picual, and Verdial de Badajoz. With respect to ripening stage, there were only differences between the green and the other two stages (spotted and ripe).

In all cases, the total sterol content was above the established minimum limit. There were, however, major differences between varieties, from the lowest level in Cacereña (1108 mg/kg) to the highest in Corniche (2080 mg/

Table 2
Standardized canonical discriminant function coefficients

	Function					
	1	2	3	4	5	6
Cholesterol	0.241	0.396	0.552	0.155	−0.286	0.556
24-Metilencholesterol	0.608	0.118	−0.658	0.335	0.561	0.077
Campesterol	0.015	−1.254	0.391	0.204	0.238	−0.043
Campestanol	0.728	0.147	−0.363	−1.447	−1.226	−0.261
Stigmasterol	−0.412	−0.059	−0.338	0.408	−0.249	0.272
Δ-7-Campesterol	0.548	0.092	−0.419	−1.088	0.161	0.067
Clerosterol	0.059	0.308	0.45	1.066	−0.433	0.25
Sitostanol	−0.222	−0.166	−0.501	0.284	0.997	0.075
Δ-5-Avenasterol	−0.193	0.219	0.884	−0.054	0.032	−0.308
5,24-Stigmastadienol	0.174	−0.148	−0.17	1.209	0.362	0.116
Δ-7-Stigmastenol	0.642	0.857	0.077	−0.536	0.391	0.573
Δ-7-Avenasterol	−0.329	−0.029	0.494	0.439	−0.248	−0.56
Total sterols	1.54	0.133	−0.082	−0.372	−0.17	−0.134
Erythrodiol + uvaol	0.106	0.122	0.14	1.087	0.1	−0.356

Table 3
Eigenvalues

Function	Eigenvalue	Variance (%)	Accumulated (%)	Canonical correlation
1	13.935[a]	41.4	41.4	0.966
2	12.003[a]	35.6	77	0.961
3	30.177[a]	9.4	86.5	0.872
4	3.142[a]	9.3	95.8	0.871
5	0.930[a]	2.8	98.6	0.694
6	0.487[a]	1.4	100	0.572

[a] The first 6 canonical discriminant functions were used in the analysis.

kg). With respect to ripening stage, there was a tendency for the levels to decline with increasing ripeness index. This has been noted by other workers (Gutiérrez et al., 1999; Mariani et al., 1991), and is understood as being due to the synthesis of the sterols occurring in the first stages of development of the fruit: with ripening, the sterols become diluted as more oils are produced.

With respect to the levels of erythrodiol + uvaol, there were clear differences between the high values of the varieties Carrasqueña, Corniche, and Verdial de Badajoz (2.6–3.3%), the intermediate values of Cornezuelo and Morisca (2.42%), and the low values of Cacereña and Picual (1.3–1.5%). There were no significant differences in this parameter by ripening stage, despite the apparent slight increase from green to spotted.

Not included in the results of the analysis of variance in Table 1 is a calculated parameter the 'apparent sitosterol' expressed by the sum of the content of sitosterol and another five chromatographically adjacent phytosterols. It is important to emphasize that the levels of this parameter were above 93% in all the oils analysed. This is the regulatory minimum limit, indicative that the sum of the remaining sterols does not surpass 7%, thereby confirming the authenticity of the corresponding oil.

3.2. Discriminant analysis

With the aim of establishing a model to classify the 63 samples as single-variety oils, we first selected the data of 42 of the samples at random and applied a discriminant analysis using 'variety' as the grouping variable and the different sterols, total sterols, and erythrodiol + uvaol as independent variables. The data of the remaining 21 samples were used to validate the model.

Initially, in order to avoid including redundant information in the model, we searched for possible correlations between the independent variables. We found significant correlations between the sterols Δ-5-avenasterol and sitosterol ($r = 0.970$) and between sitosterol and 24-methylenecholesterol ($r = 0.758$). We therefore decided not to consider sitosterol as an independent variable in the discriminant analysis.

Table 2 lists the standardized coefficients of the six canonical discriminant functions obtained, which include cholesterol, 24-methylenecholesterol, campesterol, campestanol, stigmasterol, Δ-7-campesterol, clerosterol, sitostanol, Δ-5-avenasterol, 5,24-stigmastadienol, Δ-7-stigmastenol, Δ-7-avenasterol, total sterols, and erythrodiol + uvaol. One notes the importance of the content in total sterols, campestanol, and Δ-7-stigmastenol, especially in the first function, as well as the weight of campesterol and Δ-7-stigmastenol in the second. These first two functions explain 77.0% of the variance, as is indicated by the eigenvalues given in Table 3.

The results of the classification of the 42 samples with these functions presented a 100% success rate for all the varieties (Table 4).

In order to validate the model, these same functions were used to classify the 21 remaining samples, representing each of the varieties and each stage of ripening. The data again were the sterol profile, total sterols, and

Table 4
The classification obtained and the validation of the discriminant analysis

		Variety	Forecast membership group							Total
			Carrasqueña	Cacereña	Cornezuelo	Corniche	Morisca	Picual	Verdial de Badajoz	
Selected cases[a]	Count	Carrasqueña	6	0	0	0	0	0	0	6
		Cacereña	0	6	0	0	0	0	0	6
		Cornezuelo	0	0	6	0	0	0	0	6
		Corniche	0	0	0	6	0	0	0	6
		Morisca	0	0	0	0	6	0	0	6
		Picual	0	0	0	0	0	6	0	6
		Verdial de Badajoz	0	0	0	0	0	0	6	6
	%	Carrasqueña	100	0	0	0	0	0	0	100
		Cacereña	0	100	0	0	0	0	0	100
		Cornezuelo	0	0	100	0	0	0	0	100
		Corniche	0	0	0	100	0	0	0	100
		Morisca	0	0	0	0	100	0	0	100
		Picual	0	0	0	0	0	100	0	100
		Verdial de Badajoz	0	0	0	0	0	0	100	100
Unselected cases[b]	Count	Carrasqueña	3	0	0	0	0	0	0	3
		Cacereña	0	3	0	0	0	0	0	3
		Cornezuelo	0	0	3	0	0	0	0	3
		Corniche	0	0	0	3	0	0	0	3
		Morisca	0	0	0	0	2	0	1	3
		Picual	0	0	0	0	0	3	0	3
		Verdial de Badajoz	0	0	0	0	1	0	2	3
	%	Carrasqueña	100	0	0	0	0	0	0	100
		Cacereña	0	100	0	0	0	0	0	100
		Cornezuelo	0	0	100	0	0	0	0	100
		Corniche	0	0	0	100	0	0	0	100
		Morisca	0	0	0	0	66.7	0	33.3	100
		Picual	0	0	0	0	0	100	0	100
		Verdial de Badajoz	0	0	0	0	33.3	0	66.7	100

[a] 100% correct classification of the selected original grouped cases.
[b] 90.5% correct classification of the unselected original grouped cases.

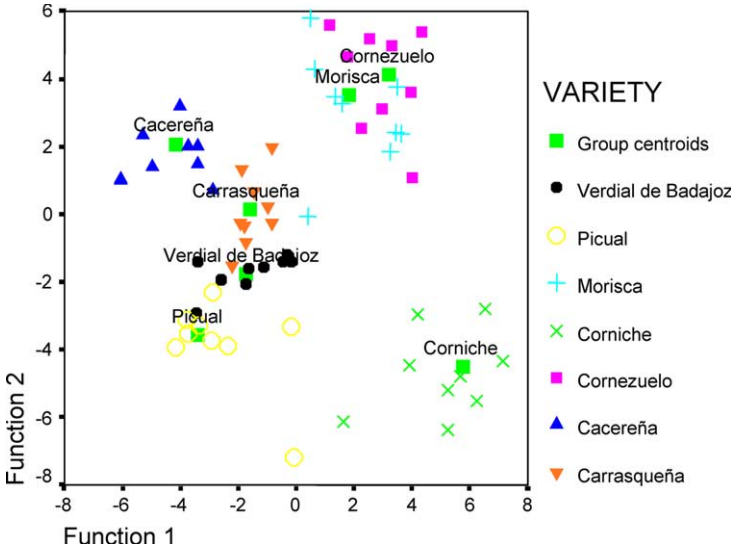

Fig. 2. Canonical discriminant functions.

erythrodiol + uvaol. The classification was 100% correct for all the varieties except for Morisca and Verdial de Badajoz which gave 66.7% of the samples correctly classified. The mean classification obtained in this validation procedure represented greater than 90% of the samples correctly classified.

Fig. 2 shows the different groups of oils classified by the first two functions of the proposed model, visually confirming the aforementioned success rates.

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References

Albi, T., Lanzón, A., Cert, A., & Aparicio, R. (1970). Valores de eritrodiol en muestras de aceites de oliva virgenes andaluces. *Grasas y Aceites, 2*, 167–170.

Christopoulou, E., Lazaraki, M., Alexiou, F., Synouri, S., & Frangiscos, E. (1996). Influence of certain factors on the composition of olive–pomace oils. Part II sterol, triterpenic dialcohols and aliphatic alcohols. *La revista italiana delle sostanze grasse, LXXIII*(Maggio), 201–211.

De Blas, O. J., & del Valle, A. (1996). Determination of sterols by capillary column gas chromatography. Differentiation among different types of olive oil: Virgin, refined and solvent-extracted. *JAOCS, 73*(12), 1685–1689.

De la Torre, M. C., López, M. C., & Coleil, J. (1985). Evolución de la fracción esterólica durante la maduración de las aceitunas. *Grasas y Aceites, 36*(3), 198–202.

Duarte, H. M. P., & Martins, R. M. B. S. (1976). Insaponifiable des corps gras. Fraction stérolique des huiles d'olive vierges portugaises. *Grasas y Aceites, 27*(2), 101–106.

EC Regulation 2568. (1991). On the characteristics of olive oil and olive residue oil and on the relevant methods of analysis. *Official Journal L*248, pp. 6–47.

El Antari, A., Hital, A., Boulouha, B., & El Moudni, A. (2000). Estudio de la influencia de la variedad, los factores ambientales y la técnicas de cultivo en las características de los frutos y la composición química del aceite de oliva virgen extra de Marruecos. *Olivae, 80*, 29–36.

Fiorino, P., & Nizzi, F. (1991). Maduración de las aceitunas y variaciones de algunos componentes del aceite. *Olivae, 35*, 25–33.

Gracia, M. S. (2001). Composición química de distintas calidades de aceites de oliva virgen de la variedad "Empetre" en el bajo Aragón. *Grasas y Aceites, 52*(1), 52–58.

Gutiérrez, F., Jimenez, B., Ruíz, A., & Albi, M. A. (1999). Effect of olive ripeness on the oxidative stability of virgin olive oil extracted from the varieties Picual and Hojiblanca and on the different components involved. *Journal of Agricultural and Food Chemistry, 47*, 121–127.

Gutierrez, F., Varona, I., & Albi, M. A. (2000). Relation of acidity and sensory quality with sterol content of olive oil from stored fruit. *Journal of Agricultural and Food Chemistry, 48*, 1106–1110.

Hermoso, M., Uceda, M., Garcia-Ortiz, A., Morales, B., Frias, L., Fernandez, A. (1991). Elaboracio n de aceite de oliva de calidad. *Junta de Andalucia. Consejería de Agricultura y Pesca*. ISBN: 84-87564-54-2. p. 173.

Hajana, H., El-Antari, & Hafifi, A. (1998). Fatty acids sterol evolution during the ripening of olives from the Moroccan Picholine cultivar. *Grasa y Aceites, 49*(5-6), 405–410.

Harwood, J., Aparicio, R. (2000). *Handbook of olive oil. Analysis and properties*. Aspen Publishers, Inc. ISBN 0-8342-1633–7.

Koutsaftakis, A., Kotsifaki, F., & Stefanoudaki, E. (1999). Effect of extraction system, stage of ripeness, and kneading temperature on the sterol composition of virgin olive oils. *JAOCS, 76*(12), 1477–1481.

Koutsaftakis, A., Kotsifaki, F., Stefanoudaki, E., & Cert, A. (2000). Estudio sobre las variaciones de determinados parámetros químicos y de los componentes menores de los aceites de oliva virgen obtenidos de aceitunas recolectadas en distintas fases de maduración. *Olivae, 80*(Febrero), 22–27.

Määtta, K., Lampi, A. M., Petterson, J., Fogelfors, B. M., Pironen, V., & Kamal-Eldin, A. (1999). Phytosterol content in seven oat cultivars grown at three locations in Sweden. *Journal of Science of the Food and Agriculture, 79*, 1021–1027.

Mariani, C., Fedeli, E., Grob, K., & Artho, A. (1991). Indagine sulle variazioni dei componenti minori liberi ed esterificati di oli ottenuti da olive in funzione della maturazione e dello stoccaggio. *La revista italiana delle sostanze grasse, LXVIII*(Aprile), 179–187.

Martinez Suarez, J. M., Muños Aranda, E., Alba, J., & Lanzaón, A. (1975). Informe sobre la utilización del analizador de rendimientos "Abencor". *Grasas y Aceites, 26*, 379–385.

Moreda, W., Pérez Camino, M. E., & Cert, A. (1995). Determinación de algunos parámetros de pureza en aceites de oliva. Resultados de un estudio colaborativo. *Grasas y Aceites, 46*(fasc. 4-5), 279–284.

Pasqualone, A., & Catalano, M. (2000). Free and total sterols in olive oils. Effects of neutralization. *Grasas y Aceites, 51*(3), 180–182.

Piironen, V., Lindsay, D., Miettinen, T., Toivo, J., & Lampi, A. M. (2000). Plant sterols: Biosynthesis, biological function and their importance to human nutrition. *Journal of Science of the Food and Agriculture, 80*, 939–966.

Reina, R. J., White, K. D., & Jahngen, E. G. E. (1997). Validated method for quantitation and identification of 4,4-desmethylsterols and triterpene diols in plant oils by thin-layer-chromatography-high resolution gas chromatography-mass spectrometry. *Journal of AOAC International, 80*(6), 1272–1280.

Stefanoudaki, E., Chartzoulakis, K., Koutsaftakis, A., & Kotsifaki, F. (2001). Effect of drought on qualitative characteristics of olive oil of cv Koroneiki. *Grasas y Aceites, 52*(3-4), 202–206.