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Investigation and Comparison of cv. Koroneiki and cv. Mastoides Extra Virgin Olive Oils Cultivated in the Southern Region of Peloponnese, according Their Sterolic and Fatty Acid Profile †

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Abstract: Greece is ranked third among olive oil-producing countries, after Spain and Italy, with the region of Peloponnese representing one of the most important olive oil producing regions. However, very little information is available regarding the profile of the two major olive cultivars (cv. Koroneiki and cv. Mastoides) cultivated in the southern part of Peloponnese. Analysis of variance of the 112 analyzed olive oil samples showed substantial compositional differences in the fatty acid and sterolic profile between Koroneiki and Mastoides cultivars demonstrating that those chemical parameters could be a potential indicator for olive oil discrimination in terms of their botanical origin. In addition, this work is the first systematic attempt focusing on the profile of Messinian (cv. Koroneiki) olive oils so as to evaluate to what extent they comply with the recent EU regulations in order to be classified as "Kalamata Protected Designation of Origin (PDO)"certified products. Detailed analysis of the examined Messinian samples revealed major fluctuations from the relevant EU regulatory limits. Results showed low concentrations of total sterols, with 66.7% of the examined samples being below the regulated set limits for Kalamata PDO status; high concentrations of campesterol, with a total of 21.7% exceeding the legal maximum limit of 4.0%; and a slight tendency of high total erythrodiol content.

Keywords: EVOO; cv. Koroneiki; Kalamata PDO; cv. Mastoides; south peloponnese; fatty acids; sterols; botanical origin

1. Introduction

Olive oil is a key element of the Mediterranean diet as well as an exceptional lipid source [1,2]. The consumption of extra virgin olive oil is steadily increasing due to its unique sensory, nutritive qualities, biological properties and health promoting effects [3].

Olive cultivation is greatly spread in central Greece, with almost 40% of olive oil production being centered in Peloponnese with the prefecture of Messinia being the major olive growing area [4]. In southern Peloponnese, among the predominant monovarietal olive oils produced are cv. Koroneiki and cv. Mastoides [5]. Although there are many research publications related to cv. Koroneiki in different areas in Greece, no systematic work has been carried out on olive oil analysis from the Messinia region. Meanwhile, in August 2015, the European Commission approved the extension of the "Kalamata PDO olive oil," from the former province of Kalamata to the rest Regional Unit of Messinia, considerably enlarging the area covered by the PDO. On this basis, the new

"Kalamata PDO olive oil" introduces more stringent criteria than those laid down in the EC Regulation 2568/91 for extra virgin oil [6,7].

The first aim of this study was to profile the qualitative and chemical parameters of extra virgin olive oils obtained from the Messinia region as well as to report and evaluate to what extent olive oils of cv. Koroneiki meet the requirements of the amended EU regulation in order to be classified as "Kalamata Protected Designation of Origin (PDO)" certified olive oils. The second aim of this study was to evaluate and compare the chemical characteristics of Koroneiki and Mastoides olive oils originated from the southern region of Peloponnese emphasizing on the potential of their discrimination in terms of their botanical origin.

2. Materials and Methods

2.1. Geographical Distribution and Sampling

A total of one hundred and twelve (n = 112) extra virgin olive oil samples were collected during the harvesting period 2014–2015 from two neighborhood regions in the southern region of Peloponnese in Greece. In particular, sixty nine (69) olive oil samples of Koroneiki cultivar originated from the region of Messinia and forty three (43) olive oil samples of Mastoides cultivar from the southeast part of Lakonia. All regions are characterized by similar climatic conditions. Olive samples were transferred to local oil mills for olive oil extraction under the same post-harvest conditions. The obtained olive oil samples were stored at 4 °C until further analysis. All the examined chemical parameters were determined in triplicate.

2.2. Determination of the Qualitative and Chemical Parameters

Free fatty acid, peroxide value and spectroscopic indices (K232 and K268) were carried out, following the analytical methods described in the Regulation EEC/2568/91 of the European Commission and later amendments. Individual sterols, total sterols and triterpene dialcohols were determined according to the method adopted by EEC/2568/91 regulation, Annexes V and Annex VI respectively. In accordance, fatty acid composition was determined according to the official method of the Regulation EEC/2568/91, Annex IV

2.3. Statistical Analysis

Results were expressed as mean values ± standard deviation (SD). Data were evaluated using MINITAB 18 software. Differences between means were tested for statistical significance using analysis of variance (ANOVA).

3. Results and Discussion

3.1. Physico-Chemical Parameter of the Two Major Olive Cultivars (cv. Koroneiki and cv. Mastoides) of Southern Peloponnese

Free fatty acid, peroxide value and spectrophotometric absorption were examined in the studied olive oils. As shown in Table 1. it is clear that all analysed samples obtained from the two examined cultivars in the southern region of Peloponnese are classified in the highest quality category as "Extra Virgin Olive Oil (EVOO)" as they fullfil the demands of the current EU Regulation 2568/91[8], depicting the overall high quality of south Peloponesse olive oil production.

Table 1. Qualitative parameters from the two major olive cultivars of southern Peloponnese.

	cv. Koroneiki (*N* = 69)		cv. Mastoides (*N* = 43)		EEC Limit for EVOO Category
Parameter	**Mean ±SD**	**Min–Max**	**Mean ±SD**	**Min–Max**	
Free acidity (%)	0.34 ± 0.13	0.17–0.76	0.39 ± 0.13	0.15–0.77	≤0.80
Peroxide value (meqO_2/kg)	7.24 ± 1.88	3.64–11.96	6.96 ± 2.31	2.88–14.70	≤20
K_{232}	1.55 ± 0.14	1.33–2.14	1.63 ± 0.11	1.33–2.02	≤2.50
K_{268}	0.13 ± 0.01	0.08–0.21	0.12 ± 0.02	0.08–0.17	≤0.22

Results are expressed as means ± standard deviation (SD). *N* = 112 [9].

3.2. Sterolic Profile of the Messinian Olive Oils (cv. Koroneiki) in Southern Peloponesse

Phytosterols are important components of the unsaponifiable fraction of olive oil beneficial for the human health and nutrition. Numerous studies have shown that each variety has a characteristic sterol "fingerprint" [10]. Therefore, those minor components can be considered as an important and useful tool for detecting oil adulteration and/or classifying virgin olive oils in accordance with their variety [11,12].

Although several studies have been conducted for cv Koroneiki in other regions of Greece, mainly in Crete [13–15], very little information is available in the literature regarding the sterolic profile of cv Koroneiki in Peloponnese generally and more precisely in the Messinia region.

In the present study, we evaluated the sterolic composition of the examined Messinian olive oils. As shown in Table 2, the main sterols detected were β–sitosterol, Δ5-avenasterol and campesterol, with mean values of 80.73%, 12.28% and 3.71%, respectively. Apparent β-sitosterol, falls within the established limits, with a mean value of 94.63%. Finally cholesterol and Δ7-stigmastenol values were low and quite below the limits set by EU regulation (0.5%), with a mean value of 0.11% and 0.19% of total sterols, respectively (Table 2).

Table 2. Sterolic profile and triterpene diols determined in Messinian (cv. Koroneiki) olive oil, Greece.

Sterols and Triterpene Diols	**Mean ± SD**	**EEC Limit**	**PDO Limit**
Cholesterol (%)	0.11 ± 0.03	≤0.5	≤0.5
24-methylene-cholesterol%	0.32 ± 0.09		
Campesterol%	3.71 ± 0.38	≤4.0	≤4.0
Campestanol%	0.05 ± 0.03	<campesterol	<campesterol
Stigmasterol%	0.74 ± 0.19		
Chlerosterol%	0.85 ± 0.07		
β-Sitosterol%	80.73 ± 3.73		
Sitostanol%	0.37 ± 0.30		
Δ-5-avenasterol%	12.28 ± 3.96		
Δ-5,24-stigm/dienol%	0.29± 0.10		
Δ-7-stigmastenol%	0.19 ± 0.09	≤0.5	≤0.5
Δ-7-avenasterol%	0.28 ± 0.11		
Apparent b-Sitosterol%	94.63 ± 1.07	≥93.0	≥93.0
Total erythrodiol%	2.85 ± 1.25	≤4.5	≤4.5
Total sterols (mg/kg)	1033.3 ± 150.1	≥1000	>1100

Results are expressed as the means ± standard deviation (SD). *N* = 69 [16].

In contrast, several major deviations were observed in the sterolic profile of the Messinian olive oils (cv. Koroneiki). Most importantly, as illustrated in Figure 1, 43.5% of the examined olive oil samples did not surpass the required limit of 1000 mg/kg in total sterols according to the EEC Reg. 2568/91. In addition, a really high percentage (66.7%) of the examined samples was below the established PDO limit of 1100 mg/kg (Figure 1). A similar case was observed in campesterol, where a total of 21.7% of the examined samples exceeded the legal maximum of 4%, with a mean value of 3.71% and ranged from 2.78 to 4.70%. Finally, as far as total erythrodiol content is concerned, the mean value was 2.85% (Table 2). However, a small but noteworthy percentage of 8.06% of the

examined samples exceeded the upper set limit of 4.5% as shown in Figure 1. In that case, a possible assumption may be the inappropriate higher degree of olive crushing during the extraction process, leading to an increase in erythrodiol levels from the olive's exocarp.

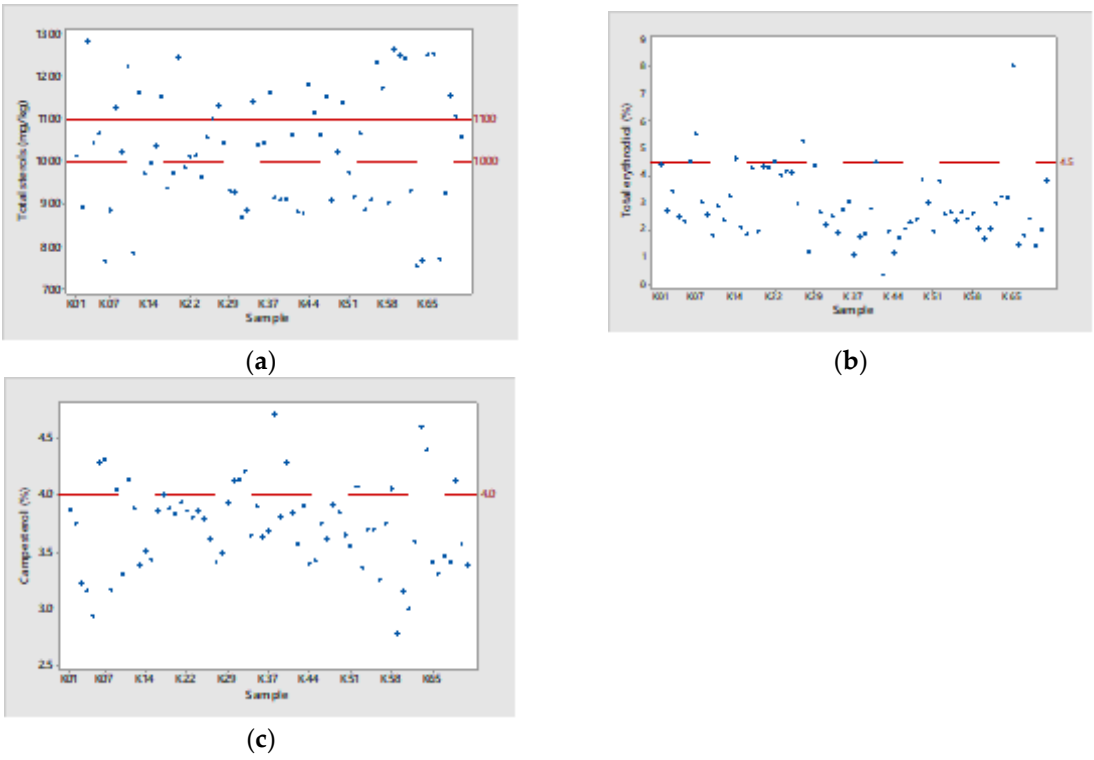

Figure 1. (**a**) Scatter plots visualizing the chemical parameters of Messinian (cv. Koroneiki) olive oils samples: (**a**) total sterols (**b**) campesterol (**c**) total erythrodiol Note. Dotted line: limits according to EEC/2568/91 for the EVOO category; straight line: limits according to Council Regulation (EC) 510/2006 for Kalamata PDO olive oil. [16].

Although no information exists in the literature regarding Kalamata PDO olive oils, results show that cv Koroneiki in the Messinian region shows a clear tendency of low concentrations of total sterols and high concentrations of campesterol. Low mean values on total sterol concentration for cv Koroneiki were reported earlier in Crete, in 2001, by Stefanoudaki et al., who studied the effect of drought stress on olive oil characteristics, without giving emphasis on the mentioned tendency [14]. In general, such problems (fluctuations from EU regulations) could inevitably raise questions regarding the authenticity of Kalamata PDO extra virgin olive oils in the olive oil sector and so they certainly require further investigation.

3.3. Evaluation and Discrimination of the Examined cv. Koroneiki and cv. Mastoides Olive Oils From the Southern Region of Peloponnese according to Their Fatty Acid and Sterolic Profile

Many researchers have used fatty acid composition and sterol composition in order to group olive oils according to the origin of the cultivar [17–20]. In the present study, the GC-FID analysis of the 112 olive oil samples from Koroneiki and Mastoides cultivars showed their complete fatty acid composition. As shown in Table 2, olive oils of Koroneiki cultivar had a mean value of 76.70% for C18:1 compared to olive oils of Mastoides cultivar which had a mean value of 75.93% ($p < 0.05$). Moreover, olive oils of Koroneiki presented a higher concentration with respect to C18:3 with a mean value of 0.68% compared to cv. Mastoides (0.55%). On the other hand, olive oils of cv. Mastoides were characterized by a clearly higher concentration in C17:0 with a mean value at 0.14% and in C17:1 with a mean value at 0.25% compared to the olive oils of cv. Koroneiki which had almost a three-fold lower concentration, with mean values 0.05% and 0.08%, respectively. Analysis of variance on the fatty acid composition data of the 112 olive oil samples revealed that, apart from C14:0, C16:0, C16:1 and C18:2,

substantial differences were observed between Koroneiki and Mastoides cultivars in all the rest analyzed fatty acids ($p < 0.05$) (Table 2).

Table 2. Fatty acid profile of cv. Koroneiki and cv. Mastoides cultivated in southern Peloponnese.

	cv. Koroneiki (*N* = 69)		cv. Mastoidis (*N* = 58)		Calculated	EEC Limit for EVOO Category
Parameter	**Mean ± SD**	**Min–Max**	**Mean ± SD**	**Min–Max**	***p*-value**	
Myristic C14:0 (%)	0.01 ± 0.00	0.00–0.02	0,01 ± 0.00	0.00–0.02	n.s	≤0,03
Palmitic C16:0 (%)	12.02 ± 0.74	9.54–13.56	12.29 ± 0.77	9.96–13.28	n.s	7.50–20.00
Palmitoleic C16:1 (%)	0.92 ± 0.13	0.64–1.43	0,92 ± 0.10	0.64–1.08	n.s	0.30–3.50
Heptadecanoic C17:0 (%)	0.05 ± 0.02	0.03–0.15	0.14 ± 0.02	0.08–0.17	0	≤0.40
Heptadecenoic C17:1 (%)	0.08 ± 0.04	0.06–0.24	0.25 ± 0.03	0.16–0.29	0	≤0.60
Stearic C18:0 (%)	2.53 ± 0.19	1.98–3.12	2.64 ± 0.16	2.35–2.99	0.001	0.50–5.00
Oleic C18:1 (%)	76.70 ± 1.96	70.67–81.40	75.93 ± 1.27	73.15–79.44	0.024	55.00-83.00
Linoleic C18:2 (%)	6.09 ± 1.60	4.20–12.01	6.44 ± 0.69	5.11–8.13	n.s	2.50–21.00
Linolenic C18:3 (%)	0.68 ± 0.07	0.51–0.86	0.55 ± 0.04	0.49–0.66	0	≤1.00
Arachidic C20:0 (%)	0.44 ± 0.03	0.33–0.50	0.39 ± 0.02	0.35–0.44	0	≤0.60
Eicosenoic C20:1 (%)	0.31 ± 0.02	0.27–0.35	0.27 ± 0.02	0.23–0.32	0	≤0.50
Behenic C22:0 (%)	0.14 ± 0.01	0.09–0.17	0.10 ± 0.01	0.07–0.12	0	≤0.20
Lignoceric C24:0 (%)	0.05 ± 0.00	0.03–0.08	0.04 ± 0.007	0.03–0.06	0	≤0,20

Results are expressed as means ± standard deviation (SD). n.s = not-significant. The statistical significance level was set at $p < 0.05$ [9].

As far as the sterolic profile of the examined mono-cultivars, Table 3 lists the mean values expressed as percentage of the individual sterols and total sterols concentration. In general, Mastoides oils exhibited higher mean value for β-sitosterol (84.12%) and lower mean value for Δ-5-avenasterol (9.85%) and total erythodiol content (1.40%) compared to the relative values for Koroneiki olive oils (Table 3). In addition, higher concentration in the mean total sterols was observed in Mastoides olive oils (1219.6 mg/kg) compared to the olive oils of Koroneiki cultivar. Finally, analysis of variance on the sterolic profile of the 112 olive oil samples revealed that substantial differences were observed between Koroneiki and Mastoides cultivars ($p < 0.05$) (Table 3). The calculated *p*-value was in most cases close to 0.00 ($p \approx 0.00$), indicating a strong botanical effect.

Table 3. Sterol profile of cv. Koroneiki and cv. Mastoides cultivated in southern Peloponnese.

	cv. Koroneiki (*N* = 69)	cv. Mastoidis (*N* = 43)	Calculating *p*-value	EEC Limit for EVOO Category
Sterols and Triterpene Diols	**Mean ± SD**	**Mean ± SD**		
Cholesterol (%)	0.11 ± 0.03	0.12 ± 0.03	0.017	≤0.5
24-methylene-cholesterol %	0.32 ± 0.09	0.19 ± 0.05	0.00	
Campesterol %	3.71 ± 0.38	3.14 ± 0.16	0.00	≤4.0
Campestanol %	0.05 ± 0.03	0.04 ± 0.02	n.s	<campesterol
Stigmasterol %	0.74 ± 0.19	0.64 ± 0.18	0.01	
Chlerosterol %	0.85 ± 0.07	0.94 ± 0.07	0.00	
β-Sitosterol %	80.73 ± 3.73	84.12 ± 2.69	0.00	
Sitostanol %	0.37 ± 0.30	0.31 ± 0.08	n.s	
Δ-5-avenasterol %	12.28 ± 3.96	9.85 ± 2.66	0.001	
Δ-5, 24-stigm/dienol %	0.29 ± 0.10	0.22 ± 0.06	0.00	
Δ-7-stigmastenol %	0.19 ± 0.09	0.18 ± 0.09	n.s	≤0.5
Δ-7-avenasterol %	0.28 ± 0.11	0.22 ± 0.06	0.001	
Apparent b-Sitosterol %	94.63 ± 1.07	95.45 ± 0.29	0.00	≥93.0
Total Erythrodiol %	2.85 ± 1.25	1.40 ± 0.52	0.00	≤4.5
Total sterols (mg/kg)	1033.3 ± 150.1	1219.6 ± 109.2	0.00	≥1000

Results are expressed as means ± standard deviation (SD). n.s = not-significant. The statistical significance level was set at $p < 0.05$ [9].

4. Conclusions

Major fluctuations in the Messinian (cv. Koroneiki) olive oils were observed from the established EU regulatory limits (EEC 2568/91 & EC Reg. 510/2006 for Kalamata PDO olive oil). Messinian extra

virgin olive oils show a clear tendency of low concentration in total sterols depicting a "special characteristic" for Koroneiki cultivar. Fatty acid compositional data and sterols have a high differentiation potential and can be suggested as possible authenticity indicators.

Author Contributions: V.S. designed and performed the experiments; P.T. performed statistical analysis; S.A., V.S., T.V. and E.S. wrote, edited and reviewed the paper. Please turn to the CRediT taxonomy for the term explanation. All authors have read and agreed to the published version of the manuscript.

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