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Fatty Acid and Sterolic Profile as Possible Indicators for Origin Discrimination of Mono-Cultivar Extra Virgin Olive Oils, Cultivated in the Coastline Part of North-Western Greece †

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Abstract: The objective of this study was to evaluate and discriminate monovarietal extra virgin olive oils of the two dominant olive cultivars, Lianolia Kerkyras and Koroneiki, produced in the coastline part of north-western Greece, based on their chemical characteristics, followed by statistical analysis in order to profile for the first time the typical characteristics of Lianolia Kerkyras as well as to identify possible markers for authenticity purpose. A higher concentration in the mono-unsaturated oleic acid characterize olive oils of cv. Koroneiki compared to cv. Lianolia Kerkyras, while a clearly higher concentration in the poly-unsaturated linoleic acid was observed in olive oils of cv. Lianolia Kerkyras. As far as the profile of the individual sterols is concerned, Lianolia Kerkyras samples exhibited higher mean value for the total sterol content as well as for β-sitosterol, the major phytosterol in olive oils, compared to the relative values of Koroneiki. Significant differences in the sterolic and fatty acid composition of the examined olive oil samples were shown by means of statistical analysis demonstrating a strong botanical effect and depicting that those compositional markers can be suggested as possible authenticity tools.

Keywords: olive oil; cv. Lianolia Kerkyras; cv. Koroneiki; fatty acid methyl esters; sterols; authenticity; quality

1. Introduction

In an increasingly globalized world, extra virgin olive oil quality and authenticity is an important issue in order to assure consumer's protection, prevent unfair competition and disrupt the national economy by a false declaration of origin [1–3]. As a result, the authenticity efforts are focused on identifying their botanical origin as well as their adulteration with lower quality or less costly cultivars of lower commercial value [1]. Up to now, extended scientific attempts have been carried out on the examination of one or more constituents present in the olive oils

(major and minor components) able to provide useful information on olive cultivars and differentiate among their botanical origin [2,3]. The objective of this study was to evaluate and characterize monovarietal extra virgin olive oils of cv. Lianolia Kerkyras produced in the coastline part of Western Greece and compare them with olive oils of Koroneiki variety produced in the same area. Emphasis was given on the potential of their discrimination for authenticity purpose in terms of their botanical origin.

2. Materials and Methods

1. *Geographical distribution and sampling:* A total of one hundred and four (N = 104) virgin olive oil samples were collected during the harvesting period 2019–2020 from the coastline part of Western Greece. In particular, sixty (60) samples of cv Lianolia Kerkyras and forty four (44) olive oil samples of Koroneiki cultivar were originated from the following regional units: Preveza, Parga and Thesprotia. 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 duplicate.
2. *Determination of the quality and chemical parameters*: Free fatty acid, peroxide value and spectroscopic indices (K_{232} and K_{268}) were carried out, following the analytical methods described in the Regulation EEC/2568/91 of the European Commission and later amendments [4].The individual sterols, total sterols and triterpene dialcohols were determined according to the method adopted by EEC/2568/91 regulation, Annexes V. In accordance, fatty acid composition was determined according to the official method of the Regulation EEC/2568/91, Annex IV [4].
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. Quality Parameters of the Examined Olive Oils

Table 1 shows that all analyzed samples obtained from the two examined cultivars in the coastline part of Western Greece belong to the highest quality category of "extra virgin olive oil" since they satisfy the specifications set by EU Regulation 2568/91 [4]. More specifically, the mean free fatty acid was 0.24% and 0.27%, respectively for Koroneiki and Lianolia Kerkyras olive oils. Likewise, the mean peroxide value for cv. Koroneiki olive oils was 6.64 $meqO_2\ kg^{-1}$ whereas for cv. Lianolia Kerkyras the mean peroxide value was 5.21 $meqO_2\ kg^{-1}$. Similarly, both monovarietal olive oils had K_{232} and K_{268} mean values quite below the limit set by the EU Regulation 2568/91. The results depict that both cultivars had an overall high quality profile in that crop year as far as their basic qualitative parameters is concerned.

Table 1. Quality indices for the examined Koroneiki and Lianolia Kerkyras olive oils from the coastline region of Western Greece.

	cv. Koroneiki (N = 44)		**cv. Lianolia Kerkyras (N = 60)**		**EEC Limit for EVOO Category**
Parameter	**Mean ± SD**	**Min–Max**	**Mean ± SD**	**Min–Max**	
Free acidity (%)	0.24 ± 0.10	0.13–0.55	0.27 ± 0.12	0.12–0.75	≤0.80
Peroxide value ($meqO_2/kg$)	6.64 ± 1.26	3.81–9.66	5.21 ± 1.12	3.41–8.64	≤20
K_{232}	1.56 ± 0.14	1.39–2.04	1.61 ± 0.15	1.25–1.95	≤2.50
K_{268}	0.14 ± 0.01	0.11–0.19	0.14 ± 0.02	0.11–0.21	≤0.22

Values are expressed as means ± standard deviation (SD). N = 104 [5].

3.2. Fatty Acid Profile of the Two Monocultivar Olive Oils

Fatty acid profile plays an important role in the quality and characterization of an olive oil as its composition reflects the nutritional properties of an olive oil [6]. Several researchers have reported that among other major components, fatty acids composition seems to represent a possible tool for varietal characterization and authentication [7–12]. Table 2 shows the mean fatty acid composition of the analyzed monovarietal olive oils. As it is shown, all fatty acids identified were found in the normal range expected for the extra virgin olive oil category for both monocultivars. With respect to the mono-unsaturated oleic acid (C18:1), olive oils of cv. Koroneiki presented a higher concentration with a mean value of 75.07% compared to cv. Lianolia Kerkyras (69.55%). Moreover, the saturated stearic acid (C18:0) concentration was higher for cv. Koroneiki with a mean value of 2.51% compared to the concentration of 2.04% for cv. Lianolia Kerkyras. On the other hand, olive oils of cv. Lianolia Kerkyras presented a clearly higher concentration of the poly-unsaturated linoleic acid (C18:2) with a mean value of 10.40% compared to the Koroneiki olive oils (6.43%) as well as a higher concentration in palmitic acid (14.46%).

Table 2. Fatty acid profile of the examined monocultivar olive oils in the coastline region of Western Greece.

Parameter	cv. Koroneiki (N = 44)		cv. Lianolia Kerkyras (N = 60)		Calculated *P*-Value	EEC Limit for EVOO Category
	Mean ± SD	Min–Max	Mean ± SD	Min–Max		
Myristic C14:0 (%)	0.009 ± 0.002	0.006–0.018	0,008 ± 0.004	0.003–0.04	n.s	≤0.03
Palmitic C16:0 (%)	13.17 ± 1.01	11.16–17.59	14.76 ± 0.91	12.97–16.71	0.000	7.50–20.00
Palmitoleic C16:1 (%)	1.07 ± 0.17	0.83–1.69	1.47 ± 0.19	0.97–1.91	0.000	0.30–3.50
Heptadecanoic C17:0 (%)	0.04 ± 0.01	0.02–0.06	0.04 ± 0.01	0.02–0.07	n.s	≤0.40
Heptadecenoic C17:1 (%)	0.07 ± 0.01	0.05–0.12	0.08 ± 0.01	0.05–0.13	0.003	≤0.60
Stearic C18:0 (%)	2.51 ± 0.24	2.03–2.98	2.04 ± 0.15	1.78–2.64	0.000	0.50–5.00
Oleic C18:1 (%)	75.07 ± 1.71	69.76–77.96	69.55 ± 1.71	65.39–73.00	0.000	55.00-83.00
Linoleic C18:2 (%)	6.43 ± 1.27	4.21–9.55	10.40 ± 0.91	8.30–12.80	0.000	2.50–21.00
Linolenic C18:3 (%)	0.72 ± 0.07	0.63–0.88	0.79 ± 0.08	0.60–0.99	0.000	≤1.00
Arachidic C20:0 (%)	0.45 ± 0.03	0.34–0.53	0.40 ± 0.02	0.30–0.49	0.000	≤0.60
Eicosenoic C20:1 (%)	0.29 ± 0.04	0.23–0.37	0.28 ± 0.03	0.20–0.33	n.s	≤0.50
Behenic C22:0 (%)	0.13 ± 0.02	0.09–0.18	0.13 ± 0.02	0.09–0.18	n.s	≤0.20
Lignoceric C24:0 (%)	0.05 ± 0.02	0.01–0.10	0.05 ± 0.01	0.03–0.09	0.009	≤0.20

Values are expressed as means ± standard deviation (SD). n.s = not-significant. The $p < 0.05$ was set at the level of statistical significance [5].

Variability in fatty acid composition between the two monocultivar olive oil samples led to the performance of an analysis of variance (ANOVA) in order to assess their differences. Table 2 shows substantial statistical differences between Lianolia Kerkyras and Koroneiki samples in almost all the analyzed fatty acids ($p < 0.05$). The analysis of variance applied to the 13 GC analyzed variables allowed the variables with the highest discriminant power to be determined. The more discriminant variables are C18:2 (F = 343.19), C18:1 (F = 264.70), C18:0 (F = 149.88), C16:1 (F = 125.54), C16:0 (F = 71.21), C20:0 (F = 65.71), and C18:3 (F = 22.28).

Those results are in agreement with our previously published data as well as other relevant studies, demonstrating that fatty acid profile plays a crucial role in the classification of virgin olive oils according to their cultivar [7–12].

3.3. Sterolic Profile of the Two Monocultivar Olive Oils

Phytosterols and triterpenic dialcohols belong to the unsaponifiable fraction of olive oil and constitute one of its minor components with an important health beneficial impact [12–14]. Many researchers have shown that each variety has a characteristic sterol "fingerprint," revealing that the

sterolic profile can be used as a reliable indicator with a high discrimination potential for olive oil classification [15–20].Taking into account the unexplored chemical characteristics of Lianolia Kerkyras, we employed the present study to determine and compare the sterolic profile of the Koroneiki and Lianolia Kekryras olive oils obtained from the coastline region of north-western Greece. The percentage of individual sterols as well as the concentrations of total sterols for the examined monovarietal olive oil samples are presented in Table 3.

Table 3. Sterol profile of the extra virgin olive oils examined from cv. Koroneiki and cv. Lianolia Kerkyras, cultivated in the coastline region of western Greece. Results are expressed as means ± standard deviation (SD). n.s = not-significant. The statistical significance level was set at $p < 0.05$ [5].

.	**cv. Koroneiki (*N* = 44)**	**cv. Lianolia Kerkyras (*N* = 60)**	**Calculating *P*-Value**	**EEC Limit for EVOO Category**
Sterols and Triterpene Diols	**Mean ± SD**	**Mean ± SD**		
Cholesterol (%)	0.10 ± 0.08	0.12 ± 0.06	n.s	≤0.5
24-methylene-cholesterol %	0.23 ± 0.09	0.08 ± 0.04	0.00	
Campesterol %	3.82 ± 0.35	3.42 ± 0.17	0.00	≤4.0
Campestanol %	0.07 ± 0.03	0.04 ± 0.02	0.00	<campesterol
Stigmasterol %	0.63 ± 0.18	0.49 ± 0.15	0.00	
Chlerosterol %	0.81 ± 0.20	0.81 ± 0.16	n.s	
β-Sitosterol %	85.95 ± 2.68	89.21 ± 1.27	0.00	
Sitostanol %	0.48 ± 0.24	0.69 ± 0.17	0.00	
Δ-5-avenasterol %	6.93 ± 2.38	4.31 ± 1.27	0.001	
Δ-5, 24-stigm/dienol %	0.29 ± 0.14	0.22 ± 0.11	0.002	
Δ-7-stigmastenol %	0.32 ± 0.15	0.29 ± 0.11	n.s	≤0.5
Δ-7-avenasterol %	0.25 ± 0.16	0.26 ± 0.11	n.s	
Apparent b-Sitosterol %	94.63 ± 0.70	95.28 ± 0.35	0.00	≥93.0
Total Erythrodiol %	2.76 ± 1.07	1.43 ± 0.45	0.00	≤4.5
Total sterols (mg/kg)	1020.8 ± 120.7	1343.7 ± 115.1	0.00	≥1000

In general, total sterols concentration and individual sterols content of cv. Lianolia Kerkyras olive oil samples comply with the up to date EU legislation [22]. In contrast, olive oil samples of cv. Koroneiki showed lower concentration in total sterols with a mean value of 1020.8 mg/kg compared to olive oils of cv. Lianolia Kerkyras (1343.7 mg/kg). As far as the individual sterols profile, Lianolia Kerkyras olive oils samples showed a higher mean value for the major phytosterol β-sitosterol (89.21%) and for sitostanol (0.69%) compared to the relative values of Koroneiki olive oil samples (Table 3). Moreover, Lianolia Kerkyras exhibited lower mean values for the most abundant sterols, namely Δ-5-avenasterol (4.31%), campesterol (3.48%), stigmasterol (0.49%), as well as for total erythodiol content (1.43%). Although no previous reported data for the sterolic profile of cv. Lianolia Kerkyras is available, to the best of our knowledge, our results depict that this local Greek olive variety presents higher percentage mean values in β-sitosterol and total sterols compared to the most-known and exploited Koroneiki variety. Comparison of the two monocultivar olive oils according to their sterolic profile, as shown in Table 3, by means of the calculated *p*-value, shows it to be in most cases close to 0.00 ($p \approx 0.00$), indicating a strong botanical effect. Thus, the dataset of individual and total sterols can enable the classification of the examined olive oils according to their cultivar and indicate them as a possible compositional marker for olive oil authentication.

4. Conclusions

1. Both cultivars (cv. Koroneiki and Lianolia Kerkyras) in the coastline region of north-western Greece had an overall high quality profile.
2. The fatty acid and sterolic profile data set can permit the discrimination of Koroneiki and

Lianolia Kerkyras olive oil samples according to their botanical origin.

3. The obtained results can contribute in the future to the establishment of a possible "Greek Authentic Olive Network" of indigenous, local and less exploited monovarietal olive oils.

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