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Chemical and Sensory Characterization of Nine Spanish Monovarietal Olive Oils: An Emphasis on Wax Esters

Clara Diarte [1,2], Agustí Romero [3], María Paz Romero [2,4], Jordi Graell [2,4] and Isabel Lara [1,2,*]

1 Chemistry Department, Universitat de Lleida, 25198 Lleida, Spain; clara.diarte@udl.cat
2 AGROTÈCNIO-CERCA Center, 25198 Lleida, Spain; mariapaz.romero@udl.cat (M.P.R.); jordi.graell@udl.cat (J.G.)
3 Oliviculture, Oil Science and Nuts, Institut de Recerca i Tecnologia Agroalimentàries (IRTA), IRTA Mas Bové, 43120 Constantí, Spain; agusti.romero@irta.cat
4 Food Technology Department, Universitat de Lleida, 25198 Lleida, Spain
* Correspondence: isabel.lara@udl.cat; Tel.: +34-973-702526

Abstract: Olive oil is an essential part of the so-called "Mediterranean diet", purportedly one of the healthiest gastronomic traditions in the world. The wax content in olive oil is regulated under European Union directives, and it is used as a purity parameter for extra-virgin and virgin olive oils. The wax profile may also help the characterization of monovarietal olive oils. In this study, monovarietal oils were extracted from the fruits of nine native Spanish olive varieties ('Arbequina', 'Argudell', 'Empeltre', 'Farga', 'Manzanilla', 'Marfil', 'Morrut', 'Picual' and 'Sevillenca'), and their chemical and sensory attributes were determined. Total wax content in oil was cultivar-dependent and ranged widely between 26 ('Manzanilla') and 144 mg kg^{-1} ('Arbequina'), while it was negligible in 'Picual' oil. The wax ester fraction was comprised largely of phytol-containing diterpene esters, with phytyl vaccinate and phytyl arachidate being the most common components of this non-polar fraction in all nine olive oils assessed. A direct relationship between phytyl esters and the sensory perception of "ripe fruit" notes was also observed.

Keywords: chemical properties; *Olea europaea*; olive oil; phytyl esters; sensory attributes; wax

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(. . .) *oleum saporis egregii, dum viride est, intra annum corrumpitur.*
Lucius Iunius Moderatus, a.k.a. Columella
De re rustica (Book V)

1. Introduction

Olive (*Olea europaea* L.) oil is one of the key products characterizing the Mediterranean diet and displays matchless characteristics as a food fat regarding organoleptic, chemical and health-promoting attributes. High contents of monounsaturated fatty acids and the presence of phenolic acids reportedly confer olive oil valuable antioxidant and anti-cancer properties, as well as protective activity against heart diseases, osteoporosis and cognitive impairment [1–3]. The European Union (EU) is the main olive- and olive oil-producing area in the world and has established official standards and analytical methods for the classification of olive oils according to their physical, chemical and sensory characteristics [4]. According to EU regulations, the acidity of extra-virgin olive oil should not exceed 0.8% and the total wax content should be below 150 mg kg^{-1}. For peroxide value, K_{232} and K_{270}, which are used as indicators of oxidative processes, the legal limits are 20 mEq O_2 kg^{-1}, 2.50 and 0.22, respectively. As for sensory attributes, the official requirements include freedom from defects and the presence of fruity notes.

Epidermal cells of fruits and other aerial, non-lignified plant organs produce and secrete cuticular waxes, an important component of the cuticle surrounding and protecting the organ. Cuticular waxes comprise a mixture of aliphatic very-long-chain fatty acid (VLCFA) derivatives and variable amounts of triterpenoids and phenylpropanoids [5].

During the mechanical extraction of olive oil, a part of cuticular waxes from the intact fruit may be transferred into the oil in small quantities, which might affect some quality and purity characteristics of the product. For example, cuticular waxes in olive fruit are particularly rich in triterpenoid acids, with relative percentages over total waxes ranging from 58% to roughly 81% [6], contingent upon cultivar and maturity stage [7]. The presence of particular compounds in olive oil could provide information on the cultivar and extraction method employed and, thus, be helpful as a tool to authenticate oil origin [8,9]. Similarly, leaf admixture in extra-virgin olive oils may lead to significantly different *n*-alkane profiles in comparison with oils free from leaf material [10], as *n*-alkanes present in olive leaves display higher average chain lengths (ACLs) than those in fruits [10,11]. Moreover, when oil is extracted in an organic solvent such as *n*-hexane, wax esters contained in the olive paste are transferred in high quantities to the product [12], and hence, wax ester contents will be higher in crude pomace olive oil than in virgin olive oils. Additional pre- and post-harvest factors that may impact wax concentration in olive oil include maturity stage of the fruit, environmental conditions, harvesting period or centrifuge procedures during oil extraction [13]. The amount of waxes in olive oil will also depend on olive fruit integrity and will be higher in oil obtained from soft and degrading fruit tissues [14]. Accordingly, olive oils of inferior quality contain more waxes than high-quality oils; wax content in extra-virgin olive oil should not exceed 150 mg kg^{-1} [4], while that in refined or lampante oils ranges from 300 to 350 mg kg^{-1}. Consequently, it is possible to detect frauds in extra-virgin and virgin olive oils—for example, adulterations with lower-quality oils such as refined olive pomace oil or cheaper vegetable oils [15,16]. Wax ester content is, hence, a helpful indicator of the purity and high quality of olive oils, such as cold-pressed extra-virgin olive oil [17].

Wax esters found in olive oil are generally long, straight-chain fatty alcohols esterified with fatty acids, but wax esters containing a phytol (a diterpenic alcohol) group have been reported, and the C_{40} wax ester has been shown to contain phytyl behenate [18]. More recent research confirmed that although phytyl esters dominated the wax ester fraction in olive oil, these could be accompanied by variable amounts of geranylgeraniol esters [14]. A few previous studies have analyzed the wax esters in monovarietal olive oils from different Spanish and Italian cultivars [19–21]. In the present study, wax ester profile was determined in monovarietal olive oils obtained from nine different cultivars (‘Arbequina’, ‘Argudell’, ‘Empeltre’, ‘Farga’, ‘Manzanilla’, ‘Marfil’, ‘Morrut’, ‘Picual’ and ‘Sevillenca’). ‘Marfil’ is the only white-skinned olive cultivar in Spain, while the rest were chosen on the basis of their importance in the producing area. The chemical and sensory attributes of the oil samples were also determined.

2. Materials and Methods

2.1. Plant Material and Oil Extraction

Monovarietal olive oils were obtained mechanically from defect-free fruits of nine native Spanish varieties (‘Arbequina’, ‘Argudell’, ‘Empeltre’, ‘Farga’, ‘Manzanilla’, ‘Marfil’, ‘Morrut’, ‘Picual’ and ‘Sevillenca’), harvested on 3 December 2018 at the IRTA-Mas Bové experimental orchard in Constantí (41°09′ N, 1°12′ E; altitude 100 m), within the geographical area covered by the Protected Designation of Origin “Siurana”. The annual rainfall in 2018 was 310 mm, and the trees were supplied with drip irrigation. Fertilization and cultural practices were as usual in the surrounding producing area. Maturity indices at harvest (50 olives per cultivar) were determined visually according to skin and flesh color on a 0–7 scale according to standard procedures [22], and results were expressed as the weighted average of the 50 fruits within each sample. Oil was extracted immediately after harvest with an Abencor® system (MC2 Ingeniería y Sistemas, S.L., Seville, Spain), which involves mechanical extraction in a hammer mill, followed by mixing of the paste under controlled temperature to increase oil extraction efficiency and then by centrifuging to eventually separate the oil from water and solid residues. The olive oils were stored at 4 °C for 4 months in the dark until analysis. Acidity, peroxide values, K_{232} and K_{270} indices, wax esters and sensory profiles were determined in the oil samples according to official methods as briefly described below [4].

2.2. Chemical Characterization

All procedures were carried out in triplicate. For the determination of free fatty acid content, samples (5 g) of olive oil were dissolved in 50 mL diethyl ether and 95% ethanol (1:1, *v/v*) and titrated with ethanolic potassium hydroxide (0.1 mol L^{-1} KOH in 95% ethanol). Acidity was expressed as the percentage of oleic acid.

For the analysis of peroxide value, oil samples (2 g) were mixed with 10 mL chloroform, 15 mL acetic acid and 1 mL saturated potassium iodide and allowed to react for 5 min in the dark at room temperature. Distilled water (75 mL) was then added, and free iodine titrated with 0.01 N sodium thiosulphate. Results were given as milliequivalents (mEq) active oxygen kg^{-1} oil.

Specific extinction coefficients of oil oxidation products (K_{232} and K_{270}) were determined by UV spectrophotometry (Jenway™ 6715 series, Cole-Palmer©, Stone, Staffordshire, UK) on filtered samples (0.1 and 0.2 g, respectively) dissolved in 25 and 10 mL cyclohexane, respectively.

Oxidative stability was also assessed through the Rancimat method, an accelerated aging test measuring the increase in conductivity of deionized water (60 mL) as a consequence of the absorption of volatile secondary compounds produced in the course of fatty acid oxidation. Oil samples (3 g) were loaded onto the Rancimat equipment (743 Rancimat, Metrohm AG, Switzerland) at 120 °C and with a 20-mL min^{-1} air flow rate. Stability data were expressed as hours.

2.3. Wax Ester Profiles

For the analysis of wax contents, olive oil samples (500 mg) were added to 2 mL *n*-hexane and lauryl arachidate as the internal standard. The mixture was pre-purified through a silica gel column and eluted with *n*-hexane/ethyl ether (99:1, *v*/*v*). The percolated sample (180 mL) was evaporated completely under vacuum, resuspended in 2 mL n-heptane and injected (1 µL) for subsequent analysis of total wax contents in a gas chromatograph equipped with a flame ionization detector (GC-FID) (Agilent 7890N, Santa Clara, CA, USA) and a capillary column (ZB–1HT, 15 m × 0.32 mm × 0.25 µm; Zebron™ Phenomenex Inc., Torrance, CA, USA). The chromatographic conditions were adapted from the official method: the oven program was initially set at 80 °C, and this temperature was raised by 30 °C min^{-1} to 250 °C, then by 5 °C min^{-1} to 340 °C, and was then held for 15 min at this final temperature. Helium was used as the carrier gas at a flow rate of 4 mL min^{-1}. The injector and detector were held at 80 and 340 °C, respectively. Total wax contents were expressed as mg kg^{-1} oil, and the reported data represent the average of three replicates.

The identification of individual wax compounds was carried out in a gas chromatography–mass spectrometry (GC-MS) system coupled with a quadrupole mass selective detector (Agilent 5973N, Santa Clara, CA, USA). The capillary column and the chromatographic conditions were the same as in the GC-FID analyses. The mass spectra obtained from samples were compared with those from a mass spectral library (NIST 11 MS, Gaithersburg, MD, USA). The concentration of each detected ester was given as mg kg^{-1} oil.

2.4. Sensory Analysis

The sensory analysis was carried out by the Official Tasting Panel of Virgin Olive Oils of Catalonia (Panell de Tast Oficial d'Olis Verges d'Oliva de Catalunya), according to European Union Standard Methods [4]. This panel is accredited under ISO 17025 and is recognized by the International Olive Oil Council. Each oil sample was analyzed by eight tasters who scored the official sensory descriptors using a 10-cm scale anchored on zero. In addition, the presence of secondary sensory attributes and defects was determined by the percentage of panelists able to perceive each odor note using an open generic profile [23,24].

Finally, the median intensities of sensory attributes were used for the calculation of the global sensory score on a 0–9 scale (0, very bad quality; 9, highest quality) with an algorithm developed by IRTA [23]. Global scores facilitate the comparison of the sensory quality of different samples. As a reference, global sensory scores for olive oils within the extra-virgin category should be at least 6.5 points.

2.5. Statistical Analysis

Sensory attribute scores were expressed as the median. The rest of the data were submitted to analysis of variance, with cultivar as the factor. Means were calculated and compared with the Fisher's least significant difference (LSD) test ($p \leq 0.05$) using the JMP® Pro 14 software (SAS version 9.4, Cary, NC, USA). Finally, principal component analysis (PCA) was used to help visualize possible relations among the parameters. The Unscrambler software, version 9.1.2 (CAMO ASA, Oslo, Norway), was used to develop PCA models. Data were centered and weighed by the inverse of the standard deviation of each variable in order to avoid dependence on measuring units, and full cross-validation was run as a validation procedure.

3. Results and Discussion

3.1. Physicochemical and Organoleptic Quality Characteristics

The physicochemical parameters of all nine monovarietal olive oils assessed are shown in Table 1. The nine olive cultivars used for oil extraction display different ripening patterns [25], with 'Manzanilla', 'Empeltre' and 'Sevillenca' being the earliest varieties to attain maturity, whereas the rest of cultivars ripen later.

Table 1. Weight and maturity index of olives used for oil extraction and chemical characteristics of monovarietal oils studied.

Cultivar	Weight (g)	Maturity Index	Acidity (% Oleic Acid)		Peroxide Value (mEq O_2 kg^{-1})		K_{232} Index		K_{270} Index		Oxidative Stability (h)		Wax Content [1] (mg kg^{-1})	
'Arbequina'	1.69	2.4	0.14	h	6.89	d	1.68	c	0.07	f	8.53	d	143.97	a
'Argudell'	3.10	3.2	0.16	g	9.40	a	1.94	b	0.11	c	8.34	d	51.20	c
'Empeltre'	1.41	5.0	0.64	b	3.76	h	1.81	bc	0.06	g	8.27	d	65.95	b
'Farga'	2.20	3.6	0.21	e	5.52	f	1.67	c	0.08	e	8.53	d	60.04	bc
'Manzanilla'	5.79	6.4	0.51	c	4.44	g	1.53	d	0.10	d	22.11	b	25.85	d
'Marfil'	1.98	1.9	0.18	f	7.35	c	2.12	a	0.13	a	17.45	c	35.30	d
'Morrut'	2.98	3.1	0.45	d	2.57	i	1.68	c	0.08	e	8.45	d	68.77	b
'Picual'	3.61	2.3	0.14	h	8.89	b	1.70	c	0.13	a	33.24	a	nd	
'Sevillenca'	4.04	4.7	1.60	a	5.74	e	1.81	bc	0.12	b	4.43	e	67.87	b

Maturity index (0–7) values represent the weighted average of 50 fruits per cultivar [22]. Weight was determined jointly for 50 olives per cultivar, and values were divided by 50 to obtain the average weight per fruit. Values for the rest of the parameters represent means of three technical replicates (nd, non-detectable). Different letters within each column denote significant differences among the different monovarietal olive oils at $p \leq 0.05$ (Fisher's LSD test). [1] Wax content data comprise C_{42}, C_{44} and C_{46} aliphatic compounds uniquely (European Union regulation [4]).

In contrast, 'Marfil' olives were still quite green when harvested in early December and just beginning to turn white, this being the only white-skinned olive cultivar in Spain. These differences in ripening patterns were reflected in the different maturity indices found in each case at the picking date (Table 1). In all cases, oil was extracted at once after harvest, hence limiting fermentative and oxidative processes. Based on the analytical parameters considered herein, all the monovarietal oils studied could be classified as extra-virgin olive oils according to European Union regulations [4], with the exception of 'Sevillenca' oil due to its high acidity values (1.6%) exceeding the regulated limit (0.8%). On this basis, 'Sevillenca' oil had to be classified as virgin olive oil, for which the maximum acidity value is higher (2.0%). This agrees with the "fusty" defect detected by the panelists, possibly contributing to the low global sensory score of 'Sevillenca' oil in comparison to the rest of the monovarietal oils evaluated (Table 2). On the contrary, 'Arbequina' and 'Picual' oils contained the lowest acidity (0.14%).

Table 2. Sensory attributes of nine Spanish monovarietal olive oils.

Cultivar	Fruity	Bitter	Pungent	Global Sensory Score
'Arbequina'	4.40	2.55	3.40	6.6
'Argudell'	4.65	3.15	3.60	7.0
'Empeltre'	4.10	2.70	3.25	6.7
'Farga'	5.05	2.85	3.80	6.8
'Manzanilla'	4.30	4.05	4.45	6.5
'Marfil'	5.75	4.70	5.15	7.6
'Morrut'	4.35	3.45	3.90	7.0
'Picual'	6.15	5.20	5.15	7.4
'Sevillenca'	3.75	3.45	4.30	6.1

Sensory attributes were scored on a 10-cm scale. Global sensory scores were calculated from sensory data on a 0–9 scale (0, very bad quality; 9, highest quality) as described in [23]. Values represent the median of eight trained panelists from an official panel (Panell de Tast Oficial d'Olis Verges d'Oliva de Catalunya).

Peroxide values ranged from 2.56 ('Morrut' oil) to 9.40 ('Argudell' oil) mEq O_2 kg^{-1}. As regards the K_{232} index, 'Marfil' and 'Manzanilla' oils were statistically different in comparison with the rest and showed the highest (2.12) and the lowest (1.53) values, respectively. Peroxide value and K_{232} are indicators of primary oxidation in olive oil, consisting in the addition of oxygen to fatty acids at the double bond position to form peroxides, and thus, these data confirm the good quality of the oils considered in the present study. This fact was corroborated by the K_{270} values, which ranged within values below the legal limit (from 0.06 in 'Empeltre' to 0.13 in 'Marfil' and 'Picual' oils) and hence illustrated the absence of secondary oxidation, which would produce volatile compounds affecting oil taste and off-flavor, in accordance with the lack of rancidity found during sensory analyses. The wide range of values observed for each parameter, together with previous reports for other cultivars [26,27], suggests these attributes be largely cultivar-specific.

Oxidative stability showed considerable variation across all nine monovarietal oils considered and ranged widely from 4.43 ('Sevillenca') to 33.24 h ('Picual'). These data might be related to the content of phenolics, which enhance oxidative stability [28], and in which olive oil from 'Picual' is particularly rich [29] while that from 'Sevillenca' is reportedly not [30]. Accordingly, 'Manzanilla' and 'Marfil' oils also showed high stability against oxidation (22.11 and 17.45 h, respectively) in agreement with previous reports on high phenolic levels in oils obtained from these varieties [31,32]. The rest of the olive oils analyzed had similar oxidative stability values (roughly 8.50 h). Although total phenolics in oil samples were not assessed in this study, data obtained in previous producing seasons for oils extracted from the same cultivars at the same experimental orchard (Supplementary Table S1) support a relationship between higher contents of total phenolics and superior oxidative stability (Table 1).

The analysis of sensory attributes indicated the lowest fruitiness scores for 'Sevillenca' virgin olive oil, together with a "fusty" defect (some tasters reported "winy" as well). Extra-virgin oils obtained from the rest of the varieties showed no sensory defects, which is an additional indicator of their high-quality character (Table 2, Figure 1). 'Picual', 'Marfil' and, to a lesser extent, 'Manzanilla' oils were perceived as particularly bitter and pungent (Table 2, Figure 1). It has been suggested that bitter and pungent sensations are highly correlated to the total content of phenolics [33–35]. This agrees with data obtained in preceding years at IRTA-Mas Bové, showing that 'Marfil' and 'Picual' oils also displayed the highest contents of total phenolics (Supplementary Table S1). 'Picual' and 'Marfil' oils also scored higher than the rest regarding fruity and green notes (Figure 1), while 'Empeltre' oil was, in contrast, one of the softest, as indicated by low bitterness (2.70) and pungency (3.25). For 'Empeltre', a relationship has been observed between low phenolic contents in oil and low scores (2 to 4) for fruitiness, bitterness and pungency [36], which results in a soft olive oil.

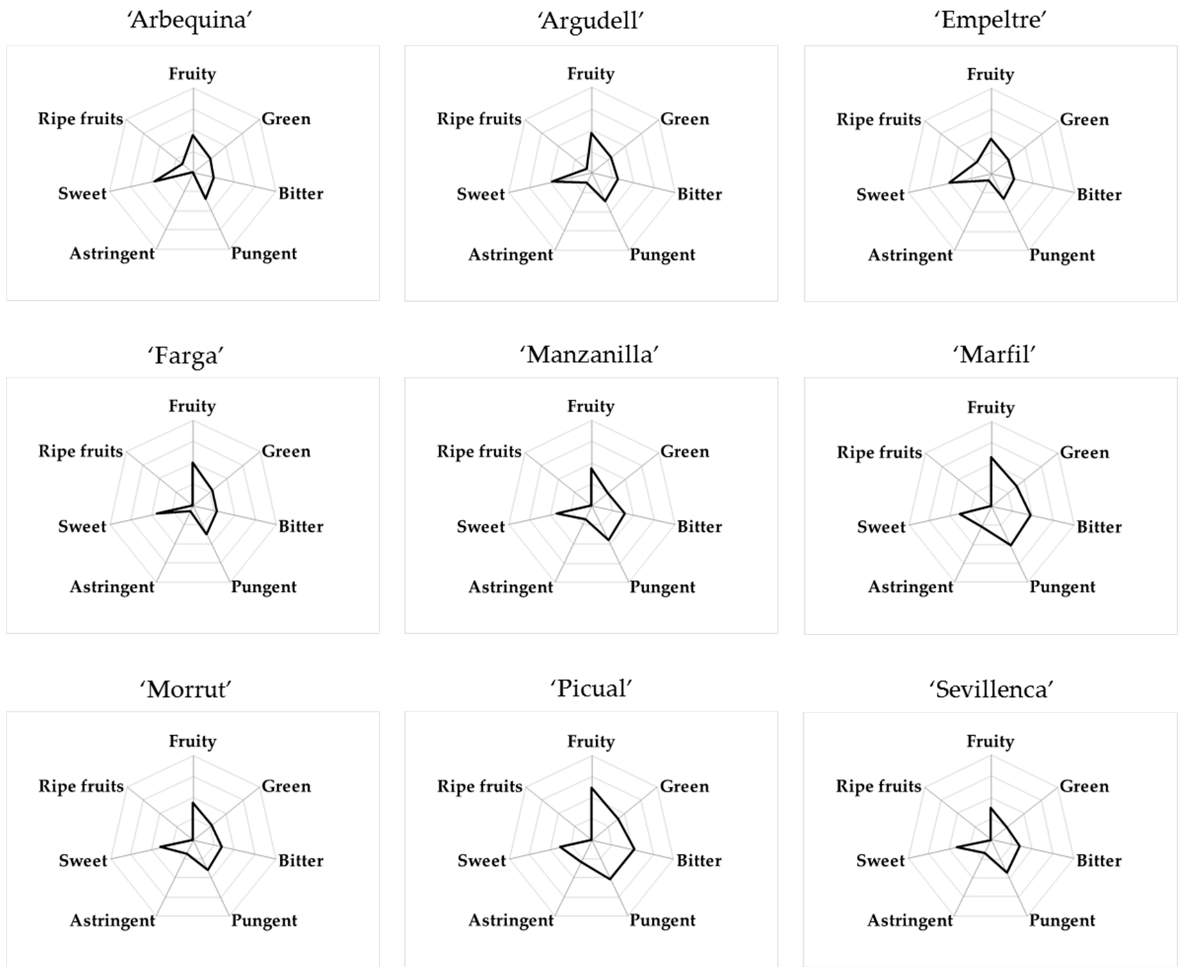

Figure 1. Radar chart of sensory parameters of nine monovarietal olive oils. Sensory attributes were scored on a 10-cm scale (chart center, 0; outer heptagon, 10). Values represent the median of eight trained panelists from an official panel (Panell de Tast Oficial d'Olis Verges d'Oliva de Catalunya).

3.2. Wax Content and Wax Ester Profiles

Olive oil waxes are used as a purity parameter. For the determination of wax content in high-quality (extra-virgin or virgin) olive oils, and according to European Union olive oil regulation [4], only C_{42}, C_{44} and C_{46} esters are considered. The wax content of all of the monovarietal olive oils studied herein was below the regulated limit, established at 150 mg kg^{-1} (Table 1). This is important, as the formation of wax esters continues during the shelf life of olive oils, and thus, the initial wax content has a significant impact on the subsequent evolution of the product. The results showed a wide range of wax content levels among the nine olive oils analyzed, suggesting that this parameter could be cultivar-dependent, as reported previously for Italian olive oils [20]. Only 'Arbequina' oil approached (144 mg kg^{-1}) the maximum established value (Table 1), with the wax content being two- to fourfold higher than that in the rest of the samples. These data are in agreement with earlier observations by Aragón et al. [19] that monovarietal 'Arbequina' olive oil displayed one of the highest wax contents in comparison with oils obtained from other cultivars.

The typically small size of the 'Arbequina' fruit as compared with other genotypes suggests that the high wax content in oil extracted from this cultivar might have arisen from the larger fruit surface area relative to fruit volume, and indeed, a negative correlation ($r = -0.74$) was found in this work between wax content in oil and fruit weight (Figure 2). This trend, though, did not hold for all the studied cultivars, particularly for 'Marfil', which displayed low wax content in oil together with an average fruit weight below 2 g (Table 1).

'Arbequina' fruits also exhibit considerable cuticle and cuticular wax contents per surface area together with high cuticle thickness by the usual time when they are harvested for oil extraction [6]. For these reasons, legal regulation of wax content may prove controversial among olive oil producers and traders, as some genotypes may naturally display higher concentrations and thus easily reach values close to or above the legal limits.

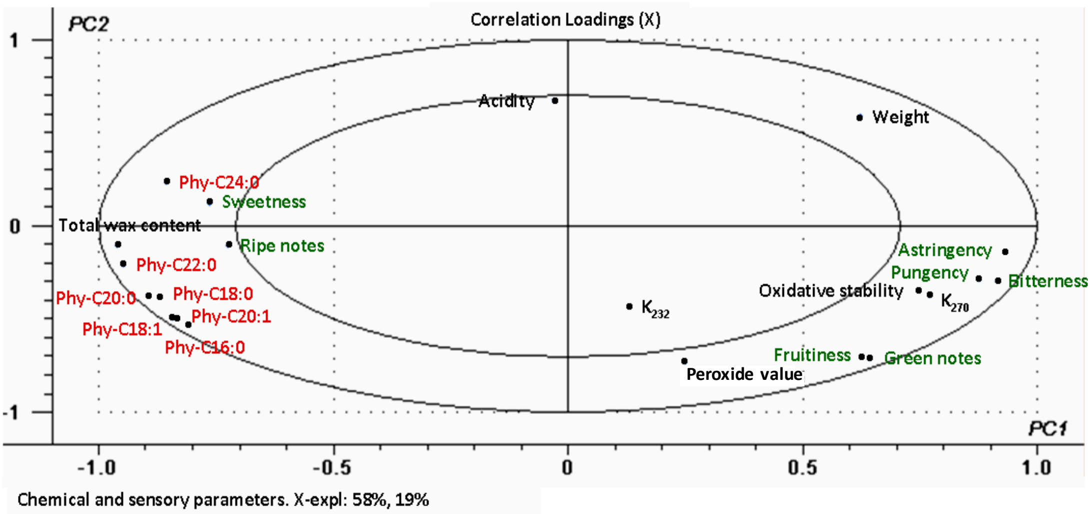

Figure 2. Correlation loadings plot of PC1 vs. PC2 corresponding to a Principal Component Analysis (PCA) model for chemical and sensory parameters assessed in nine monovarietal olive oils. * Abbreviations: Phy-C16:0, phytyl palmitate; Phy-C18:0; phytyl stearate; Phy-C18:1, phytyl vaccinate; Phy-C20:0, phytyl arachidate; Phy-C20:1, phytyl eicosenate; Phy-C22:0, phytyl behenate; Phy-C24:0, phytyl lignocerate.

The wax ester types identified in the olive oils were in the range of C_{36} to C_{44} (Table 3). In quantitative terms, C_{38} and C_{40} esters were prominent, whereas the concentrations of C_{36} and C_{44} esters were less important. For 'Picual' oil, though, C_{36}, C_{42} and C_{44} esters were undetectable, consistent with the observation that the total wax content as defined by European Union regulations [4] was negligible in this oil (Table 1). This finding agrees with previous reports for monovarietal 'Picual' oil [8], showing that wax content was not high, even though in that work, low concentrations of C_{36}, C_{42} and C_{44} esters could be identified and quantified. C_{46} esters are present in negligible quantities in extra-virgin and virgin olive oils [21], and accordingly, no C_{46} esters were detected in this work. The absence of C_{46} esters also could be attributed partially to their possible co-elution with sterol compounds, which would pose further difficulty in their identification. Indeed, the mass spectra retrieved in this work suggested that some signals detected at the last part of the chromatograms might correspond to sterol-type compounds (see Supplementary Figure S1 for an example).

GC-MS results revealed that the wax compounds identified in the non-polar fraction corresponded to diterpene esters composed of phytyl groups esterified to different fatty acids, including palmitic (16:0), stearic (18:0), vaccenic (18:1), arachidic (20:0), eicosenoic (20:1), behenic (22:0) and lignoceric (24:0) acids (Table 3). Low concentrations of geranyl-geraniol esters, also diterpenic compounds, have been occasionally identified in olive oils [14,37], but none were detected in the present study, maybe in connection with the experimental difficulty of retrieving the mass spectra when compound concentration is very low [17]. Diterpenic esters are basically found in the pulp of the olive, and it has been suggested that the official methodology for the analysis of wax content as established by the European Union may cause them to elute together with cuticular wax esters [14]. Even so, no diterpenes were detected in the cuticular waxes of olive fruits [6]. Additionally, the olive oils analyzed in this work were extracted by mechanically crushing the fruits and then centrifuging the olive paste, and no solvents whatsoever were used for the extraction. The presence of phytyl fatty acid esters might be related to fruit ripening-associated chlorophyll degradation [38]. In agreement, phytyl fatty acid esters were detected in red and yellow, but not in green, bell pepper fruits [39], indicating that they accumulated mainly during fruit ripening.

Table 3. Wax compound [1] types (mg kg^{-1}) identified in nine Spanish monovarietal olive oils.

	'Arbequina'		'Argudell'		'Empeltre'		'Farga'		'Manzanilla'		'Marfil'		'Morrut'		'Picual'		'Sevillenca'	
Ester C36																		
Phy-C16:0	61.09	a	18.73	b	15.78	b	10.82	c	4.38	d	19.25	b	8.54	c	nd		7.88	cd
Ester C38																		
Phy-C18:0	92.95	a	22.79	bc	27.09	b	19.28	c	13.83	d	22.46	bc	23.64	bc	1.51	e	20.76	c
Phy-C18:1	434.46	a	117.99	b	132.96	b	86.97	c	32.01	de	125.59	b	65.96	c	2.79	e	56.24	cd
Ester C40																		
Phy-C20:0	307.84	a	95.43	b	95.63	b	79.31	c	32.33	d	77.00	c	79.40	c	2.21	e	86.08	bc
Phy-C20:1	150.46	a	45.78	bc	42.96	bc	37.18	cd	8.26	e	48.77	b	29.04	d	nd		29.06	d
Ester C42																		
Phy-C22:0	117.05	a	40.09	b	48.15	b	40.51	b	11.94	d	26.59	c	45.22	b	nd		46.96	b
Ester C44																		
Phy-C24:0	26.92	a	11.11	d	17.87	c	19.53	c	7.29	e	8.71	de	23.55	b	nd		20.90	bc

Values represent means of three technical replicates (nd, non-detectable). Different letters within each row denote significant differences among the different monovarietal olive oils at $p \leq 0.05$ (LSD test). [1] Abbreviations: Phy-C16:0, phytyl palmitate; Phy-C18:0; phytyl stearate; Phy-C18:1, phytyl vaccinate; Phy-C20:0, phytyl arachidate; Phy-C20:1, phytyl eicosenate; Phy-C22:0, phytyl behenate; Phy-C24:0, phytyl lignocerate.

In all the analyzed oil samples, phytyl vaccinate and phytyl arachidate dominated the wax ester fraction. Both compounds also stood out quantitatively among diterpene esters identified in monovarietal Kalamata olive oil [17]. 'Arbequina' oil displayed the highest phytyl vaccinate concentration (434.46 mg kg^{-1}), in agreement with previous studies showing that this ester represented about 8–10% of total phytyl wax esters detected in oil from this cultivar [21]. The concentrations of phytyl vaccinate in the nine monovarietal olive oils considered herein amounted for as much as 21–38% of total phytyl esters. In 'Picual', this compound practically amounted to around 43%, although this percentage corresponded to a concentration of only 2.79 mg kg^{-1}. The high content of phytyl vaccinate is noticeable, taking into account that oleic acid (18:1 Δ^9), not vaccenic acid (18:1 $\Delta^{trans-11}$), is the most common 18:1 fatty acid component of olive oil triacylglycerols (around 70% and 3% in extra-virgin oil, respectively) [40]. Additionally, unsaturated fatty acids are common in triacylglycerols present in olive oil, but in contrast, a substantial percentage (47% to 68%) of fatty acid constituents of diterpene esters identified herein were saturated (Table 3). These data agree with previous reports [21] and suggest the presence of a dedicated biosynthetic pathway for these esters.

The data were used to characterize the oil samples by means of a PCA model, and the corresponding correlation loadings plot (Figure 2) shows that the two first principal components (PC1 and PC2) explained up to 77% of sample variability. Samples were separated mainly along PC1, which accounted alone for 58% of total variability. An interesting association was found between phytyl esters and the perception of "ripe fruit" notes in the sensory analysis. Phytyl ester content has been suggested as a feasible marker for the maturity stage of bell pepper fruits [39]. The PCA model also revealed that the sensory perceptions of bitterness, pungency and "fruity" and "green" notes were associated to oxidative stability and K_{270} and correlated negatively to the wax content and the perception of "ripe" notes. In contrast, acidity and primary oxidation indicators (peroxide value and K_{232}) were apparently unrelated to wax ester content or the sensory perception of "sweet" and "ripe fruit" notes.

4. Conclusions

The bulk of results reported in this study illustrate the existence of cultivar-related differences in wax contents and profiles of monovarietal olive oils. The highest concentration of waxes was found for 'Arbequina' oil, which was at least twofold the amount observed for the rest of the monovarietal oils studied in this work, while, conversely, 'Picual' oil displayed the lowest wax contents. The data also show the relevance of phytyl esters as the main components of the wax ester fraction in extra-virgin and virgin olive oils and confirm vaccenic acid as a major fatty acid constituent thereof. In contrast, the data do not support the hypothesis that cuticular waxes may be transferred to the oil during mechanical extraction, as no relationship was found between wax profiles in olive oils and those in the fruit cuticle. On the basis of the data, diterpenic esters in extra-virgin and virgin olive oils appear a promising topic for future investigations, with a focus on improving the knowledge of the metabolic origins of phytol and of less common fatty acids. A wider range of cultivars and agronomic conditions should be considered for such future studies.

Supplementary Materials: The following are available online at https://www.mdpi.com/2077-0472/11/2/170/s1, Figure S1: GC-FID chromatogram from 'Arbequina' olive oil wax esters. Table S1: Average contents of total phenolics (TPC) in nine Spanish monovarietal oils (IRTA-Mas Bové experimental station, period 1993–1998).

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