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ORIGINAL PAPER

Molecular characterization of Dalmatian cultivars and the influence of the olive fruit harvest period on chemical profile, sensory characteristics and oil oxidative stability

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Abstract Four Dalmatian autochthonous olive cultivars (*Buhavica*, *Drobnica*, *Lastovka* and *Oblica*) were molecularly characterized by analyzing length variability of genomic DNA sequences encompassing 15 microsatellite repeats. Furthermore, several important parameters of olive oils were analyzed in relation to the harvest period. An analysis of major phenolics secoiridoids was done by qNMR, while the fatty acid profile of oils and squalene content was determined by GC–FID. Oxidative stability was evaluated by the Rancimat method and sensory evaluation was carried out by a trained professional panel. The results indicate that the effect of the harvest period on the phenolic profile of oils depends on the olive cultivar and is related to its genetic profile. *Drobnica* oil from the late harvest contained an extremely high concentration of oleocanthal + oleacein (966 mg/kg). The longest oxidative stability was achieved by *Drobnica* and *Lastovka* oils from the early harvest period (20.95 and 18.65 h). Squalene had no effect on the oil oxidative stability. This study shows that the content of phenolic secoiridoids depends mainly on the cultivar. In addition, some cultivars, such as *Drobnica* did not show significant change of phenolic secoiridoids content in relation to the harvest period.

Keywords Monovarietal olive oil · Secoiridoids · Fatty acids · Rancimat · Sensory evaluation · Molecular characterization

Introduction

Due to its specific chemical composition and direct extraction method, which includes only mechanical processes without any refining, and in relation to other plant-derived oils, olive oil is recognized as a natural functional food. Therefore, in the Mediterranean olive oil market there is a tendency to enhance the consumers' awareness of its health effects as well as to raise the price of extra virgin olive oils. Beside high levels of monounsaturated fatty acids, olive oil contains a broad spectrum of biologically active compounds such as phenolics (phenolic acids, phenolic alcohols, flavonoids and secoiridoids), tocopherols, volatile compounds, squalene, sterols and carotenoids [1–3]. The content of these minor components from diverse chemical groups of compounds in olive oils is a matter of great interest as is the case that usually beneficial health effects of olive oils are attributed to their presence. Furthermore, it is well known that these compounds are major contributors to the olive oil nutritive value, oxidative stability, long shelf-life and sensory characteristics [4, 5].

The content of these valuable phytochemicals in olive oils is strongly influenced by different factors such as olive cultivar, growing area, climate and environmental factors,

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stage of ripening and harvest time as well as extraction technology [3, 4, 6]. However, it has been proved that the maturity of olives at the time of harvest causes highest variations in content of phenolics and consequently their antioxidant properties and oil stability. The fruit ripening results in increased softness of the fruit as well as in modification and degradation of cell wall components which ensures better extraction of phenolics from the fruit [2]. The activity of the hydrolytic enzymes throughout the fruit maturation should not be neglected [3]. Also, decrease of squalene content during olive maturation has been reported [7].

Croatian olive oil market is still relatively small in comparison to other Mediterranean countries so it is rarely included in the list of significant olive oil-producing countries. However, there is a long-standing tradition of olive cultivation and the production of high quality olive oil in Croatian costal regions of Dalmatia and Istria [8]. In the last few years, considerable efforts have been taken to increase the number of olive orchards, so the Croatian Bureau of Statistics reports an increase in production of olives by 220% in 2015 [9], which is in accordance with a global increase of the world production of olives and olive oil [10]. Also, great attention has been given to native varieties of olives that are cultivated throughout the country and have been genetically characterized by several authors [11–14]. While Istrian olive oils are the object of numerous studies, there are few studies on chemical, biological and sensory analyses of the oils from Dalmatian olive cultivars [15–18]. To the best of the author's knowledge, to date there is no detailed study on the influence of the harvest time on chemical and sensory characteristics of Dalmatian olive oils, and consequently their oxidative stability.

The objective of this study is to determine the effect of the harvest period (early harvest of green fruits at the beginning of October and late harvest of ripe fruit at the end of November) on the content of phenolics (secoiridoids), fatty acid composition and sensory characteristics of monovarietal olive oils from four native Dalmatian cultivars (*Buhavica, Drobnica, Lastovka* and *Oblica*), which were molecularly characterized using microsatellite-based genotyping. An analysis of phenolics secoiridoids was done by quantitative Nuclear Magnetic Resonance (qNMR) spectrometry, fatty acid profile and squalene content were determined using Gas Chromatography with Flame-Ionization Detection (GC–FID), while the oxidative stability was tested by the Rancimat method. Finally, sensory evaluation was carried out by a trained professional panel. The approach based on a comparison of chemical composition of olive oils extracted from fruits in different maturity stage, provides valuable information about the influence of the investigated parameters on oil sensory properties and oxidative stability (Fig. 1).

Materials and methods

Molecular characterization

The total DNA was isolated from young olive leaves and flower buds of *Olea europaea* var. *europaea* from four Dalmatian native cultivars: *Buhavica* (Brač Island, Central Dalmatia), *Drobnica* and *Lastovka* (Korčula Island, South Dalmatia) and *Oblica* (Kaštela, Central Dalmatia), following the olive DNA isolation method previously described in Štambuk et al. [10]. Olive specimens were characterized using the following 15 microsatellite-based markers: UDO99-008, UDO99-012, UDO99-019, UDO99-024, UDO99-028, UDO99-031, UDO99-039, UDO99-043 [19], ssrOeUA-DCA3, ssrOeUA-DCA8, ssrOeUA-DCA9, ssrOeUA-DCA10, ssrOeUA-DCA14, ssrOeUA-DCA16 [20], and EMO3 [21] according to the procedure described in detail in the cited paper. Polymerase chain reactions were carried out in a volume of 12.5 µL containing 1.5 mM $MgCl_2$ (for all ssrOeUA markers and EMO3), 2 mM $MgCl_2$ (for all UDO99 markers except for UDO99-008) and 2.5 mM $MgCl_2$ (for UDO99-008 marker), 0.2 mM of each dNTP (Applied Biosystems, USA), GeneAmp 10 × PCR

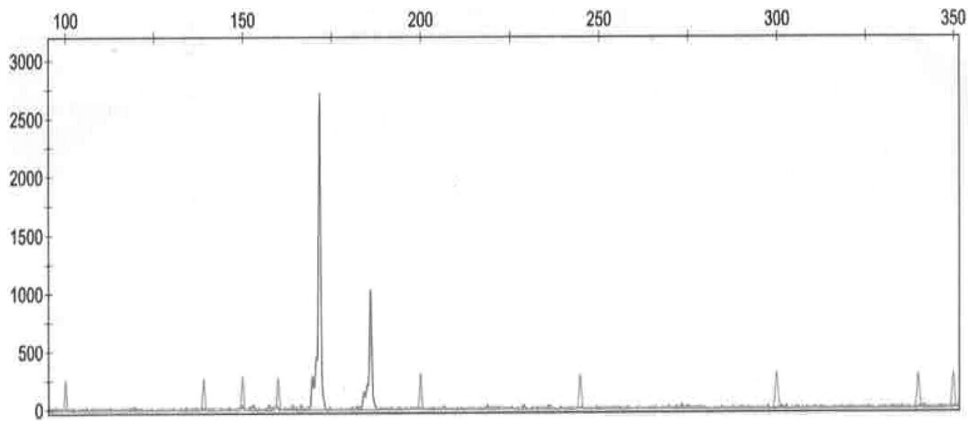

Fig. 1 The electropherogram of olive DNA obtained in this study by applying UDO99-024 microsatellite based marker

Buffer II [(1.25 µL for ssrOeUA-DCA3, ssrOeUA-DCA14, ssrOeUA-DCA16), (1.5 µL for ssrOeUA-DCA8, ssrOeUA-DCA9, ssrOeUA-DCA10, EMO3, UDO99-019, UDO99-043), (1.75 µL for UDO99-008, UDO99-012, UDO99-024, UDO99-028, UDO99-031, UDO99-039 markers) Applied Biosystems], relevant primer pairs and 0.25 U AmpliTaq Gold DNA polymerase (Applied Biosystems). PCR reactions were performed in the Applied Biosystems thermocycler under the following conditions: a step of 10 min at 95 °C, followed by 35 cycles of 45 s at 94 °C, 1 min at the appropriate annealing temperature of the primer, and 1 min at 72 °C, and a final extension at 72 °C for 30 min. PCR products were analyzed in an automated sequencer (ABI Prism 3130 Genetic Analyzer, Software v3.2, Applied Biosystems) and fragment lengths were determined using Genescan 500 Liz internal size standard (Applied Biosystems).

All PCR reactions were repeated at least three times if the results were perfectly concordant, and up to six times if there was a discrepancy in the first three amplifications, until obtaining at least three concordant results. Such discrepancies occurred on the average in 25% of cases of total amplification for each microsatellite-based marker, but were resolved in further three amplifications.

Olives harvest and processing

Olive oils from the fruit of *Buhavica, Drobnica*, *Lastovka* and *Oblica* cultivars were extracted from the olive fruits collected at the early stage of the harvest period (green fruit, beginning of October 2016) and at the late period of the harvest (ripe fruit, end of November 2016) from the olive orchards located at Brač Island (the village of Selca, *Buhavica* cultivar), Korčula Island (the village of Vela Luka, cultivars *Drobnica* and *Lastovka*), and the town of Kaštela (Split region, *Oblica* cultivar). Olives were hand-picked and only healthy and undamaged fruits were processed. All samples were processed immediately after harvesting under the same conditions (malaxation temperature was 26 °C and duration was 35 min), using a small-size olive mill Abencor (MC2, Ingenierias y Sistemas, Sevilla, Spain) which simulates commercial oil-extraction systems. At the exit of the hammer mill a sieve with 5 mm holes was used. The paste was centrifuged at 3000 rpm and filtered on a cotton layer. All oil samples were stored in dark glass bottles at the temperature between 18 and 20 °C until analyses were conducted.

Secoiridoids profile

Phenolics secoiridoids in investigated oils were detected by qNMR technique. Oil samples for NMR analysis were prepared according to procedure described by Karkoula et al. [22].

To 5.0 g of olive oil, 20 mL of cyclohexane and 25 mL of acetonitrile was added and the mixture was homogenized using a vortex mixer for 30 s. The layers were separated by centrifugation for 5 min at 4000 rpm. The acetonitrile phase was collected and mixed with 1.0 mL of a syringaldehyde solution (0.5 mg/mL) in acetonitrile. The samples were evaporated by rotary evaporator and the residue was dissolved in 750 µL of deuterated chloroform ($CDCl_3$). From the prepared solution, the volume of 550 µL was transferred to a 5 mm NMR tube. ^{1}H-NMR spectra were recorded at 600 MHz (Bruker Avance 600) and 400 MHz (Bruker DRX 400). Typically, 50 scans were collected into 32 K data points over a spectral width of 0–16 ppm with a relaxation delay of 1 s and an acquisition time of 1.7 s. Prior to Fourier transformation (FT) an exponential weighing factor corresponding to a line broadening of 0.3 Hz was applied. The spectra were phase corrected and accurate integration was performed manually for the peaks of interest using TOPSPIN as described by Karkoula et al. [22, 23] and by Diamantakos et al. [24].

Fatty acid composition and squalene content

Analysis of fatty acid methyl esters and squalene in the same run was carried out by gas chromatograph (model 3900; Varian Inc., Lake Forest, CA, USA) with FID detection using capillary column RTX 2330 (30 m × 0.25 mm i.d., coating thickness 0.25 µm, Restek, Bellefonte, PA, USA).

In a 5 mL screw-top test tube 0.1 g of the oil sample was weighted. Oil was dissolved in 2 mL of heptane and additionally 0.2 mL of the 2 M methanolic solution of potassium hydroxide was added. The tube was capped and shaken vigorously for 30 s. The solution was than left to stratify until the upper layer, heptane solution containing methyl esters, became clear and ready for injection into the chromatograph. Carrier gas was helium and the column flow rate was 3 mL/min. Injector and detector temperatures were held at 220 °C. The initial oven temperature was 140 °C and the final 210 °C, and the temperature was ramped at a rate of 5 °C/min for 16 min. The injection volume was 1 µL and the split ratio was 1:40. The amounts of detected components are expressed as a percentage by mass of methyl esters, by determining the percentage represented by the area of the corresponding peak relative to the sum of the areas of all the peaks. Squalene was identified by the retention time of corresponding analytical standard, while the quantification was made by external calibration.

Oxidative stability testing

The oxidative stability of the investigated olive oils was evaluated using Rancimat 743 (Metrohm, Herisau, Switzerland) instrument to monitor the progress of accelerated oxidation at high temperatures. The olive oil samples (3 g) were tested at a temperature of 120 °C ($\Delta T = 1.4$ °C) with the constant air flow of 20 L/h. The conductivity was measured as a function of time and the results are expressed as induction time (in hours). All determinations were performed in triplicate, and the results are presented as mean value ± standard deviation.

Sensory evaluation

Sensory evaluation of investigated olive oils was performed by a trained panel of experts, according to the Official International Olive Oil Council methodology [25] and the European Communities Regulation No. 2568/91 [26]. Quantitative descriptive evaluation of different olfactory descriptors (olive fruity, other ripe fruits, apple, green grass or leaves), taste descriptors (bitter, pungent and sweet) were quantified using a ten-point intensity ordinal rating scale from 0 (no perception) to 10 (extreme). The final results are expressed as a median of eight assessments for each descriptor.

Statistical analysis

Statistical analysis was performed using GraphPad InStat3 (GraphPad Software, San Diego, USA). The relationship between the obtained parameters was described using Pearson correlation coefficient "*r*". Differences at $p < 0.05$ were considered to be statistically significant.

Results and discussion

Molecular characterization

The results of molecular characterization of the selected autochthonous Dalmatian olive cultivars are shown in Table 1. *Oblica* is the most popular and prevalent olive cultivar in Croatia and it is growing almost everywhere along the coast [8]. *Buhavica* is an olive autochthonous cultivar from the Island of Brač (Central Dalmatia), while *Lastovka* is a characteristic olive cultivar from the Island of Korčula (South Dalmatia). *Drobnica* is the oldest cultivar on the island of Korčula [27]. The mentioned olive varieties were characterized on the basis of the analysis of length variability of genomic DNA sequences encompassing 15 microsatellite repeats. The microsatellite-based DNA sequences range among the most appropriate genetic markers used in olive cultivar

Table 1 The lengths of DNA sequences encompassing 15 microsatellites as a results of genotyping native Dalmatian olive cultivars *Buhavica, Drobnica, Lastovka* and *Oblica*

Cultivar/marker	UDO8	UDO12	UDO19	UDO24	UDO28	UDO31	UDO39	UDO43	DCA3	DCA10	DCA14	DCA16	DCA8	DCA8	EMO3
Buhavica	162 168	156 165	130 145	187 187	127 135	113 113	107 178	178 217	230 247	219 241	188 188	153 177	125 138	165 165	209 209
Drobnica	162 162	156 165	130 145	173 187	135 173	110 110	180 180	180 217	230 237	160 241	188 188	153 158	125 136	206 212	205 213
Lastovka	157 165	158 165	100 130	185 189	135 135	110 110	107 180	172 221	230 230	154 194	188 188	153 158	132 140	189 196	211 213
Oblica	167 167	156 156	130 145	173 187	135 153	110 110	178 181	178 180	237 251	94 219	179 188	153 177	136 138	165 206	209 213

UDO-No refer to UDO99-No., *DCA-No.* refer to ssrOeUa-DCANo

characterization, especially due to their good resolution power [28, 29]. Several microsatellites have been isolated from olives [19–21, 29–31], while their characteristics, distribution in genome, function, and mechanism of their creation and control justify their utilization in genotyping analysis [32].

Genotype analysis that was done in this study indicates that the most similar allele content appears in *Oblica* and *Drobnica* cultivars. They contain 12 out of 30 alleles in common, while *Buhavica* and *Drobnica* as well as *Buhavica* and *Oblica* contain 13 and 10 alleles in common, respectively. Divergence of *Lastovka* from the other three cultivars is greater than any of their mutual divergences and it has only 11 alleles in common with *Drobnica*, and only 6 alleles in common with *Buhavica* and *Oblica.* According to the available literature, this is the first report on the genetic profile of *Buhavica* and *Drobnica* cultivars.

Chemical characterization

As a part of the chemical composition analysis of investigated olive oils, the effects of the harvest period on phenolic secoiridoids content, fatty acid profile and squalene concentration were studied. The phenolic profile of olive oil extracted from the olives at different ripening stages evidences significant differences between the investigated cultivars. The results of qNMR analysis of major secoirdioids detected in olive oil are presented in Table 2.

The obtained results warrant the conclusion that total phenolic content as well as the change of phenolics secoiridoids composition in the oil from early and late period of harvest is related to olive cultivar. In comparison with *Buhavica, Lastovka* and *Oblica* olive oils that showed higher concentration of total phenolic content at the early harvest period than at the late harvest period, the total phenolic content in *Drobnica* oil was not significantly different at both harvest periods. The concentration of phenolics in *Lastovka* and *Oblica* oils was more than twofold higher in the samples from the early harvest period which is in accordance with the result of the study reported by Gouvinhas et al. [3].

D1 index (oleocanthal + oleacein content) of *Drobnica* oil was higher (966 mg/kg of oil from the late harvest period) than the results obtained in the study by Karkoula et al. [22] in which among 175 monovarietal Greek and Californian olive oils the highest D1 index was 501 mg/kg in the oil of *Koroneiki* variety (Paros). Except for *Drobnica* oil, a very high D1 index was detected for *Lastovka* from early harvest (563 mg/kg) as well as for *Oblica* cultivar from both periods (532 and 651 mg/kg).

As expected, the fatty acid composition, especially the amount of oleic acid which ranged from 62% in *Oblica* oil to 71% in *Lastovka* oil, was almost unchanged or only

Table 2 ^{1}H-NMR analysis of major secoirdioids (in mg/kg of oil) in *Buhavica, Drobnica, Lastovka* and *Oblica* olive oils obtained from fruits from different maturity stages. For all measurements SD value was lower than 5%

Oil sample	Oleocanthal	Oleacein	Oleuropein aglycon (monoaldehyde form)	Oleuropein aglycon (dialdehyde forms)	Ligstroside aglycon (monoaldehyde form)	Ligstroside aglycon (dialdehyde forms)	D1 (Oleocanthal + Oleacein)	Total tyrosol derivatives	Total hydroxytyrosol derivatives	Total measured phenols
Buhavica										
Early harvest	137	281	77	63	6	129	418	219	473	692
Late harvest	110	191	96	63	12	92	301	218	347	565
Drobnica										
Early harvest	74	230	258	383	51	584	304	384	1197	1581
Late harvest	269	697	181	151	25	261	966	475	1109	1584
Lastovka										
Early harvest	26	20	129	201	28	261	46	183	482	665
Late harvest	201	362	258	164	35	395	563	495	921	1416
Oblica										
Early harvest	274	258	660	154	74	449	532	1008	861	1869
Late harvest	261	390	77	23	0	153	651	338	565	903

Table 3 Fatty acid profile of *Buhavica, Drobnica, Lastovka* and *Oblica* olive oils obtained from fruits from different maturity stages

Fatty acid (%)	*Oil sample*							
	Buhavica		*Drobnica*		*Lastovka*		*Oblica*	
	Early harvest	Late harvest	Early harvest	Late harvest	Early harvest	Late harvest	Early harvest	Late harvest
Palmitic ($C_{16:0}$)	11.37 ± 0.01	13.18 ± 0.79	15.50 ± 0.66	16.43 ± 0.23	16.41 ± 0.68	15.17 ± 0.09	19.18 ± 0.03	19.09 ± 0.32
Palmitoleic ($C_{16:1}$, n-9)	0.13 ± 0.00	0.15 ± 0.13	0.10 ± 0.00	0.13 ± 0.00	0.10 ± 0.01	0.09 ± 0.00	0.10 ± 0.00	0.11 ± 0.00
Palmitoleic ($C_{16:1}$, n-7)	0.43 ± 0.00	0.96 ± 0.07	1.24 ± 0.07	1.78 ± 0.03	1.90 ± 0.06	2.19 ± 0.00	0.90 ± 0.00	0.62 ± 0.02
Heptadecanoic ($C_{17:0}$)	0.26 ± 0.02	0.20 ± 0.02	0.02 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.05 ± 0.00
Heptadecenoic ($C_{17:1}$)	0.37 ± 0.00	0.31 ± 0.01	0.07 ± 0.00	0.11 ± 0.00	0.09 ± 0.00	0.08 ± 0.00	0.07 ± 0.00	0.07 ± 0.00
Stearic ($C_{18:0}$)	3.35 ± 0.00	3.16 ± 0.07	2.55 ± 0.07	1.85 ± 0.00	2.26 ± 0.01	2.21 ± 0.02	2.40 ± 0.00	2.04 ± 0.01
Vaccenic ($C_{18:1}$ *trans*)	n.d.	0.01 ± 0.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Oleic ($C_{18:1\ n\text{-}9}$)	69.14 ± 0.01	67.11 ± 0.94	67.19 ± 0.81	67.03 ± 0.58	70.99 ± 0.78	70.39 ± 0.14	62.92 ± 0.16	62.45 ± 0.20
Linoleic ($C_{18:2}$)	12.03 ± 0.03	12.22 ± 0.14	10.09 ± 0.09	9.72 ± 0.01	6.58 ± 0.05	7.05 ± 0.01	11.57 ± 0.02	13.31 ± 0.05
18:2 *trans–cis*	0.02 ± 0.00	0.02 ± 0.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
18:2 *cis–trans*	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Linolenic ($C_{18:3}$)	0.81 ± 0.00	0.71 ± 0.01	0.75 ± 0.00	0.79 ± 0.00	0.74 ± 0.00	0.55 ± 0.00	0.78 ± 0.00	0.64 ± 0.00
Arachidic ($C_{20:0}$)	0.52 ± 0.01	0.46 ± 0.03	0.39 ± 0.03	0.33 ± 0.01	0.34 ± 0.00	0.32 ± 0.00	0.39 ± 0.00	0.35 ± 0.00
Gadoleic ($C_{20:1}$)	0.37 ± 0.01	0.34 ± 0.02	0.28 ± 0.02	0.31 ± 0.01	0.27 ± 0.01	0.24 ± 0.00	0.29 ± 0.00	0.30 ± 0.00
Behenic acid ($C_{22:0}$)	0.14 ± 0.00	0.13 ± 0.00	0.12 ± 0.01	0.12 ± 0.00	0.10 ± 0.00	0.08 ± 0.00	0.11 ± 0.00	0.10 ± 0.00
Lignoceric acid ($C_{24:0}$)	0.06 ± 0.02	0.06 ± 0.00	0.19 ± 0.01	0.16 ± 0.01	0.15 ± 0.01	0.04 ± 0.00	0.11 ± 0.01	0.03 ± 0.01

Table 4 Squalene content in *Buhavica, Drobnica, Lastovka* and *Oblica* olive oils from different harvest periods

Oil sample	Squalen (mg/100 g oil)	
	Early harvest	Late harvest
Buhavica	987.89 ± 24.36	565.88 ± 11.60
Drobnica	1285.95 ± 10.72	874.82 ± 22.12
Lastovka	602.88 ± 53.93	519.64 ± 4.87
Oblica	984.58 ± 67.82	878.48 ± 43.85

Table 5 Oxidative stability (induction time) of olive oils from *Buhavica*, *Drobnica*, *Lastovka* and *Oblica* cultivars from early and late stage of harvest, compared to synthetic antioxidants

Oil sample	Induction time (h)
Buhavica	
Early harvest	14.90 ± 0.11
Late harvest	13.98 ± 0.28
Drobnica	
Early harvest	20.95 ± 0.31
Late harvest	16.79 ± 0.09
Lastovka	
Early harvest	18.65 ± 0.55
Late harvest	12.80 ± 0.40
Oblica	
Early harvest	12.27 ± 0.01
Late harvest	7.71 ± 0.01

slightly changed in relation to the period of the harvest (Table 3). Although there are no significant changes in fatty acid profile of oils from early and late harvest, the oleic acid level slightly decreased during ripening in all cultivar oils which contradicts the results obtained in the study of Brkić-Bubola et al. [14] on the two Istrian olive varieties, *Buža* and *Črna*.

Squalene, terpenoid hydrocarbon, is a minor constituent of the olive oil which makes up for more than 90% of the oil hydrocarbon fraction [7]. Generally, the content of squalene in investigated olive oils decreased at the late harvest period. However, the results presented in Table 4 do not confirm its rapid decrease during the stage of olive maturity as it was reported by D'Imperio et al. [33]. The highest content of squalene was detected in *Drobnica* oil (1285.95 mg/100 g oil) from the early harvest period. The contents of squalene in *Buhavica* and *Oblica* oils from early harvest were almost the same, while *Lastovka* oil contained more than twofold lower concentration of squalene than *Drobnica* oil. Although decrease in squalene content was detected in oils from late harvest in all cultivars, the differences in its content in *Buhavica* and *Drobnica* as well as in *Drobnica* and *Oblica* were minimal.

Oxidative stability

Results presented in Table 5 show the oxidative stability of investigated olive oils extracted from fruits harvested

in the early and late period. The oxidative stability testing was performed to find the connection between chemical composition of oils and their degradation under accelerated conditions [6]. With the exception of *Buhavica* oil, all other oils showed a higher oxidative stability at the early stage of fruit maturity. *Drobnica* oil from the early harvest period showed the highest oxidative stability (induction time was 20.95 h) while high stability was also detected for *Lastovka* oil from the early harvest period (18.65 h) in comparison with oils of *Oblica* and *Buhavica*.

If we compare the obtained results for oxidative stability with the content of total phenolic secoiridoids, we can conclude that the highest content of these compounds did not result in the highest oxidative stability (the case of *Oblica* oil). Obviously, it is important to point out that the composition of phenolics secoiridoids (or their mutual ratio) is a more important factor for the prolongation of the oxidative stability. To corroborate this state, we statistically analyzed the correlation between the content of detected secoirdioid compounds and oil oxidative stability, and significant positive correlation between the content of oleuropein aglycon (dialdehyde forms) contents and induction periods was detected ($r = 0.7248$; $p = 0.02419$). In that sense, *Drobnica* oil with the highest oxidative stability showed low content of oleocanthal (74 mg/kg at the early stage). The shortest induction time was detected for *Oblica* oil (12.27 and 7.71 h). The effect of tyrosol and hydroxytyrosol on olive oil oxidative stability has already been reported [6]. Furthermore, different authors have researched the influence of oleic and linoleic acid content on oxidative stability of olive oils and the obtained results indicate that the ratio of monounsaturated and polyunsaturated fatty acids is one of the factors that also affect oil oxidative stability [16, 34]. The ratio of oleic and linoleic acid from *Buhavica*, *Drobnica*, *Lastovka* and *Oblica* oils did not differ significantly in the frame of the harvest period and a slight decrease of that value in the samples from late harvest was recorded for all samples. The highest values were detected for *Drobnica* (ratio 6.66 and 6.89) and *Lastovka* (10.79 and 9.98) oils which resulted the longest induction period, while the shortest induction period (7.71 h) was recorded for *Oblica* oil from late harvest in which oleic and linoleic acid ratio was only 4.69.

Psomiadou and Tsimidou [35] have investigated the potential influence of squalene content and olive oil oxidative stability and have concluded that this compound plays a limited role in olive oil stability, while Naziri et al. [36] have investigated the oxidation products of squalene and their prooxidant activity. Although squalene is considered as a relatively stable molecule under autoxidation conditions, its degradation products actively participate in propagation reactions [37]. *Lastovka* oil had the lowest concentration of squalene and its oxidative stability after the early harvest period was longer than the stability of oils with higher content of squalene (*Buhavica* and *Oblica*). In this study, the oxidative stability of olive oil enriched with squalene (0.83 v/v%, 1.66 v/v%, and 3.33 v/v%) was measured, and the results showed that squalene did not have any effect on the prolongation of oil oxidative stability.

Sensory evaluation

Descriptive sensory analysis of investigated olive oils was used to define differences in intensities of certain sensations regarding harvest time for each olive cultivar. The early *Drobnica* and *Lastovka* oils showed the highest content of oleuropein aglycon, which is in concordance with rather high bitterness detected in these oils, since this phenolic compound is responsible for bitter taste of olive oils. In the late harvested *Drobnica* oil the decreasing of bitter taste, as well as intensity of other typical sensory attributes (artichoke, chicory, coffee, and leaf) is detected. On the other hand, the later harvested *Lastovka* oil showed almost unchanged intensity of bitterness, astringent and pungent sensation, as well as some typical varietal descriptors (chicory, leaf, almond and coffee). The early harvested *Buhavica* oil exhibited a good balance of bitter and pungent sensations, which decreased in the later harvested oil, as well as bitter almond sensation and persistency. It is interesting to notice that in *Buhavica* oil from late harvest a typical sensation of boiled vegetable was perceived. *Oblica* oils had the lowest detected levels of bitterness and pungency which is linked to the lowest oxidative stability measured for this oil. However, *Oblica* oil from early harvest had a well-balanced intensity of bitterness and pungency, along with bitter almond and artichoke sensation, while in the late harvested *Oblica* oil bitterness and pungency significantly decreased, and apple and tomato fruitiness were predominant sensory attributes, along with the lower intensity of bitter almond.

The sensory analyses of monovarietal olive oils showed particular attributes of taste and aroma that is featured exclusively by olive cultivar. Sensory characteristics of the samples vary depending on the olive variety and have certain properties for each monovarietal olive oil.

Conclusion

Oblica and *Drobnica* are molecule-wise most similar cultivars, while *Lastovka* cultivar diverges from the other tested Croatian autochthonous olive cultivars. Concerning the amount of phenolic secoiridoids in relation to early and late harvest periods, *Drobnica* showed the highest amount of phenolic secoiridoids after both harvest periods. This study has proved the poor correlation between the phenolic

content as well as squalene content in the oil and the oil oxidative stability.

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Compliance with ethical standards

Conflict of interest None.

Compliance with ethics requirements This article does not contain any studies with human or animal subject.

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