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Morphological and Molecular Characterization of Bova Olive Cultivar and Aroma Fingerprint of Its Oil

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Summary

This interdisciplinary study aims to contribute to the characterization of Istrian (Croatia) olive cultivars and oil, giving for the first time the morphological and genetic profile of Bova cultivar, and chemical and sensorial characteristics of its oil. Morphological features of Bova cv. were determined according to the International Olive Council methodology, while molecular characterization was performed using eleven microsatellite markers. Bova cultivar was morphologically and genetically different from other described Istrian olive cultivars. The microsatellite profile of Bova was also unique when compared to more than 200 different Italian genotypes using the same set of markers. In order to characterize the oil from Bova cv., fruits from three trees at the same ripening stage were harvested and processed separately under the same conditions. Volatile composition of the obtained oil samples was determined using solid-phase microextraction with gas chromatography/mass spectrometry. About 50 volatiles were detected, mostly hydrocarbons (34.69 % of total peak area), followed by aldehydes (25.80 %), alcohols (22.24 %), ketones (8.76 %), organic acids (4.08 %), terpenes (2.10 %), esters (2.18 %) and furans (0.26 %). Bova oil was rich in total C6 (39.87 %) and C5 volatiles (13.85 %), biogenerated through the lipoxygenase pathway. The most prevalent volatile compound was C6 aldehyde *E*-2-hexenal. Quantitative descriptive sensory analysis of the investigated olive oil samples was carried out by a sensory panel. The sensory profile was characterized by medium intensities of olive fruity, green leaves and grass, light tomato and aromatic herbs flavours with mild apple and other ripe fruit notes. The taste was characterized by medium to strong bitterness, followed by medium pungency and mild sweetness.

Key words: *Olea europaea* L., morphological characteristics, DNA fingerprinting, olive oil, volatiles, sensory characteristics

Introduction

Autochthonous olive cultivars (*Olea europaea* L.) are important not only for biodiversity preservation but also for their specific adaptation to local growing conditions (*1*). According to the Croatian National list of fruit varieties from 2012 (*2*), 15 autochthonous cultivars are present in the country and six of them are concentrated on a relatively small area (3132 km^2) along the Istrian peninsula. Up to date a larger number of autochthonous cultivars have been morphologically and genetically identified in Istria (*1,3–5*). Bova cultivar, outspread in the western

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and northern parts of the peninsula (*3,4*), is relatively unknown and has not been widely investigated so far.

Virgin olive oil is highly appreciated for its nutritional benefits, but its delicious taste and aroma are in fact the key characteristics for consumer acceptability (*6*). Virgin olive oil aroma consists of a complex mixture of various classes of volatile compounds, which include mainly hydrocarbons, aldehydes, alcohols, ketones and esters (*7*). However, its particularity and delicacy are primarily ascribed to C6 and C5 volatiles, biogenerated from polyunsaturated fatty acids through the lipoxygenase pathway. The biosynthesis of these compounds starts at the moment of cell disruption during crushing of olive fruits and continues during oil extraction (*6*). Aroma formation of olive oil is a dynamic process and depends on a number of factors, among which genotype (cultivar) is probably the most important (*8–11*).

Deep study and characterization of olive cultivars is quite important because each of them can have specific agronomic features or produce specific olive oil, which can be exploited to differentiate the production in different regions. Considering its relatively high oil yield, regional exclusivity and branding potential, very few investigations of Bova cultivar at the morphological level have been performed up to date (*3,4*). Because of that, and because a complete lack of genetic, volatile aroma and sensory analysis data regarding Bova cultivar and its oil in the literature, we have conducted this investigation to give the first morphological and genetic description, as well as monovarietal olive oil characterization of this local cultivar.

Materials and Methods

Morphological characterization of olive cultivar

Morphological description of Bova cultivar was performed according to the International Olive Council (IOC) methodology (*12*) on olive leaves, fruits and stones obtained from three trees grown in the collection of Institute of Agriculture and Tourism in Poreč (Istria, Croatia). Flesh/stone ratio was calculated according to the pomological characterization procedure proposed by the IOC (*13*).

DNA extraction and microsatellite genotyping

Fresh leaves of Bova cultivar were sampled from the collection orchard of the Institute of Agriculture and Tourism in Poreč, Istria. Plant material from 17 local accessions was sampled from 12 locations in the western and northern parts of Istrian peninsula. These accessions to be used in comparison with Bova cv. were selected on the basis of previous research made in the area (unpublished data). The list includes plants with uncertain attribution, local names and areal distribution analogous to Bova cultivar. Total genomic DNA was extracted from 100 mg of fresh leaves using DNeasy Plant Mini Kit (Qiagen, Venlo, the Netherlands) following the manufacturer's instructions. The analysis was performed using 11 simple sequence repeats (SSRs) developed by several authors and selected based on their high levels of polymorphism: ssrOeUA-DCA03, ssrOeUA-DCA04, ssrOeUA-DCA14, ssrOeUA-DCA16, ssrOeUA-DCA17, ssrOeUA-DCA18 (*14*), UDO99-43 (*15*), EMOL (*16*), GAPU45, GAPU71b and GAPU103 (*17*). These markers, characterized by a very high discrimination power, were chosen among those used in the framework of the Italian interregional project OLVIVA funded by the Interregional Programme 'Rural development', subprogramme 'Innovation and research' No. 25279 in 2003.

Amplification by polymerase chain reaction (PCR) was carried out in a total volume of 25 μL containing 25 ng of genomic DNA, 0.625 units of Thermo Scientific DreamTaq DNA Polymerase, 1× PCR buffer (DreamTaq buffer, Fermentas, Thermo Scientific, Waltham, MA, USA), 200 mM of each CleanAmp dNTP (Sigma-Aldrich Chemie GmbH, Munich, Germany), and 2 mM of forward (fluorescently labelled) and reverse primers. Reactions were performed in a thermal cycler (Eppendorf Mastercycler Gradient, Hamburg, Germany) starting with a denaturation step at 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s at the annealing temperature ($t_{annealing}$ as indicated in Table 1) and 72 °C for 30 s, plus a final extension for 8 min at 72 °C. A volume of 2 μL of each PCR product were mixed with 0.25 μL of Et400-R size standard (GE Healthcare, Milwaukee, WI, USA), and 4.9 μL of deionised H_2O, centrifuged, denatured at 95 °C for 2 min, cooled in ice and separated on a MegaBACE 500 capillary sequencer (GE Healthcare).

Table 1. List of SSR markers and annealing temperatures used in this study

SSR	$t_{annealing}$/°C
GAPU103	58
GAPU71b	57
GAPU45	57
UDO99-43	57
ssrOeUA-DCA03	50
ssrOeUA-DCA04	55
ssrOeUA-DCA14	50
ssrOeUA-DCA16	50
ssrOeUA-DCA17	55
ssrOeUA-DCA18	50
EMOL	55

Dye-labelled amplicons were automatically sized using internal standards and the Fragment Profiler v. 1.2 software (GE Healthcare) and then visually inspected. To avoid variation in allele sizing, reference genotypes with specific alleles for each locus were used.

To clarify the genetic relationships of Bova cv. with accessions and varieties native to Istria (Croatia) and Italy, traditionally closely related by reproductive material trade, the fingerprinting of Bova cv. was compared to other 16 Istrian accessions spread in the same area and more than 200 accessions from Italy, analyzed with the same set of markers. The genetic distance of Bova cv. from other olive accessions was calculated on the basis of the number of shared bands (*18*) using the NTSYSpc package (Exeter Software, East Setauket, NY, USA) (*19*). Sequential agglomerative hierarchical nested cluster analysis with unweighted pair group method of clustering

(UPGMA) was successively applied and the tree plot procedure of the same package was finally used to provide a graphic representation of Lynch's similarity index data, from which relationships among accessions can be deduced.

Preparation of virgin olive oil samples

Approximately 1.5 kg of olive fruits from each of the three trees of Bova cv. with the same ripening index (RI=3) were handpicked in November 2010. RI of fruits was determined applying the method described by Garcia and Yousfi (*20*), which is based on the evaluation of the olive skin and pulp colour. Olive fruits from each tree were processed separately using an Abencor system (MC2 Ingenieria y Sistemas, Sevilla, Spain) within 12 hours after harvesting. Fruits were crushed with a hammer mill. Olive paste samples were malaxed with a thermomixer at (25±1) °C for 45 min. After centrifugation at 1370×*g* for 60 s, extracted olive oil samples were decanted and stored at room temperature in taped dark bottles filled with nitrogen until analyses.

Analysis of volatile compounds

Volatile composition of Bova olive oil samples was determined using headspace solid-phase microextraction with gas chromatography/mass spectrometry (HS-SPME/GC-MS). A virgin olive oil sample (4.0 g) was placed in a 10-mL vial containing a microstirring bar, and sealed with a PTFE/silicone septum (Restek, Bellefonte, PA, USA). Before extraction, the headspace in the vial was stabilized by equilibration at 40 °C for 10 min, and by gentle stirring for 3 min with a magnetic stirrer. The extraction was carried out at 40 °C for 40 min using the SPME holder for manual sampling and a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre, 1 cm length, 50/30 μm film thickness (Supelco, Bellefonte, PA, USA). Thermal desorption of the volatiles was achieved by inserting the fibre into the injection port of a GC/MS system equipped with a 0.80-mm i.d. SPME liner in splitless mode at 245 °C for 3 min. GC/MS analyses were performed using a Varian 3900 gas chromatograph coupled to a Varian Saturn 2100T ion trap mass spectrometer (Varian Inc., Harbour City, CA, USA) equipped with a 60 m×0.25 mm i.d., 0.25 μm film thickness, capillary column Rtx-WAX (Restek). Initial oven temperature was 40 °C, raised linearly to 245 °C at 1 °C/min. Transfer line and ion trap temperatures were 180 and 120 °C, respectively. Mass spectra were acquired in the electron impact mode (70 eV) at 1 scan per s, using full scan with a mass acquisition range of 30–450 atomic mass units (amu). Helium was used as a carrier gas with a flow rate of 1 mL/min. The identification of volatile compounds was performed by comparing their mass spectra with those of pure standards and to mass spectra from NIST05 library. Additionally, the identification of twenty volatile compounds was performed by comparing their retention times with those of pure standards. All standards had a GC purity ≥95 % and were purchased from Sigma-Aldrich (Steinheim, Germany), Fluka (Buchs, Germany), and Merck KGaA (Darmstadt, Germany). Moreover, Kováts retention indices (KI) were determined on the polar Rtx-WAX column by injection of a standard mixture containing the homologous series of normal alkanes (C7–C24) in pure dichloromethane, and compared with retention indices of the compounds available in the literature (*21–25*). The relative proportions of the volatile compounds were obtained by peak area normalization. For each volatile compound, mean proportions of three independent repetitions and proportion ranges are reported.

Sensory analysis

Quantitative descriptive analysis of olive oil samples was performed by a panel of twelve assessors trained for virgin olive oil sensory analysis. Different positive odour descriptors (olive fruity, other ripe fruits, apple, green grass or leaves) and taste descriptors (bitter, pungent and sweet) as well as unpleasant attributes (winey/vinegary, rough, metallic, musty, muddy sediment, fusty, rancid) were quantified using a six-point intensity ordinal rating scale from 0 (no perception) to 5 (extreme).

Results and Discussion

Morphological characteristics

Morphological characteristics of Bova cultivar are presented and compared to the most widespread Istrian autochthonous cultivars in Table 2 (*5,12,26*). The leaves had medium length ((6.9±0.9) cm) and medium width ((1.3±0.2) cm). The length of fruits was (2.7±0.1) cm, while the width was (2.1±0.1) cm, and according to length/width ratio, the fruits were classified as ovoid (Table 2). Stone length was (1.8±0.1) cm, while the width was (1.1±0.1) cm and after calculating length/width ratio, the stone shape was classified as ovoid (Table 2). Compared to other previously investigated Istrian cultivars (*1*), Bova cultivar had the heaviest fruits ((5.7±0.6) g) and stones ((0.9±0.1) g). However, the fruit/stone mass ratio calculated for Bova cv. samples (6.8±1.0) was higher than the ratio previously reported for the same cultivar by other authors (*3,4*). Differences in fruit/stone mass ratio of the same cultivar could be caused by different growing conditions (*27,28*). Therefore, stone morphological traits including surface texture and shape, which are less influenced by environmental conditions than the leaves and drupes, are a helpful tool for cultivar characterization and identification (*29–31*). Bova cultivar stone (Table 2) was morphologically different compared to other investigated Istrian autochthonous cultivars (*5,26*).

Molecular characterization

Amplification was successful with all eleven SSR markers assayed. They were all polymorphic but EMOL, which presented only a single allele in all the accessions. The number of alleles ranged from four at locus DCA14 to ten at loci DCA16 and GAPU45, with an average number of 7 alleles per locus in the 17 examined accessions. SSR profiles of the analysed accessions are presented in Table 3. Bova cultivar fingerprinting profile was different from all the other Istrian accessions used for comparison, sharing 9–15 out of 22 observed alleles. The differences between Bova cv. and the other studied accessions can be seen in the dendrogram produced on

Table 2. Morphological characteristics according to the International Olive Council standards (*12*) of Bova cultivar compared to the most widespread Istrian autochthonous cultivars

Cultivar	Characteristic									
	Leaf*		Fruit*							
	shape	longitudal curvature of the blade	shape	symmetry	position of max. transverse diameter	apex	base	nipple	presence of lenticels	size of lenticels
Bova	eliptic-lanceolate (2)	flat (2)	ovoid (2)	asymmetric (3)	central (2)	rounded (2)	truncate (1)	tenuous (2)	many (2)	large (2)
Reference cultivars										
Buža (*26*)	eliptic-lanceolate (2)	flat (2)	spherical (1)	symmetric (1)	central (2)	rounded (2)	truncate (1)	absent (1)	many (2)	small (1)
Buža puntoža (*26*)	eliptic-lanceolate (2)	flat (2)	ovoid (2)	symmetric (1)	central (2)	pointed (1)	truncate (1)	tenuous (2)	many (2)	small (1)
Istarska bjelica (*26*)	eliptic-lanceolate (2)	helicoid (4)	spherical (1)	symmetric (1)	central (2)	rounded (2)	truncate (1)	absent (1)	many (2)	small (1)
Rosinjola (*26*)	elliptic (1)	flat (2)	ovoid (2)	symmetric (1)	central (2)	rounded (2)	truncate (1)	absent (1)	few (1)	small (1)
Bjelica (*5*)	eliptic-lanceolate (2)	flat (2)	ovoid (2)	symmetric (1)	central (2)	n.a.	n.a.	n.a.	n.a.	n.a.
Črna (*5*)	eliptic-lanceolate (2)	flat (2)	ovoid (2)	symmetric (1)	central (2)	n.a.	n.a.	n.a.	n.a.	n.a.
Črnica (*5*)	eliptic-lanceolate (2)	flat (2)	ovoid (2)	symmetric (1)	central (2)	n.a.	n.a.	n.a.	n.a.	n.a.
Karbonera (*5*)	eliptic-lanceolate (2)	flat (2)	ovoid (2)	symmetric (1)	central (2)	n.a.	n.a.	n.a.	n.a.	n.a.
Drobna (*5*)	eliptic-lanceolate (2)	flat (2)	spherical (1)	symmetric (1)	central (2)	n.a.	n.a.	n.a.	n.a.	n.a.
Karbonaca (*5*)	eliptic-lanceolate (2)	flat (2)	ovoid (2)	symmetric (1)	central (2)	n.a.	n.a.	n.a.	n.a.	n.a.
Moražo (*5*)	elliptic (1)	flat (2)	spherical (1)	symmetric (1)	central (2)	n.a.	n.a.	n.a.	n.a.	n.a.
Bilica (*5*)	eliptic-lanceolate (2)	flat (2)	ovoid (2)	symmetric (1)	central (2)	n.a.	n.a.	n.a.	n.a.	n.a.
Belica (*5*)	eliptic-lanceolate (2)	flat (2)	ovoid (2)	symmetric (1)	central (2)	n.a.	n.a.	n.a.	n.a.	n.a.

Cultivar	Stone*										Diff.
	shape	symmetry (position A)	symmetry (position B)	position of max. transverse diameter	apex	base	surface	number of grooves	distribution of grooves	termination of apex	
Bova	ovoid (2)	slightly asymmetric (2)	symmetric (1)	towards apex (3)	rounded (2)	rounded (3)	rugose (2)	medium (2)	regular (1)	with mucro (2)	
Reference cultivars											
Buža (*26*)	ovoid (2)	slightly asymmetric (2)	symmetric (1)	central (2)	rounded (2)	rounded (3)	rugose (2)	medium (2)	regular (1)	with mucro (2)	1
Buža puntoža (*26*)	elliptic (3)	symmetric (1)	symmetric (1)	central (2)	pointed (1)	rounded (3)	scabrous (3)	medium (2)	regular (1)	with mucro (2)	5
Istarska bjelica (*26*)	ovoid (2)	symmetric (1)	symmetric (1)	central (2)	rounded (2)	rounded (3)	scabrous (3)	medium (2)	regular (1)	with mucro (2)	3
Rosinjola (*26*)	ovoid (2)	symmetric (1)	symmetric (1)	towards apex (3)	rounded (2)	pointed (2)	rugose (2)	medium (2)	regular (1)	with mucro (2)	2
Bjelica (*5*)	elliptic (3)	n.a.	n.a.	central (2)	rounded (2)	rounded (3)	rugose (2)	n.a.	n.a.	n.a.	2
Črna (*5*)	ovoid (2)	n.a.	n.a.	central (2)	rounded (2)	pointed (2)	rugose (2)	n.a.	n.a.	n.a.	2
Črnica (*5*)	ovoid (2)	n.a.	n.a.	towards apex (3)	rounded (2)	rounded (3)	scabrous (3)	n.a.	n.a.	n.a.	1
Karbonera (*5*)	ovoid (2)	n.a.	n.a.	central (2)	rounded (2)	rounded (3)	rugose (2)	n.a.	n.a.	n.a.	1
Drobna (*5*)	spherical (1)	n.a.	n.a.	central (2)	rounded (2)	rounded (3)	scabrous (3)	n.a.	n.a.	n.a.	3
Karbonaca (*5*)	ovoid (2)	n.a.	n.a.	central (2)	rounded (2)	rounded (3)	scabrous (3)	n.a.	n.a.	n.a.	2
Moražo (*5*)	spherical (1)	n.a.	n.a.	central (2)	rounded (2)	rounded (3)	rugose (2)	n.a.	n.a.	n.a.	2
Bilica (*5*)	elliptic (3)	n.a.	n.a.	towards apex (3)	rounded (2)	rounded (3)	scabrous (3)	n.a.	n.a.	n.a.	2
Belica (*5*)	ovoid (2)	n.a.	n.a.	central (2)	rounded (2)	rounded (3)	rugose (2)	n.a.	n.a.	n.a.	1

*Numbers in brackets represent the category code of each characteristic; n.a.=not analyzed; Diff.=number of differences between Bova and reference cultivars (*5,26*)

Table 3. Genotypes of analysed Croatian accessions at eleven microsatellite loci (allele sizes in bp)

Locus / Accession	DCA3	DCA4	DCA14	DCA16	DCA17	DCA18	EMOL	GAPU45	GAPU71b	GAPU103	UDO99-43
1	241:251	134:165	189:189	150:164	115:115	173:173	202:202	163:179	183:183	136:170	162:178
2	241:251	134:167	189:189	150:150	115:115	173:173	202:202	183:185	181:183	174:176	999:999
3	241:251	134:167	189:189	150:174	115:115	163:191	202:202	163:179	121:141	136:160	999:999
4	241:249	134:167	189:189	150:174	115:115	173:197	202:202	163:179	183:183	160:172	178:180
5	241:251	134:167	189:189	140:174	115:115	173:177	202:202	179:179	121:141	136:160	180:218
6	241:249	134:167	189:189	150:174	115:115	173:177	202:202	179:187	121:141	136:160	176:180
7	241:251	134:167	189:189	150:166	115:115	173:177	202:202	179:187	121:999	136:160	178:188
8	235:245	134:167	149:189	144:154	125:127	159.177	202:202	163:179	121:127	136:160	180:220
9 (Bova)	**235:241**	**130:167**	**189:189**	**154:174**	**115:115**	**173:177**	**202:202**	**163:179**	**127:141**	**150:160**	**180:180**
10	235:241	132:134	189:189	146:174	115.177	177:185	202:202	183:183	127:141	136:176	178:188
11	241:255	134:167	189:189	150:174	115:115	173:185	202:202	163:215	127:141	136:176	216:216
12	235:241	130:134	189:189	152:152	115:115	173:177	202:202	179:217	121:141	160:196	218:218
13	235:251	130:134	191:191	146:174	115:115	173:177	202:202	179:217	121:141	150:160	218:218
14	235:251	132:134	189:189	156:174	115:115	173:177	202:202	292:292	121:141	160:196	218:218
15	241:245	165.165	180:189	150:154	115:115	185:219	202:202	296:296	167:177	160:196	178:218
16	245:245	134.134	180:189	146:174	161:179	185:219	202:202	185:195	124:185	160:196	218:218
17	239:255	132.167	180:189	156:174	117:177	177:219	202:202	183:185	185:185	160:196	999:218

999=not identified

the basis of the genetic similarity calculated taking into consideration the shared bands (Fig. 1). As it can be seen, microsatellite profile of Bova cv. can be grouped in a cluster with other two accessions (numbers 10 and 11), sharing 10 and 13 out of 22 observed alleles. Both 10 and 11 are unknown varieties, morphologically clearly distinguished but originating from a restricted area on the west Istrian coast, with a coefficient of similarity of 70–75 %. The level of similarity and the differences in morphological traits of accessions 10 and 11 from Bova cv. indicate that, although probably originating from common ancestors within the area of cultivation, they represent in fact well differentiated cultivars and can be taken into consideration for further propagation and valorisation separately from Bova cultivar. All Istrian accessions were well separated in a single cluster when compared to more than 200 different Italian genotypes (data not shown). Croatian olive plants presented a low coefficient of similarity with the Italian cultivars (13 %), showing the specificity of the genetic origin and at the same time the strict relationship among them.

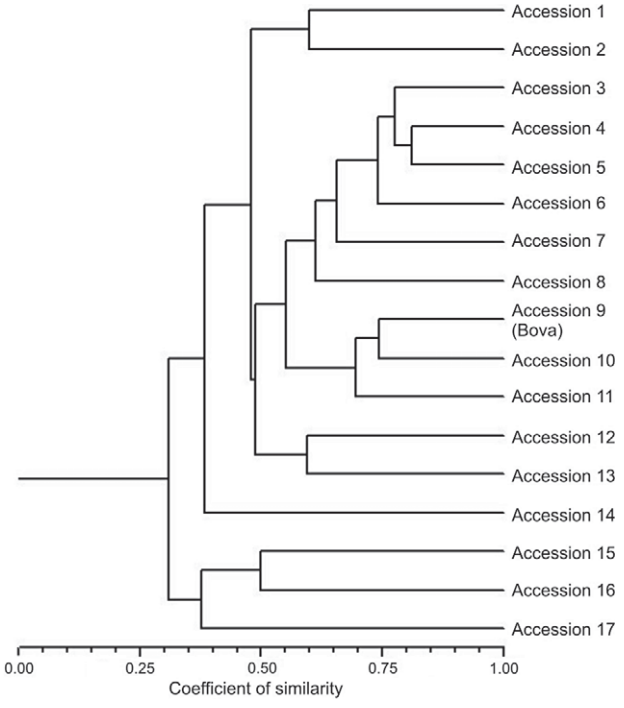

Fig. 1. Dendrogram of 17 Istrian accessions based on Lynch's genetic similarity index data

Volatile compounds and sensory profile

Fifty volatile compounds, which belong to hydrocarbons, aldehydes, alcohols, ketones, organic acids, terpenes, esters and furans, were isolated and characterized by HS-SPME/GC-MS analysis (Table 4). Bova oil samples were rich in total C6 (39.87 % of total peak area) and C5 volatiles (13.85 % of total peak area). The C6 volatiles, originating in the lipoxygenase pathway, contribute to green olive oil aroma, while C5 volatiles, which originate in the additional branch of the lipoxygenase pathway (*32*), contribute to pleasant aroma and positively correlate with bitterness and pungency of virgin olive oil (*6*). The most prevalent volatile compound in Bova oil was C6 aldehyde *E*-2-hexenal (Table 4), which is in accordance with the literature data on other investigated Istrian monovarietal types of olive oil (*33*). *E*-2--hexenal contributes to green, fruity, bitter and astringent sensory characteristics of olive oil (*8*). Generally, aldehydes are major contributors to olive oil odour because they have very low thresholds, and are usually related to positive sensory characteristics, such as green, fruity and bitter (*8*). The most abundant alcohols in Bova oil were *Z*-3-hexen-1-ol and *E*-2-hexen-1-ol (Table 4). Alcohols in olive oil are related to positive sensory characteristics, such as olive fruity, other fruits, green and aromatic, but since they have higher threshold values, they have lower sensory significance than aldehydes (*8*). In

Table 4. Identification, proportion ranges and mean proportion values of peak area of volatile compounds in Bova cultivar virgin olive oil

	Compound	Identification method	KI*	KI_{ref}	Mean value**/%	Range	
						Min.	Max.
	Aldehydes						
1	Hexanal	KI, MS, RT	1068	1074[a], 1080[b], 1073[c]	2.76	1.44	3.90
2	*Z*-3-Hexenal	KI, MS	1126	1137[a], 1115[b]	0.69	0.32	1.12
3	Heptanal	KI, MS	1171	1184[a], 1190[b]	0.04	0.01	0.07
4	*E*-2-Hexenal	KI, MS, RT	1213	1216[a], 1225[b], 1129[c]	20.04	10.09	39.10
5	Octanal	KI, MS, RT	1277	1288[a], 1296[b], 1297[c]	0.10	0.03	0.23
6	(*E*,*E*)- or (*E*,*Z*)-2,4-Hexadienal	KI, MS	1376	1397[a], 1441[b], 1402[c]	0.66	0.34	0.92
7	(*E*,*E*)- or (*E*,*Z*)-2,4-Hexadienal	KI, MS	1379	1397[a], 1441[b], 1402[c]	1.30	n.i.	2.99
8	*E*-2-Octenal	KI, MS	1410	1425[a], 1432[d]	0.02	n.i.	0.06
9	(*E*,*E*)-2,4-Heptadienal	KI, MS	1443	1463[a]	0.19	n.i.	0.39
	Alcohols						
10	Ethanol	KI, MS	935	932[a], 935[c]	0.63	0.13	1.41
11	1-Penten-3-ol	KI, MS	1148	1164[a], 1166[b], 1163[c]	1.29	1.23	1.40
12	1-Pentanol	KI, MS	1240	1250[a]	0.11	n.i.	0.33
13	*E*-2-Penten-1-ol	KI, MS, RT	1302	1320[b], 1333[c]	0.97	0.58	1.21
14	*Z*-2-Penten-1-ol	KI, MS, RT	1310	1320[a], 1329[b], 1321[c]	2.72	2.41	3.06
15	Hexanol	KI, MS, RT	1344	1357[a], 1362[b], 1360[c], 1354[d]	1.81	0.83	2.74
16	*E*-3-Hexen-1-ol	KI, MS, RT	1353	1366[a], 1372[b], 1372[c]	0.42	0.04	0.98
17	*Z*-3-Hexen-1-ol	KI, MS, RT	1372	1385[a], 1392[b], 1385[c], 1388[d]	8.31	n.i.	19.67
18	*E*-2-Hexen-1-ol	KI, MS, RT	1396	1408[a], 1414[b], 1407[c]	5.77	0.10	15.86
19	Benzyl alcohol	KI, MS	1845	1883[a], 1890[b]	0.01	n.i.	0.03
20	Phenylethyl alcohol	KI, MS	1877	1919[a]	0.11	0.06	0.16
21	Phenol	KI, MS	1971	2020[b], 2035[c]	0.09	0.04	0.21
	Esters						
22	Butyl acetate	KI, MS, RT	1061	1077[d]	0.12	n.i.	0.34
23	3-Methylbutyl acetate	KI, MS, RT	1115	1120[a]	1.88	0.34	3.45
24	*Z*-3-Hexenyl acetate	KI, MS, RT	1308	1316[a], 1326[b], 1325[c]	0.17	n.i.	0.38
	Ketones						
25	3-Pentanone	KI, MS, RT	983	979[a], 983[b], 980[c]	2.56	0.03	6.97
26	1-Penten-3-one	KI, MS, RT	1010	1016[a], 1008[c]	6.21	n.i.	9.61
	Hydrocarbons						
27	n.i. unsaturated hydrocarbon (*m*/*z*=41,67,69,95,109)	–	963		1.04	0.17	1.53
28	n.i. unsaturated hydrocarbon (*m*/*z*=41,67,69,95,109)	–	969		1.42	1.30	1.50
29	Decane	KI, MS	1000	1001[b], 1000[d]	4.86	1.36	10.98
30	3-Ethyl-1,5-octadiene (*E* or *Z*)	KI, MS	1007	1012[a], 1013[b]	7.38	6.53	8.99
31	3-Ethyl-1,5-octadiene (*E* or *Z*)	KI, MS	1019	1018[a], 1027[b]	7.73	6.95	9.05
32	n.i. unsaturated hydrocarbon (*m*/*z*=41,67,69,95,109)	–	1176		2.83	2.41	3.24
33	n.i. unsaturated hydrocarbon (*m*/*z*=41,67,69,95,109)	–	1078		4.85	4.52	5.68
34	n.i. unsaturated hydrocarbon (*m*/*z*=41,67,69,95,109)	–	1084		4.07	3.42	4.62
35	*m*-Xylene	KI, MS	1124	1133[a], 1135[b], 1131[c], 1142[e]	0.02	n.i.	0.03
36	*o*-Xylene	KI, MS	1166	1187[b], 1174[a], 1189[e], 1182[d]	0.10	0.06	0.18
37	1,3,5-Trimethyl benzene	KI, MS	1228	1251[b]	0.03	n.i.	0.05
38	Styrene	KI, MS	1241	1265[a], 1261[d]	0.24	n.i.	0.73
39	1,2,3-Trimethyl benzene	KI, MS	1262	1274[a], 1287[b]	0.08	0.03	0.13
40	n.i. aromatic hydrocarbon (*m*/*z*=91,119,134)	–	1412		0.01	n.i.	0.02

Table 4 – continued

	Compound	Identification method	KI*	KI_{ref}	Mean value**/%	Range Min.	Range Max.
	Terpenes						
41	α-Pinene	KI, MS	1154	1143[a]	0.02	0.02	0.03
42	Eucalyptol (1,8-cineole)	KI, MS	1195	1198[d]	0.04	0.02	0.06
43	*E*-β-Ocimene	KI, MS	1242	1260[b]	0.66	0.35	0.86
44	α-Copaene	KI, MS	1472	1481[a], 1500[b], 1505[c]	0.06	0.05	0.07
45	Linalool	KI, MS	1540	1554[d]	0.02	n.i.	0.02
46	α-Farnesene	KI, MS	1739	1757[b]	1.31	0.98	1.55
	Organic acids						
47	Acetic acid	KI, MS	1429	1448[a]	4.08	1.17	9.16
	Furans						
48	2-Ethyl furan	KI, MS	953	945[d], 957[e]	0.16	0.08	0.39
49	2-Penthyl furan	KI, MS	1223	1240[d], 1242[e]	0.02	n.i.	0.04
50	Furfural (2-furfuraldehyde)	KI, MS	1441	1485[c], 1474[d]	0.09	0.03	0.19

**Results are expressed as mean values of 3 independent repetitions. Identification methods: RT=identification by comparison with retention times and mass spectra of pure standards; MS=identification by comparison with mass spectra from NIST05 library; KI=identification by comparison with Kováts retention indices from literature (KI_{ref}) ([a](*21*), [b](*22*), [d](*23*), [e](*24*), [c](*25*)); KI*=Kováts retention indices on Rtx-WAX capillary column; n.i.=not identified

Bova oil, low amounts of esters were determined (Table 4). This fact indicates a low content of alcohol acyltransferase, which catalyzes their formation (*34*). Esters are usually connected to fruity odours (*8*). 3-Methylbutyl acetate, which is related to banana odour notes (*8*), was the most abundant ester detected in Bova cultivar oil (Table 4). Ketones are linked to fruity, pungent and etheric sensory attributes (*8*). Among individual ketones, 1-penten-3-one was detected in notable amount in Bova oil (Table 4). Since 1-penten-3-one is characterized by a very low threshold, it contributes significantly to olive oil aroma (*33,35*). This compound is related to sensory characteristics such as pungent and mustard (*8*), and probably contributed to the medium intensity of pungency determined in Bova oil (Fig. 2). Furans contribute to undesirable flavours of olive oil and some of them, such as 2-penthyl furan, could be useful in detecting oil oxidation at the late stage (*32,36*). Quite low amount of furans, particularly 2-penthyl furan (Fig. 3, Table 4), were found in Bova oil samples, probably due to rapid processing of healthy fruits and proper storage of oil samples until analyses. Acetic acid is associated with microbial fermentation and other fruit handling defects, and is linked to sour and pungent sensations in olive oil (*6*). Moreover, this compound may also generate a winey-vinegary sensory defect in virgin olive oil (*37*). In Bova oil acetic acid was found in low amount (Table 4), and the corresponding defect was not perceived during sensory analysis (Fig. 2). A considerable amount of hydrocarbons (about 35 % of total peak area) was detected in the headspace of Bova cv. oil samples, but some of them were not identified (Table 4). Isomers of 3-ethyl-1,5-octa-

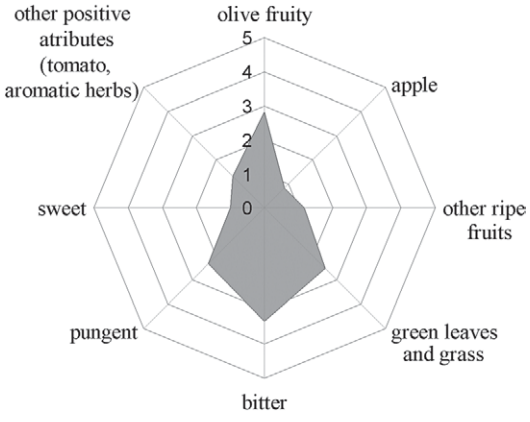

Fig. 2. Sensory profile of Bova cultivar virgin olive oil. Results are mean values of 3 independent repetitions (median of eight assessments for each descriptor). Numbers 0–5 represent perception intensity of sensory characteristics: 0=no perception, 1=scarce, 2=light, 3=medium, 4=strong, 5=extreme

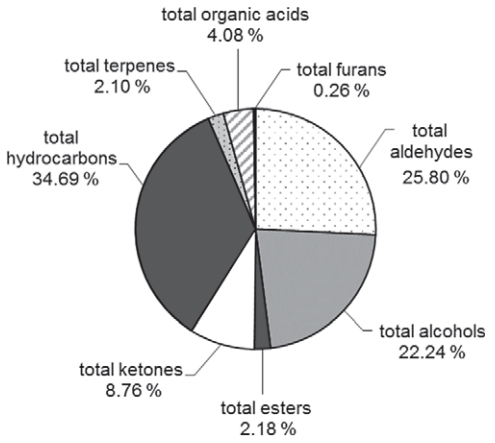

Fig. 3. The amounts of volatile compounds (percentage of total peak area) in Bova cultivar virgin olive oil. Results are expressed as mean values of peak area of three independent repetitions

diene, known as pentene dimers, derived from the enzymatic transformation of fatty acids (*38*), were the most prevalent hydrocarbons in Bova oil (Table 4). Quite low amounts of aromatic hydrocarbons, such as xylene isomers, trimethyl benzene isomers, and styrene, were found (Table 4). The origin of aromatic hydrocarbons in virgin olive oil might be from exogenous contamination and endogenous pathways (*39*). Some volatile terpenoid hydrocarbons in olive oil have been proposed as useful for geographical origin classification (*40*) and for monovarietal oil characterization (*41,42*). Six terpenoid hydrocarbons, mono- and sesquiterpenes, were found in Bova oil, and among them α-farnesene was the most abundant (Table 4). This component could play a very important role in the fragrance of virgin olive oil (*42*). α-Farnesene is derived from the enzymatic transformation of fatty acids (*38*), and is related to hot spicy flavour (*43*). Zunin *et al.* (*40*) found that olive oil from western Liguria (Italy), Spain and Tunisia showed high content of this sesquiterpene, while olive oil from Greece and Puglia (Italy) was poor in this compound.

Sensory profile of Bova cultivar oil was characterized by medium intensities of the sensory attributes olive fruity, green leaves and grass, light tomato and aromatic herbs odour with mild apple and other ripe fruits notes (Fig. 2). Sensory characteristics bitter and pungent, besides being related to some volatile compounds, are mostly dependent on the concentration of phenolic compounds in the oil (*44–46*). The medium intensities of pungent and bitter observed in Bova cultivar oil (Fig. 2) could be tentatively explained by high concentration of phenolic compounds in monovarietal oil from this cultivar (*4*).

Conclusion

This work provided a valuable morphological characterization and genetic fingerprinting of the relatively unknown autochthonous Istrian olive cultivar Bova. Among other possible purposes, it opens the possibility to apply the fingerprinting information for genetic identification of the propagation material within the nursery and along the plant production chain. The work has also expanded the knowledge about Bova cultivar virgin olive oil by reporting its detailed volatile compound profile and professional sensory description for the first time. The research demonstrates that for Croatia Bova cultivar can be a viable alternative to other traditionally cultivated olive cultivars, expanding the offer on the market both at local and international level.

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