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## Volatile Compounds and Sensory Profiles of Monovarietal Virgin Olive Oil from Buža, Črna and Rosinjola Cultivars in Istria (Croatia)

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### Summary

The volatile compounds found in virgin olive oil, mainly C6 and C5 volatile compounds biogenerated from polyunsaturated fatty acids through the lipoxygenase pathway, are responsible for their particular aroma. The composition of volatile compounds in olive oil depends on the cultivar, the ripening degree of the fruits and processing conditions. Among many different autochthonous cultivars in Istria (Croatia), some of the most prevalent are Buža, Črna and Rosinjola. The volatiles and sensory characteristics of their monovarietal virgin olive oil are little known. Therefore, fruits from these three cultivars were handpicked at the same ripening degree and processed under the same conditions. Quantitative descriptive sensory analysis of monovarietal virgin olive oil was carried out by the panel. Volatile composition was evaluated by headspace solid phase microextraction-gas chromatography, previously optimized and validated. The main parameters affecting effectiveness, time and temperature of extraction were optimized. The extraction procedure showed detection and quantification limits, as well as linear ranges adequate for the analysis of selected volatile compounds. Good precision was obtained both in terms of intra-day repeatability (relative standard deviations generally lower than 7 %) and inter-day precision. The tested types of monovarietal olive oil showed different volatile profiles, although *E*-2-hexenal was the main compound in all samples. Buža oil was the richest in total C6 and C5 volatile compounds. The results show that the most important contributors to the olive oil aroma (odour activity value >1.0) were 1-penten-3-one, *E*-2-hexenal, hexanal, hexanol, *Z*-3-hexen-1-ol and *Z*-2-penten-1-ol. These chemical findings were compared with those provided by the panel test. Buža had the highest intensity of sensory characteristic 'other ripe fruits' and Rosinjola had the highest intensity of sensory characteristic 'bitter'. All results show that olive oil aroma compounds accumulate differently depending on the cultivar, indicating a close dependence on the enzymatic pool, which is genetically dependent.

*Key words:* volatiles, SPME, sensory characteristics, olive oil, cultivars

### Introduction

The aroma of olive oil consists of a complex mixture of volatile compounds, which includes mainly aldehydes,

alcohols, ketones and esters. Until now, more than 120 volatile compounds, contributing positively or negatively to the odour of olive oil, have been identified (1). However, particular and delicate aroma of virgin olive oil is

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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 (2). It is well known that the taste of virgin olive oil is influenced primarily by phenolic compounds, but some volatile compounds have also been found to have an influence. Angerosa *et al.* (3) confirmed that 1-penten-3-one has a positive correlation with sensory characteristics 'bitter' and 'pungent', while Inarejos-García *et al.* (4) found positive correlation between the sensory characteristic 'pungent' and C6 alcohol hexanol.

According to the present knowledge, cultivar is one of the most important factors that significantly influences volatile compound composition and sensorial characteristics of virgin olive oil (5). It has been presumed that the enzymatic potential of particular olive cultivars, included in the biogenesis of the mentioned volatiles, is genetically determined (6). The composition of volatile compounds and sensory characteristics of monovarietal virgin olive oil were a subject of numerous investigations on a global level, which confirmed that monovarietal oil has specific characteristics that reflect the cultivar and its geographical origin (5,7–19). Until now, a large number of autochthonous cultivars have been morphologically and genetically identified in Istria, and Buža, Črna and Rosinjola are one of the most prevalent (20–22), so the interest in their cultivation is increasing. High oil content in these cultivars indicates their high economical potential (23). Sensory characteristics and volatile compounds of monovarietal types of olive oil in Istria have been poorly investigated (24), and no data about the characteristics of Rosinjola oil can be found up to date. Since olive oil is the most important typical product of the Istrian region in Croatia (25), the need to define and characterize specific properties of virgin olive oil obtained from the mentioned autochthonous cultivars has emerged (21). In that context, the aim of this investigation is to contribute to the characterization of volatile compounds and sensory characteristics of virgin olive oil from the three most prevalent autochthonous Istrian cultivars, Buža, Črna and Rosinjola. It must be stressed that, besides cultivar, the concentration and activity of the enzymes responsible for biogenesis of volatile compounds of virgin olive oil are influenced by other factors, especially by the stage of fruit ripening (16,26–28) and olive oil production conditions (29–32). With the aim to control these factors, the fruits were harvested at the same ripening stage and produced under the same conditions.

## Materials and Methods

### *Preparation of virgin olive oil samples*

Samples of virgin olive oil (VOO) were obtained from olive fruits from three Istrian autochthonous cultivars, Buža, Črna and Rosinjola grown in the western part of the region of Istria (Croatia). Approximately 100 kg of olive fruits from three trees per cultivar were handpicked at the same maturity index (MI=1.5–2.0) in October 2006.

Maturity index of the fruits was determined applying the method described by Gutiérrez *et al.* (33), which is based on the evaluation of olive skin and pulp colour.

Olive fruits of each cultivar were processed separately in an oil extraction plant Cultivar 500 (Oliomio, Toscana Enologica Morri, Firenze, Italy) within 24 h after the harvesting. Fruits were crushed with a knife crusher and olive paste was malaxed at (26±1) °C for 35 min in a vertical olive paste mixer with a capacity of 300 kg. The oil was separated by centrifugation through a two-phase decanter at 500 kg/h. Before and after the preparation of each different oil sample, the extraction plant was cleaned up. The extracted oil was filtered through the layer of hydrophilic cotton by means of a vacuum pump and stored at room temperature in dark bottles filled with nitrogen and sealed until analyses.

### *Analysis of volatile compounds*

Volatile compounds of the investigated olive oil samples were evaluated using headspace solid phase microextraction-gas chromatography (HS-SPME/GC), which was optimized and validated.

### *Materials*

Twenty standards of volatile compounds had a GC purity ≥95 %. Pentan-3-one, ethyl 2-methylbutyrate, butyl acetate, E-2-pentenal, E-2-hexenal, octanal, E-2-penten-1-ol, Z-3-hexenyl acetate, Z-2-penten-1-ol, E-3-hexen-1-ol and E-2-octenal were purchased from Sigma-Aldrich (Steinheim, Germany), 3-methylbutan-1-ol, 1-penten-3-one, hexanal, hexyl acetate, hexanol, Z-3-hexen-1-ol, E-2-hexen-1-ol and Z-2-hexen-1-ol were supplied from Fluka (Sigma-Aldrich, Buchs, Switzerland), while 3-methylbutyl acetate was purchased from Merck KGaA (Darmstadt, Germany).

The holder for manual SPME sampling and a fused silica fibre coated with highly cross-linked divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS), 1 cm length, 50/30 µm film thickness (Supelco, Bellefonte, PA, USA), was used for HS-SPME and preconcentration of volatiles in this study because it had been reported to be the most suitable for olive oil volatile compound analysis (34).

### *Extraction, identification and quantification of volatile compounds*

The 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 the extraction, the headspace in the vial was stabilized by equilibration at 40 °C for 10 min and gentle agitation for 3 min with a magnetic stirrer. The extraction was carried out at 40 °C for 40 min. The thermal desorption of the analytes was achieved by inserting the fibre into the injection port of the GC system equipped with an 0.80-mm i.d. SPME liner in splitless mode at 245 °C for 3 min. Before sampling, the fibre was reconditioned for 10 min in an injecting port at 245 °C, and blank runs were done periodically during the study.

GC analyses were performed using a Varian 3350 gas chromatograph (Varian Inc., Harbor City, CA, USA) e-

quipped with a flame ionization detector (FID) operated at 248 °C and a capillary column Rtx-WAX (30 m×0.25 mm i.d.×0.25 µm film thickness; Restek). Initial oven temperature was 40 °C, increased to 85 °C after 8 min at 2.5 °C/min and finally increased to 245 °C at 10 °C/min, and kept for 20 min. The carrier gas was helium at the pressure of 103 kPa (15 p.s.i.) at the column head. The identification of volatile compounds was performed by comparing their retention times with those of pure standards (Table 1). Quantification was carried out using calibration curves of pure standards dissolved in freshly refined sunflower oil. The analyses were run in triplicate.

#### Optimization of HS-SPME conditions

Before the analysis of olive oil samples, the main parameters affecting the effectiveness of the extraction, time and temperature were optimized. The influence of temperature (30 and 40 °C) and time (10, 20, 30 and 40 min) of sampling on the extraction efficiency of volatile compounds from olive oil samples was tested. For that purpose, standard model solution containing 4 mg/kg of each standard in freshly refined sunflower oil was prepared and used. The total sum of peak area counts was the highest at 40 °C and after 40 min of sampling (data not shown). Therefore, these conditions were chosen for further analysis and were strictly monitored to obtain good precision of analysis.

#### Validation of the analytical method

Validation of the method was performed in terms of limits of detection and quantification, linearity and precision (intra- and inter-day precision). The limit of de-

tection (LOD) was estimated as the mass fraction of the compound that produces a signal-to-noise ratio of 3, and the limit of quantification (LOQ) as the mass fraction of the compound producing a signal 10 times that of the noise. LOD and LOQ are represented as the mean values of the limits detected during the analyses of five standard model mixtures in refined sunflower oil containing standards of volatile compounds in the mass fraction range from 0.1 to 2 mg/kg (Table 1). LOD and LOQ were low enough to determine almost all the selected volatile compounds in the investigated olive oil samples.

To determine linearity, three replicate analyses of five standard mixtures prepared in freshly refined sunflower oil containing compounds within the ranges shown in Table 1 were performed. For most of the studied compounds, the resulting calibration curves were found to have good linearity in the tested mass fraction range, with  $R^2$  values ranging between 0.978 (hexanal) and 0.999 (*E*-2-pentenal).

The intra-day precision was determined by six replicate analyses of the same olive oil sample using the proposed method. For each assay, mean values and relative standard deviations (RSD) were calculated. Good precision was obtained in terms of intra-day repeatability: RSD generally lower than 7 %, except for butyl acetate (12 %) and hexyl acetate (17 %). Inter-day precision was determined by comparison of the results obtained for the same olive oil sample analyzed in an interval of three days. For each day, three replicate analyses were performed. There were no statistically significant differences between the fractions of volatiles determined on different days of analysis, indicating a good inter-day precision (data not shown).

Table 1. Retention time of volatile compound standards, limit of detection (LOD), limit of quantification (LOQ) and linearity of HS-SPME/GC method for determination of Buža, Črna and Rosinjola monovarietal virgin olive oil volatile compounds

Volatile compound	Retention time min	$w(\text{LOD})$ mg/kg	$w(\text{LOQ})$ mg/kg	Linearity	
				$w(\text{range})$ mg/kg	$R^2$
3-methylbutan-1-al	2.56	0.001	0.004	0.10–0.50	0.990
pentan-3-one	3.27	0.001	0.002	0.10–1.00	0.982
1-penten-3-one	3.97	0.000	0.001	0.10–2.00	0.985
ethyl 2-methylbutyrate	4.84	0.000	0.001	0.10–2.00	0.981
butyl acetate	5.39	0.002	0.006	0.10–2.00	0.993
hexanal	5.58	0.001	0.004	0.10–2.00	0.978
<i>E</i> -2-pentenal	7.24	0.003	0.011	0.10–2.00	0.999
3-methylbutyl acetate	7.41	0.003	0.011	0.10–2.00	0.970
<i>E</i> -2-hexenal	11.95	0.007	0.023	1.06–37.87	0.997
hexyl acetate	15.84	0.005	0.017	0.10–2.00	0.997
octanal	16.41	0.007	0.023	0.10–2.00	0.995
<i>E</i> -2-penten-1-ol+ <i>Z</i> -3-hexenyl acetate*	18.16	0.006	0.019	0.10–2.00	0.994
<i>Z</i> -2-penten-1-ol	18.64	0.003	0.010	0.10–2.00	0.991
hexanol	20.67	0.006	0.021	0.50–10.00	0.996
<i>E</i> -3-hexen-1-ol	21.06	0.005	0.017	0.10–2.00	0.991
<i>Z</i> -3-hexen-1-ol	22.08	0.006	0.020	0.50–10.00	0.995
<i>E</i> -2-hexen-1-ol	22.42	0.008	0.025	0.50–10.00	0.997
<i>Z</i> -2-hexen-1-ol+ <i>E</i> -2-octenal*	23.91	0.013	0.044	0.10–2.00	0.996

\*These compounds had equal retention time and their fractions were expressed as the corresponding sums

### Sensory analysis

Sensory analysis was performed by a panel of eight trained assessors according to the European Communities Regulation no. 2568/91 (35). Quantitative evaluation of different odour descriptors (olive fruity, other ripe fruits, apple, green grass or leaves), taste descriptors (bitter, pungent and sweet) and unpleasant attributes (winey/vinegary, rough, metallic, musty, muddy sediment, fusty and rancid) were quantified using a six-point intensity ordinal rating scale from 0 (no perception), 1 (scarce), 2 (mild), 3 (middle), 4 (strong) to 5 (extreme).

### Statistical analysis

Differences among samples were tested by the one-way analysis of variance at 5 % significance level. The homogeneity of variance was tested by the Lavene test and some logarithmic transformation of the original data was needed. The mean values were compared using the Tukey's honestly significant difference test ( $p \leq 0.05$ ). Statistical analyses were performed using the software package STATISTICA v. 9 (36).

## Results and Discussion

Table 2 shows the fractions of volatile compounds in the samples of virgin olive oil from the three Istrian cultivars and their odour activity values calculated on the basis of literature thresholds (5,8,37). Statistically signi-

ficant differences were found among the fractions of total aldehydes, total alcohols, total ketones (Table 2), and total volatiles, as well as of total C6 and C5 volatile compounds (Fig. 1) in the three investigated monovarietal oil samples. The highest fraction of total volatile compounds was found in Buža oil samples, and the lowest in the oil obtained from Črna cultivar (Fig. 1).

Concerning the fraction of total aldehydes, Rosinjola was the richest and Črna the poorest (Table 2). These differences in aldehyde fractions were probably caused by different activities of hydroperoxide lyase, which catalyzes the cleavage of fatty acid hydroperoxides that produce volatile aldehydes. Moreover, another reason for different fractions of aldehydes could be different acyl hydrolase activity, as well as better or poorer availability of free polysaturated fatty acids in Arbequina and Picual cultivars, as determined by Sánchez-Ortiz *et al.* (38).

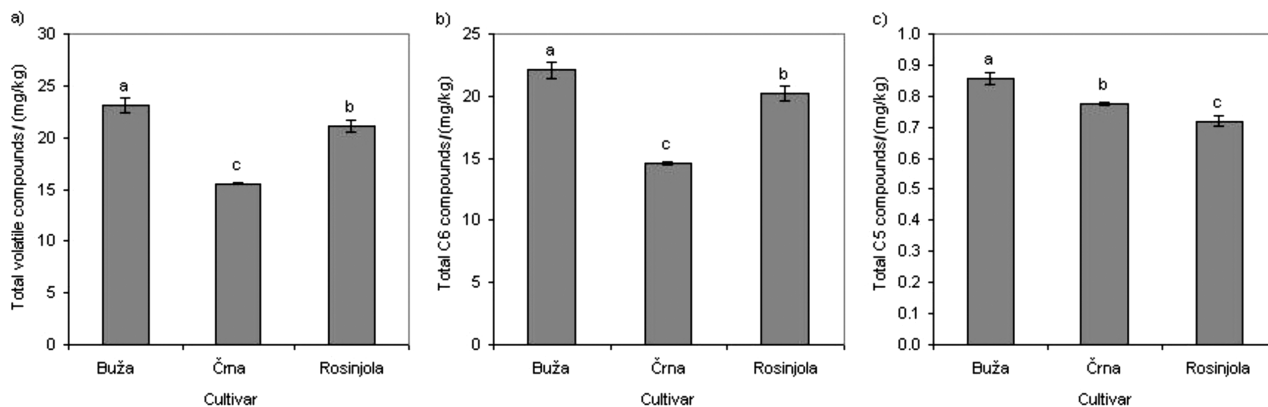
The most prevalent volatile compound in all the investigated monovarietal VOO samples was C6 aldehyde *E*-2-hexenal (Table 2), which is in accordance with the literature data on other monovarietal olive oil types published by other authors (5,16,29,38,39). In addition, fraction ranges of *E*-2-hexenal determined in the three investigated Istrian autochthonous monovarietal VOO samples were similar to the fractions of *E*-2-hexenal determined in other monovarietal VOO samples by means of HS-SPME/GC-FID methods using DVB/CAR/PDMS fibre (5,29,38). Rosinjola and Buža had around 80 and 60

Table 2. Fractions of volatile compounds and their odour activity values (OAV) in virgin olive oil from Buža, Črna and Rosinjola cultivars (MI=1.5–2.0)

Volatile compound	Buža		Črna		Rosinjola	
	<i>w</i> /(mg/kg)	OAV	<i>w</i> /(mg/kg)	OAV	<i>w</i> /(mg/kg)	OAV
3-methylbutan-1-al	(0.007±0.002) <sup>b</sup>	1.3	(0.009±0.000) <sup>a</sup>	1.7	(0.003±0.000) <sup>c</sup>	0.6
hexanal	(0.433±0.015) <sup>a</sup>	5.8	(0.296±0.003) <sup>c</sup>	4.0	(0.352±0.003) <sup>b</sup>	4.7
<i>E</i> -2-pentenal	(0.083±0.001) <sup>a</sup>	0.3	(0.075±0.000) <sup>b</sup>	0.3	(0.043±0.001) <sup>c</sup>	0.1
<i>E</i> -2-hexenal	(9.203±0.350) <sup>b</sup>	21.7	(5.886±0.060) <sup>c</sup>	13.9	(10.489±0.325) <sup>a</sup>	24.7
octanal	(0.026±0.004) <sup>b</sup>	0.5	(0.029±0.000) <sup>b</sup>	0.5	(0.055±0.005) <sup>a</sup>	1.0
<i>total aldehydes</i>	(9.753±0.366) <sup>b</sup>	–	(6.293±0.062) <sup>c</sup>	–	(10.942±0.328) <sup>a</sup>	–
<i>Z</i> -2-penten-1-ol	(0.316±0.005) <sup>c</sup>	1.3	(0.395±0.004) <sup>b</sup>	1.6	(0.427±0.014) <sup>a</sup>	1.7
hexanol	(3.824±0.137) <sup>a</sup>	9.6	(1.797±0.023) <sup>b</sup>	4.5	(0.928±0.055) <sup>c</sup>	2.3
<i>E</i> -3-hexen-1-ol	(0.116±0.002) <sup>a</sup>	0.1	(0.104±0.002) <sup>b</sup>	0.1	(0.101±0.006) <sup>b</sup>	0.1
<i>Z</i> -3-hexen-1-ol	(6.870±0.136) <sup>a</sup>	6.3	(4.927±0.044) <sup>b</sup>	4.5	(3.652±0.074) <sup>c</sup>	3.3
<i>E</i> -2-hexen-1-ol	(1.595±0.044) <sup>b</sup>	0.3	(1.574±0.021) <sup>b</sup>	0.3	(4.620±0.133) <sup>a</sup>	0.9
<i>total alcohols</i>	(12.722±0.317) <sup>a</sup>	–	(8.797±0.050) <sup>c</sup>	–	(9.727±0.277) <sup>b</sup>	–
ethyl 2-methylbutyrate	(0.002±0.000) <sup>a</sup>	2.9	n.d.	n.c.	n.d.	n.c.
butyl acetate	n.d.	n.c.	n.d.	n.c.	n.d.	n.c.
3-methylbutyl acetate	(0.011±0.002) <sup>b</sup>	n.c.	(0.027±0.002) <sup>a</sup>	n.c.	(0.015±0.001) <sup>b</sup>	n.c.
hexyl acetate	(0.050±0.009) <sup>ab</sup>	0.1	(0.021±0.014) <sup>b</sup>	0.0	(0.075±0.016) <sup>a</sup>	0.1
<i>total esters</i>	(0.063±0.008) <sup>ab</sup>	–	(0.048±0.013) <sup>b</sup>	–	(0.091±0.016) <sup>a</sup>	–
pentan-3-one	(0.234±0.006) <sup>a</sup>	0.0	(0.215±0.002) <sup>b</sup>	0.0	(0.206±0.003) <sup>b</sup>	0.0
1-penten-3-one	(0.224±0.006) <sup>a</sup>	320.0	(0.092±0.001) <sup>b</sup>	131.4	(0.043±0.002) <sup>c</sup>	61.4
<i>total ketones</i>	(0.457±0.012) <sup>a</sup>	–	(0.307±0.003) <sup>b</sup>	–	(0.249±0.004) <sup>c</sup>	–

Results are mean values of triplicate analyses±standard deviation (S.D.), the means within each row labelled with different letters are significantly different (Tukey's test,  $p < 0.05$ ); n.d.=not detected, n.c.=not calculated, MI=maturity index  
OAV were calculated on the basis of literature threshold values (5,8,37); OAV>1 indicates volatile compounds with direct contribution to olive oil aroma





**Fig. 1.** Fraction of: a) total volatile compounds, b) total C6 and c) total C5 compounds in virgin olive oil from Buža, Črna and Rosinjola cultivars (maturity index of olives was 1.5–2.0). Results are mean values of triplicate analyses  $\pm$  S.D., the means labelled with different letters are significantly different (Tukey's test,  $p < 0.05$ ). Total C6 compounds: hexanal + *E*-2-hexenal + hexanol + *E*-3-hexen-1-ol + *Z*-3-hexen-1-ol + *E*-2-hexen-1-ol + hexyl acetate. Total C5 compounds: *E*-2-pentenal + *Z*-2-penten-1-ol + pentan-3-one + 1-penten-3-one

% higher *E*-2-hexenal values when compared to Črna, respectively (Table 2). Buža showed statistically higher fraction of hexanal in relation to Črna (around 45 %) and Rosinjola oil (around 25 %). When compared to literature, fraction values of hexanal determined in Istrian autochthonous monovarietal VOO samples were similar to the fractions determined by Sánchez-Ortiz *et al.* (38) in Spanish monovarietal Picual (0.257 mg/kg) and Arbequina (0.710 mg/kg) oil samples. However, Runcio *et al.* (29) determined a significantly higher fraction of hexanal in Italian monovarietal olive oil samples, which ranged from 3.15 mg/kg (Ciciarello) to 15.42 mg/kg (Pendlino). However, it must be pointed out that hexanal can derive not only from the lipoxygenase pathway, but also from the oxidation of fatty acids (11), which is possibly one of the reasons for variations in hexanal fraction among the monovarietal VOO samples determined in different investigations.

Buža oil samples showed the highest fraction of total alcohols, probably due to higher activity of the enzyme alcohol dehydrogenase, which reduces C6 aldehydes into C6 alcohols. Angerosa *et al.* (40) suggested that the activity of alcohol dehydrogenase is genetically determined for each cultivar. In addition, differences among the proportions of individual alcohols in monovarietal VOO samples were found. *Z*-3-hexen-1-ol predominated in Buža and Črna oil samples, while in Rosinjola oil samples the most abundant alcohol was *E*-2-hexen-1-ol, with threefold higher value in relation to the other two investigated oil samples. Buža samples had higher fraction of hexanol, twice higher than Črna and four times higher than Rosinjola. When compared to literature, the fractions of the three mentioned alcohols were similar to the values determined in Italian monovarietal VOO samples (29), but higher than values established in Spanish and Tunisian monovarietal VOO samples (5,38).

Fractions of total esters in Buža, Črna and Rosinjola monovarietal oil samples were relatively low (Table 2), which is in accordance with the literature data for monovarietal VOO in general (5,38). Low concentrations of esters were probably caused by low activity of the enzyme alcohol acyltransferase (AAT), which has an optimal pH for its activity in the range from neutral to basic

(41), while regular pH values of olive paste during olive oil production are in the acidic range. Concerning individual esters, butyl acetate was not identified in the investigated monovarietal VOO samples, while ethyl 2-methylbutyrate was found only in Buža (Table 2). Buža was also distinguished from the other two oil samples by significantly higher fraction of 1-penten-3-one, which was 2.5 times higher with respect to Črna and 5 times higher than in Rosinjola (Table 2).

C5 volatile compounds were determined in all the investigated oil samples (Fig. 1), which implies the existence of an additional branch of lipoxygenase pathway that leads to their biogenesis (27). C6 and C5 volatile compounds are mainly responsible for delicate green aroma of virgin olive oil, which is characteristic of high quality virgin olive oil samples highly appreciated by consumers (42). In both cases, Buža contained significantly higher fractions in relation to the other two monovarietal VOO samples.

However, volatile compounds found in high fractions are not necessarily important contributors to the virgin olive oil aroma. Therefore, sensory significance and contribution of volatile compounds to the aroma of the investigated VOO samples were expressed as odour activity values (OAV) (Table 2), calculated as quotients of the fractions and the corresponding threshold values reported in literature (5,8,37). OAV higher than one indicates a volatile compound with a direct contribution to the olive oil aroma. Volatile compound with the highest OAV in Buža, Črna and Rosinjola oil samples was C5 ketone 1-penten-3-one (Table 2). 1-Penten-3-one had previously been associated with the sensory characteristic 'bitter' (8), while a positive correlation between 1-penten-3-one and sensory characteristics 'pungent' and 'bitter' was also found (3). In all the investigated Istrian monovarietal oil samples, mild to middle intensities of the sensory characteristic 'bitter' and middle to strong intensities of the sensory characteristic 'pungent' were perceived (Fig. 2). However, 1-penten-3-one odour activity values were not in clear correlation with the intensities of the mentioned sensory characteristics. Sensory characteristics 'bitter' and 'pungent', besides their relation with some volatile compounds, are mostly dependent on the

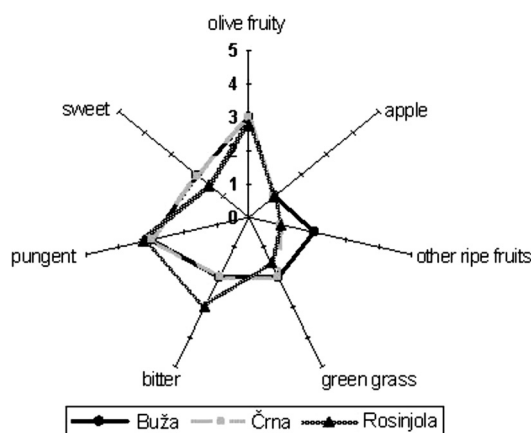


Fig. 2. Sensory profile of virgin olive oil from Buža, Črna and Rosinjola cultivars (maturity index of olives was 1.5–2.0). Results are expressed as median of eight assessments for each descriptor. Numbers 0–5 represent perception intensity of sensory characteristics: 0 (no perception), 1 (scarce), 2 (mild), 3 (middle), 4 (strong), 5 (extreme)

fraction of phenolic compounds (3,4). In a previous investigation, higher fraction of phenolic compounds was determined in Rosinjola in relation to Buža oil (21). The highest intensity of the sensory characteristic 'bitter' observed in Rosinjola oil (Fig. 2) could be explained by generally higher fraction of phenolic compounds in monovarietal oil samples from this cultivar.

Another volatile compound with an assumed significant influence on sensorial profiles of the investigated monovarietal VOO samples was *E*-2-hexenal, the compound associated with 'green', 'fruity', 'bitter' and 'astringent' sensory oil characteristics (8). Additionally, the aldehydes with a direct contribution to Buža and Črna aroma ( $OAV > 1$ ) were hexanal and 3-methylbutan-1-al, while hexanal and octanal contributed to Rosinjola oil aroma (Table 2). Hexanal contributes to sensory characteristics 'green apple' and 'green grass', 3-methylbutan-1-al to 'sweet' and 'fruity' (8), and octanal is associated with citric aroma of olive oil (37). The highest intensity of the sensory characteristic 'other fruits' observed in Buža oil (Fig. 2) was probably related to the highest odour activity values of hexanal and 3-methylbutan-1-al, as well as to relatively high values of *E*-2-hexenal, when compared to Črna and Rosinjola monovarietal VOO samples.

Considering individual alcohols, hexanol, *Z*-3-hexen-1-ol and *Z*-2-penten-1-ol had a direct contribution ( $OAV > 1$ ) to the aroma of Buža, Črna and Rosinjola (Table 2). Alcohols are associated with 'fruity', 'green' and 'aromatic' sensory characteristics of olive oil, but have lower sensory significance than aldehydes, due to their higher threshold values (8). Odour activity values of individual alcohols hexanol and *Z*-3-hexen-1-ol were the highest in Buža, and this could be one of the reasons for the highest intensity of the sensory characteristic 'other ripe fruits' observed in Buža in relation to Črna and Rosinjola (Fig. 2). Apart from that, ethyl 2-methylbutyrate, related to the sensory characteristic 'fruity' (37), had direct contribution to Buža oil aroma (Table 2). The presence of this ester in the volatile fraction of Buža oil could be one more reason for higher intensity of the sensory characteristic

'other ripe fruits', which distinguished Buža from the other two oil samples.

## Conclusion

All results put in evidence that olive oil aroma compounds accumulate differently depending on the cultivar, indicating a close dependence on the enzymatic pool, which is genetically determined. The obtained information could be very useful in focusing the efforts of researchers, but also of producers, on understanding the potential of autochthonous cultivars in the production of high quality monovarietal olive oil with specific sensory characteristics.

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