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Influence of storage temperature on quality parameters, phenols and volatile compounds of Croatian virgin olive oils

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SUMMARY: The influence of low storage temperature (+4 °C and −20 °C) and conventional storage room temperature on the quality parameters, phenolic contents and volatile profiles of Buža, Črna and Rosinjola monovarietal virgin olive oils after 12 months of storage was investigated in this study. Virgin olive oils stored at low temperatures maintained better quality parameters than oils stored at room temperature. A negligible decrease in the total phenols was detected after 12 months of storage at all investigated temperatures. The total volatile compounds, aldehydes, alcohols and esters in almost all stored samples were unchanged compared to fresh oils. Total ketones increased after storage, although at a lower temperature these changes were less notable. An increase in the oxidation indicators hexanal and hexanal/*E*-2-hexenal ratio was the lowest in oils stored at +4 °C.Storage at temperatures lower than room temperature could help to prolong the shelf-life of extra virgin olive oil by maintaining high quality parameters and preserving the fresh oil's volatile profile.

KEYWORDS: *Freezing; Low storage temperature; Shelf-life; Total phenols; Virgin olive oil; Volatile compounds*

RESUMEN: ***Influencia de la temperatura de almacenamiento sobre los parámetros de calidad, fenoles y compuestos volátiles de aceites de oliva vírgenes croatas***. Se ha estudiado la influencia, durante 12 meses, de temperaturas bajas (+4 °C y –20 °C) y convencional (ambiente), sobre los parámetros de calidad, contenido fenólico y perfil de volátiles de aceites de oliva vírgenes monovarietales Buža, Črna y Rosinjola. Los aceites de oliva vírgenes almacenados a bajas temperaturas mantienen mejores propiedades de calidad que los aceites almacenados a temperatura ambiente. Se encontró una disminución no significativa de los fenoles totales después de 12 meses de almacenamiento a todas las temperaturas estudiadas. Los compuestos volátiles totales, aldehídos, alcoholes y ésteres, en casi todas las muestras almacenadas, se mantuvieron sin cambios en comparación con los aceites frescos. Las cetonas totales incrementaron tras el almacenamiento, aunque a temperaturasmas bajas estos cambios fueron menos notables. El incremento de los indicadores de la oxidación hexanal y la relación hexanal/*E*-2-hexenal fue más bajo en los aceites almacenados a +4 °C. El almacenamiento a temperaturas inferiores a la temperatura ambiente ayuda a prolongar la vida útil de los aceites de oliva virgen extra, manteniendo la alta calidad y preservando el perfil de volátilesde un aceite fresco.

PALABRAS CLAVE: *Aceite de oliva virgen; Bajas temperaturas de almacenamiento; Compuestos volátiles; Congelación; Fenoles totales; Tiempo de conservación*

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1. INTRODUCTION

Fresh virgin olive oil (VOO) has a characteristic odor, taste and nutritive properties that distinguish it from other edible oils. The delicate and unique odor of VOO is related to the volatile compounds, mainly C5 and C6 volatile compounds, enzymatically produced by the oxidation of polyunsaturated fatty acids in the lipoxygenase pathways (Kalua *et al.*, 2007). Hydrophilic phenols are responsible for the bitter and pungent taste of VOO as well as for its oxidative stability. These natural antioxidants are also important in the prevention of severe diseases which have the highest incidence in the late aged classes, for example cancer and chronic degenerative disease (Servili *et al.* 2004).

During storage, virgin olive oil properties are liable to deteriorate and the rate of deterioration depends on the oil composition, mainly its fatty acid composition and content of minor compounds with antioxidant activity (Velasco and Dobarganes, 2002), as well as on the time of storage and storage conditions (Gómez-Alonso *et al.* 2007). The assurance of the EVOO shelf-life is a matter of great concern for the olive oil industry. The bottled oil should maintain the compositional and sensory characteristics of the commercial category reported in the label, so it is important to be studied the behavior of oil from each olive cultivar under different storage conditions in order to predict the sell-by date. Since virgin olive oil produced in one crop season is usually consumed before the next crop season (Morello *et al.* 2004), their composition and quality need to be only minimally maintained during that storage period.

The influence of storage conditions on olive oil quality has been considered in different papers. Conventional storage at room temperature (Cavalli *et al.* 2004; Gómez-Alonso *et al.* 2007; Pristouri *et al.* 2010; Méndez and Falqué, 2007; Fadda *et al.* 2012;), or under medium or high temperature accelerated storage conditions (Krichene *et al.* 2010), have already been investigated. One of the main causes of quality degradation is the chemical oxidation that leads to the formation of volatile compounds responsible for some oil defects known as *rancid*, *cucumber* and *musty* (Angerosa *et al.* 2004). According to Vichi *et al.* (2003) and Kiritsakis (1998), the occurrence of particular volatile compounds, such as nonanal or hexanal/nonanal ratio, can be a good indicator of olive oil oxidative deterioration. In addition, Cavalli *et al.* (2004) have found that the storage of virgin olive oils leads to a decrease in C6 aldehyde *E*-2-hexenal and an increase in C6 alcohols and C5 ketones, and have recommended these compounds as quality-freshness markers for virgin olive oils. The other important cause of VOO quality degradation is the decrease in the total content of hydrophilic phenols, as a consequence of their involvement in oxidative processes (Morelló *et al.* 2004; Gómez-Alonso *et al.* 2007; Méndez and Falqué, 2007).

Up to now there has been little knowledge about the stability of volatile compound composition in virgin olive oils during long term frozen storage (Mulinacci *et al.* 2013). Furthermore, to our knowledge, no data has been reported in the literature about the changes produced in the volatile profile of olive oil after long term storage in a refrigerator at +4 °C, representing a simulation of olive oil storage condition during winter.

Therefore, in this research the influence of different storage temperatures (RT - room temperature, +4 °C and –20 °C) on the trade quality parameters, phenols (total and *ortho*-diphenols) and volatile compounds of Buža, Črna and Rosinjola monovarietal VOO after 12 months of storage was investigated. Taking into account that the stability of oil depends on its initial chemical composition, the three autochthonous Croatian cultivars have been chosen because of their quantitatively different total phenol contents and volatile compound profiles (Poljuha *et al.* 2008; Brkić Bubola *et al.* 2012). The changes in VOO characteristics of the three chosen cultivars during long term storage under the above mentioned conditions have been investigated for the first time.

2. MATERIALS AND METHODS

2.1. Preparation of VOO samples

Samples of VOOs were obtained from the olive fruits of three Istrian autochthonous cultivars, Buža, Črna and Rosinjola, grownin the western part of the Istria region (Croatia). Approximately 100 kg of olive fruits from three trees per cultivar were handpicked at the same maturity index (MI=1.5–2) in October 2006. The maturity index of the fruits was determined applying the method described by Gutierrez *et al.* (1999) which is based on evaluation of the olive skin and pulp color.

Olive fruits from each cultivar were processed separately in the oil extraction plant Cultivar 500 (Oliomio, Toscana Enologica Morri, Italy) within 24 hours after harvesting. Fruits were crushed with a knife crusher and the olive paste was malaxed at 26±1 °C for 35 min in a vertical olive paste mixer. The olive oil was separated by centrifugation through a two phase decanter. Olive oil samples were filtered through a hydrophilic cotton layer and stored in tapped dark bottles filled with nitrogen until analysis. Analyses were performed immediately after oil sample production and after 12 months of storage at three different temperatures, at room temperature (RT), at +4 °C in a refrigerator and at –20 °C, in a freezer. The storage of oil samples at room temperature presented a simulation of virgin olive oil

storage in a household, and the average room temperature during storage time was 17.3 °C (ranging from 10 °C to 27 °C).

2.2. Trade quality parameters

Free fatty acids (FFA), peroxide value (PV) and spectrophotometric indices (K_{232} and K_{270}) were determined according to the analytical methods described in the European Commission Regulation (EEC, 1991) and subsequent amendments.

2.3. Total phenols and *ortho*-diphenols

The total phenols and *ortho*-diphenols were extracted following the procedure of Montedoro *et al*. (1992) and determined in the methanolic extract according to the Folin-Ciocalteu colorimetric method (Gutfinger, 1981). The results of the total phenol and *ortho*-diphenol contents were expressed as mg of caffeic acid per kg of oil.

2.4. Extraction, identification and quantification of volatile compounds

The volatile compounds of the investigated VOOs were determined using headspace solid-phase microextraction-gas chromatography (HS-SPME/GC), applying the method described by Brkić Bubola *et al*. (2012). The VOO sample (4.0 g) was placed in a 10 mL vial containing a micro stirring bar and sealed with a PTFE/silicone septum (Restek, Bellefonte, USA). Before extraction, the headspace in the vial was stabilized by equilibration for 10 min at 40 °C and gently agitated for 3 min with a magnetic stirrer. The SPME holder for manual sampling and a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber, 1 cm length, 50/30 μm film thickness (Supelco, Bellefonte, USA), were used for the HS-SPME and pre-concentration of volatiles. The extraction was carried out at 40 °C for 40 min. The thermal desorption of the analytes was achieved by inserting the fiber into the injection port of the GC system equipped with a 0.80 mm i.d. SPME liner in splitless mode for 3 min at 245 °C. GC analyses were performed using a Varian 3350 gas chromatograph equipped with a flame ionization detector (FID) operated at 248 °C and a 30 m×0.25 mm i.d. ×0.25 μm film thickness capillary column Rtx-WAX (Restek, USA). The initial oven temperature was 40 °C, raised after 8 min at 2.5 °C·min^{-1}. to 85 °C and finally increased to 245 °C at 10 °C·min^{-1} and held for 20 min. The carrier gas was helium at a pressure of 10.3 kPa at the column head. The identification of volatile compounds was performed by comparing their retention times with those of pure standards. All twenty standards of volatile compounds had a GC purity of ≥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 Aldrich (Steinheim, Germany). 3-Methylbutan-1-al, 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 (Buchs, Germany), while 3-methylbutyl acetate was purchased from Merck KGaA (Darmstadt, Germany). Quantification was carried out using the calibration curves of pure standards dissolved in freshly refined sunflower oil. *E*-2-penten-1-ol and *Z*-3-hexenyl acetate, as well as *Z*-2-hexen-1-ol and *E*-2-octenal, had equal retention times and their concentrations were expressed as the corresponding sums and added to the sum of the total volatile compounds investigated. The analyses were run in triplicate. An additional identification of volatile compounds was performed by GC/MS analysis using a Varian 3900 gas chromatograph coupled to a Varian Saturn 2100T ion trap mass spectrometer (Varian Inc., Harbour City, CA, USA). The extraction and desorption conditions, as well as the GC column, oven and injector parameters were the same as for the GC-FID analysis described in previous paragraphs. The transfer line and ion trap temperatures were 180 and 120 ºC, respectively. Mass spectra were acquired in the electron impact mode (70 eV) at 1 scan/s, using full scan with a mass acquisition range of 30–450 amu. Helium was used as carrier gas with a flow rate of 1 mL·min^{-1}. The identification of volatile compounds was performed by comparing their mass spectra with those of pure standards and to mass spectra from the NIST05 library. Kováts' retention indexes (KI) were determined on the polar Rtx-WAX column by injection of a standard mixture containing the homologous series of normal alkanes (C_7–C_{24}) in pure dichloromethane and compared with retention indexes of the compounds available in the literature (Vichi *et al*. 2003; Bianchi *et al*. 2007; Kandylis *et al*. 2011).

2.5. Statistical analysis

All parameters were determined in triplicate for each sample. Differences among samples were tested by the one-way analysis of variance at a 5% significance level. The homogeneity of variance was tested by the Levene test. The mean values were compared by the Tukey's honest significant difference test ($p \leq 0.05$). Statistical analyses were performed using the software package Statistica version 9 (Stat-Soft, Tulsa, OK, USA).

3. RESULTS AND DISCUSSION

3.1. Influence of storage temperature on VOO quality parameters

The trade quality of VOO samples was ascertained according to the analytical parameters suggested by Regulation EEC 2568/91 (EEC, 1991) as olive oil freshness indices: acidity value, PV, K_{232} and

K_{270} values (Table 1). The FFA values of the fresh oil samples of the three cultivars were quite low (from 0.10 to 0.19%). After 12 months of storage at RT, a slight, although significant, increase was observed for Črna and Rosinjola samples. Other investigation also confirmed that the acidity value could slightly increase after 12 months of storage at 22 °C in the dark (Pristouri *et al*. 2010) or even after six months of storage in five different containers at room temperature (Méndez and Falqué, 2007). After storage at low temperatures a negligible but significant increase in the FFA value was detected in Rosinjola oil, while no significant increase in Buža and Črna was observed. Similar to our results found for Buža and Črna oils stored at −20 °C, Mulinacci *et al*. (2013) reported that the acidity value of six Tuscan filtered EVOO was not affected after 9 months of frozen storage.

At the beginning of storage, the oil samples of the three cultivars had quite low levels of PV (from 4.35 to 6.12 meq $O_2 \cdot kg^{-1}$). PV is a useful parameter for monitoring the initial stage of oil oxidation. A significant increase in PV occurred in the samples stored at RT and +4 °C, but maintained within the limit for the EVOO category after 12 months of storage. Regarding storage at −20 °C, a mild increase in PV was detected only in Buža oil samples, while no significant changes were observed for the other two cultivars. An increase in PV was inversely proportional to storage temperature, indicating that the refrigeration and freezing of olive oil could slow down the oxidation rate of EVOO. Moreover, Mulinacci *et al*. (2013) found that six Tuscan EVOOs had two or three times lower PV after 9 months of frozenstorage than the corresponding oils stored at RT.

Gómez-Alonso *et al*. (2007) recommended the K_{232} value as the most relevant index for monitoring the maintenance of VOO within commercial categories. After 12 months of storage, the K_{232} value significantly increased in Črna and Rosinjola oil at RT and +4 °C, while no significant changes were observed for the Buža sample. Storage at −20 °C led to a significant increase only in the case of Rosinjola oil. With regards to K_{270}, a secondary oxidation product marker, a slight, although significant, increase was observed only for Rosinjola oil at any of the applied storage temperatures. This was not expected, considering that Rosinjola oil had the lowest starting value of K_{232} and K_{270}, accompanied by the highest content of *ortho*-diphenols (Table 1), which are known as natural antioxidants.

Considering all olive oil quality parameters, it should be pointed out that after 12 months of storage at different temperatures, all the samples remained within the EVOO category, except for Črna oil stored at RT in which the K_{232} value exceeded the limit of 2.50. Its starting value was the highest among the samples of the three cultivars (2.10),

Table 1. Quality parameters (free fatty acids - FFA, peroxide value - PV, UV spectrophotometric indices - K_{232} and K_{270}), total phenols, *ortho*-diphenols in Buža, Črna and Rosinjola virgin olive oils before (fresh) and after 12 months of storage at different temperatures

	Buža			Črna			Rosinjola		
	Fresh	Stored at		Fresh	Stored at		Fresh	Stored at	
		RT*	+4 °C	−20 °C	RT*	+4 °C	−20 °C		RT*	+4 °C	-20 °C
FFA (% oleic acid)	0.10^{a} (0.00)	0.13^{a} (0.01)	0.11^{a} (0.01)	0.12^{a} (0.00)	0.13^{b} (0.00)	0.15^{a} (0.00)	0.13^{b} (0.00)	0.12^{c} (0.00)	0.19^{c} (0.01)	0.25^{a} (0.01)	0.22^{b} (0.00)	0.21^{bc} (0.00)
PV (meq $O_2 \cdot kg^{-1}$)	5.07^{d} (0.11)	10.97^{a} (0.01)	8.23^{b} (0.03)	6.61^{c} (0.41)	6.12^{e} (0.03)	12.75^{a} (0.66)	7.67^{b} (0.36)	5.37^{c} (0.09)	4.35^{c} (0.44)	10.43^{a} (1.25)	7.40^{b} (0.19)	5.77^{bc} (0.49)
K_{232}	1.87^{a} (0.04)	2.27^{a} (0.00)	1.72^{a} (0.50)	1.94^{a} (0.02)	2.10^{c} (0.02)	2.60^{a} (0.01)	2.34^{b} (0.01)	2.01^{d} (0.02)	1.66^{c} (0.04)	2.15^{a} (0.02)	2.19^{a} (0.00)	1.94^{b} (0.05)
K_{270}	0.11^{a} (0.00)	0.14^{a} (0.02)	0.13^{a} (0.01)	0.13^{a} (0.01)	0.16^{d} (0.00)	0.16^{a} (0.00)	0.16^{a} (0.00)	0.14^{b} (0.00)	0.11^{b} (0.01)	0.14^{a} (0.00)	0.15^{a} (0.00)	0.15^{a} (0.00)
Total phenols (mg·kg^{-1}) (mg caffeic acid/kg)	231.45^{a} (4.02)	214.59^{a} (19.78)	215.66^{a} (9.01)	231.28^{a} (15.34)	241.11^{a} (3.00)	205.83^{a} (13.61)	220.20^{a} (1.57)	224.14^{a} (15.61)	364.59^{a} (2.64)	313.04^{a} (11.28)	335.20^{a} (22.24)	306.38^{a} (23.31)
Ortho-diphenols (mg·kg^{-1}) (mg caffeic acid/kg)	18.65^{a} (0.13)	17.39^{a} (0.97)	17.52^{a} (1.02)	17.95^{a} (0.46)	19.33^{a} (0.24)	16.00^{b} (0.86)	16.78^{b} (0.04)	17.39^{b} (0.14)	26.70^{a} (0.51)	24.25^{a} (1.18)	23.05^{a} (3.46)	26.48^{a} (1.43)

Results are mean values of three replicates (SD), the means within each row for single cultivar, labelled by different letters, are significantly different (Tukey's test, $p<0.05$). *RT=room temperature

indicating that storage at a temperature lower than RT would be particularly appropriate in such cases in order to maintain extra quality of VOO during long term storage.

3.2. Influence of storage temperature on total phenols and ortho-diphenol content

The negligible and not statistically significant decrease in the total phenol value was detected after 12 months of storage in all investigated samples. Many reports suggested significant losses in these components after storage. For instance, Morelló *et al*. (2004) reported important losses in the total phenol content of Arbequina cultivar VOOs after 12 months of storage at RT. Also, Gómez-Alonso *et al*. (2007) found a reduction in total phenolic compounds after 21 months of storage at RT. The slight decrease in total phenols after 12 months of storage detected in the present study was probably due to the high quality of fresh samples and favorable storage conditions used sincethe oil was packed in dark bottles filled with nitrogen that protect the product from light and oxygen.

Storage at different temperatures did not influence the *ortho*-diphenol contents in Buža and Rosinjola oil samples. However, after 12 months of storage, a slight decrease in *ortho*-diphenol content occurred in Črna oils and the degree of this decrease was inversely proportional to the storage temperature (Table 1). The observed favorable effect of the low temperature storage of VOO can be attributed to the slowing down of the kinetics in the oxidative reaction (Cerretani *et al*. 2005; Bonoli *et al*. 2005).

3.3. Influence of storage temperature on volatile profiles

Our earlier investigations confirmed that Buža, Črna and Rosinjola monovarietal oils have mainly quantitatively different volatile profiles which influence their different sensory characteristics. Buža oil had the highest amount of C6 and C5 volatile compounds, responsible for their pronounced fruity odor, and the Črna oil had the lowest (Brkić Bubola *et al*. 2012). The effect of different storage temperatures on the volatile compounds in these three monovarietal VOOs after 12 months of storage is shown in Table 2. With regards to total volatile compounds, no statistical differences were found among fresh samples and those stored at different temperatures, but some significant differences in the behavior of the individual volatile compounds in oils stored at different conditions were found (Table 2).

The concentration of total aldehydes in Buža, Črna and Rosinjola oils did not significantly change after 12 months of storage at different temperatures (Table 2). The concentration of C6 aldehyde *E*-2-hexenal, the most abundant volatile compound in three investigated monovarietal olive oils, did not significantly change (Table 2). These results are not in accordance with the results obtained by Cavalli *et al*. (2004), who detected a decrease in the *E*-2-hexenal concentration in Cailletier, Blanquettier and Arbequines monovarietal olive oil samples after 8 months of storage at ambient temperature in the dark and considered *E*-2-hexenal as a quality freshness marker of VOO. However, Cavalli *et al*. (2004) did not report excluding oxygen, which may have contributed to the loss in *E*-2-hexenal. Since *E*-2-hexenal significantly contributes to the aroma of olive oils and is related to the positive sensory characteristics of almond and green olive fruits (Luna *et al*. 2006), it could be concluded that after 12 months of storage investigated VOO could preserve the "green" aroma of fresh olive oils. According to Jiménez *et al*. (2007), a higher hexanal/*E*-2-hexenal ratio indicates a higher oxidation degree of the oil, since hexanal (except that produced in the LOX pathway during olive oil production), could also originate from auto oxidation of unsaturated fatty acids. After 12 months of storage at RT, the value of the hexanal/*E*-2-hexenal ratio remained unchanged in the Buža oil sample due to a moderate increase in hexanal and a slight, but not significant, increase in *E*-2-hexenal (Figure 1). In Črna and Rosinjola oils stored at RT this ratio significantly increased compared to fresh samples due to the increase in hexanal (Figure 1). The increase in hexanal indicated that oxidative alterations took place during the storage of all oil samples at RT. Also, after 12 months of storage in a freezer (at −20 °C) the values of hexanal/*E*-2-hexenal ratio increased in all samples (Figure 1). However, it seems that oxidative deterioration did not occur during storage in a refrigerator at +4 °C, since the value of hexanal/*E*-2-hexenal ratio remained equal in Črna and Rosinjola, or even decreased in Buža oil samples. The reason for the higher expressed oxidation changes in the freezer compared to refrigerator storage conditions could be due to a change in the physical state during the freezing process of VOO. At −20 °C, the freezing of olive oil is quick, and completely solidified oil has a porous structure due to solidification in the spherical shape. On the other hand, at +4 °C olive oil solidifies gradually and more or less a portion of solid phase remains in the continuous oil phase. The porous structure probably facilitates the penetration of air through the whole volume of the oil sample and it could be one of the reasons for significant oxidation changes, determined by the hexanal/*E*-2-hexenal ratio, which occurred in oil stored at −20 °C.

Alcohols, connected to the sensory characteristics fruity, green and aromatic, have less sensory significance than aldehydes, due to their high threshold values (Luna *et al*. 2006). Similarly to total aldehydes, changes in the concentration of total alcohols caused by 12 months of storage at three

TABLE 2. Volatile compounds (mg·kg^{-1}) in Buža, Črna and Rosinjola virgin olive oils before (fresh) and after 12 months of storage at different temperatures

Volatile compound	KI	KIref	Buža				Črna				Rosinjola			
				Stored at				Stored at				Stored at		
			Fresh	RT*	+4 °C	−20 °C	Fresh	RT*	+4 °C	−20 °C	Fresh	RT*	+4 °C	−20 °C
Aldehydes														
3-Methylbutan-1-al	925	916, 917	0.01^{c} (0.00)	0.03^{a} (0.00)	0.03^{b} (0.01)	0.02^{b} (0.00)	0.01^{b} (0.00)	0.02^{a} (0.01)	0.02^{a} (0.00)	0.01^{ab} (0.00)	0.00^{b} (0.00)	0.01^{a} (0.00)	0.01^{a} (0.00)	0.01^{a} (0.00)
Hexanal	1069	1074, 1073	0.43^{b} (0.02)	0.46^{b} (0.01)	0.38^{c} (0.02)	0.52^{a} (0.01)	0.30^{c} (0.00)	0.43^{a} (0.01)	0.28d (0.00)	0.36^{b} (0.00)	0.35^{c} (0.00)	0.50^{a} (0.02)	0.33^{c}(0.01)	0.43^{b} (0.01)
E-2-Pentenal	1114	1127, 1135	0.08^{c} (0.00)	0.18^{a} (0.01)	0.14^{ab} (0.03)	0.11^{bc} (0.00)	0.08^{b} (0.00)	0.09^{a} (0.00)	0.09^{a} (0.00)	0.09^{a} (0.00)	0.04^{b} (0.00)	0.07^{a} (0.01)	0.05^{b} (0.00)	0.06^{ab} (0.00)
E-2-Hexenal	1206	1216, 1225	9.20^{a} (0.35)	9.94^{a} (0.04)	9.95^{a} (0.09)	9.38^{a} (1.11)	5.89^{a} (0.06)	6.14^{a} (0.13)	5.62^{a} (1.12)	5.82^{a} (0.61)	10.49^{a} (0.33)	10.49^{a} (0.30)	10.22^{a} (0.32)	10.38^{a} (0.49)
Octanal	1277	1288, 1286	0.03^{a} (0.00)	0.04^{a} (0.01)	0.03^{a} (0.01)	0.02^{a} (0.00)	0.03^{a} (0.00)	0.06^{a} (0.02)	0.05^{a} (0.04)	0.04^{a} (0.00)	0.06^{b} (0.01)	0.07^{b}(0.01)	0.15^{a}(0.02)	0.07^{b} (0.03)
Alcohols														
Z-2-Penten-1-ol	1311	1323, 1321	0.32d (0.01)	0.41^{a} (0.01)	0.38^{b} (0.00)	0.35^{c} (0.02)	0.40^{a} (0.00)	0.44^{a} (0.02)	0.40^{a} (0.07)	0.42^{a} (0.01)	0.43^{a} (0.01)	0.46^{a} (0.01)	0.45^{a} (0.45)	0.42^{a} (0.01)
Hexanol	1345	1357, 1354	3.82^{a} (0.14)	4.00^{a} (0.05)	3.87^{a} (0.09)	3.83^{a} (0.10)	1.80^{a} (0.02)	1.72^{a} (0.13)	1.64^{a} (0.21)	1.78^{a} (0.01)	0.93^{a} (0.06)	0.81^{ab} (0.00)	0.78^{b} (0.04)	0.82^{ab} (0.03)
E-3-Hexen-1-ol	1354	1366, 1371	0.12^{a} (0.00)	0.10^{b} (0.00)	0.09^{c} (0.00)	0.09^{c} (0.00)	0.10^{b} (0.00)	0.21^{a} (0.03)	0.17^{a} (0.04)	0.18^{a} (0.00)	0.10^{ab} (0.01)	0.11^{a} (0.00)	0.09^{ab} (0.00)	0.09^{b} (0.01)
Z-3-Hexen-1-ol	1373	1385, 1388	6.87^{a} (0.14)	7.26^{a} (0.07)	7.03^{a} (0.19)	7.02^{a} (0.16)	4.93^{a} (0.04)	4.83^{a} (0.24)	4.78^{a} (0.65)	5.13^{a} (0.03)	3.65^{a} (0.07)	3.28^{a} (0.05)	3.42^{a} (0.17)	3.53^{a} (0.10)
E-2-Hexen-1-ol	1397	1408, 1410	1.60^{a} (0.04)	1.62^{a} (0.03)	1.59^{a} (0.02)	1.58^{a} (0.07)	1.57^{a} (0.02)	1.63^{a} (0.12)	1.56^{a} (0.17)	1.69^{a} (0.03)	4.62^{a} (0.13)	3.95^{b} (0.17)	4.04^{b} (0.19)	4.20^{ab} (0.07)
Esters														
Ethyl 2-methylbutyrate	1042	1040	tr.	tr.	tr.	tr.	n.d	n.d	n.d	n.d	n.d.	0.01^{a} (0.01)	n.d.	n.d.
Butyl acetate	1062	1077	n.d.	n.d.	n.d.	n.d.	n.d	n.d	n.d	n.d	n.d.	tr.	tr.	tr.
3-Methylbutyl acetate	1116	1120	0.01^{a} (0.00)	tr.	tr.	tr.	0.03^{a} (0.00)	0.02^{a} (0.00)	0.02^{a} (0.00)	0.02^{a} (0.00)	0.02^{a} (0.00)	0.01^{a} (0.00)	0.01^{a} (0.00)	0.02^{a} (0.00)
Hexyl acetate	1265	1274, 1269	0.05^{a} (0.01)	0.04^{ab} (0.02)	0.05^{a} (0.01)	0.02^{b} (0.00)	0.02^{b} (0.01)	0.06^{a} (0.00)	0.02^{b} (0.00)	0.02^{b} (0.00)	0.08^{a} (0.02)	0.05^{a} (0.02)	0.06^{a} (0.03)	0.06^{a} (0.02)
Ketones														
Pentan-3-one	971	979, 980	0.23^{c} (0.01)	0.51^{a} (0.02)	0.44^{ab} (0.77)	0.37^{b} (0.02)	0.22^{b} (0.00)	0.44^{a} (0.01)	0.39^{a} (0.00)	0.38^{a} (0.04)	0.21d (0.00)	0.54^{a} (0.02)	0.45^{b} (0.02)	0.39^{c} (0.02)
1-Penten-3-one	1010	1016, 1008	0.22^{a} (0.01)	0.23^{a} (0.00)	0.23^{a} (0.01)	0.20^{b} (0.01)	0.09^{a} (0.00)	0.07^{c} (0.00)	0.07^{c} (0.00)	0.08^{b} (0.01)	0.04^{b} (0.00)	0.05^{b} (0.00)	0.05^{b} (0.00)	0.07^{a} (0.00)
Total aldehydes			9.75^{a} (0.37)	10.64^{a} (0.04)	10.52^{a} (0.07)	10.05^{a} (1.12)	6.29^{a} (0.06)	6.74^{a} (0.15)	6.06^{a} (1.14)	6.32^{a} (0.61)	10.94^{a} (0.33)	11.13^{a} (0.33)	10.75^{a} (0.35)	10.94^{a} (0.50)
Total alcohols			12.72^{a} (0.32)	13.40^{a} (0.15)	12.96^{a} (0.28)	12.86^{a} (0.35)	8.82^{a} (0.05)	8.82^{a} (0.54)	8.54^{a} (1.13)	9.21^{a} (0.05)	9.73^{a} (0.28)	8.59^{b} (0.23)	8.78^{ab} (0.41)	9.05^{ab} (0.22)
Total esters			0.06^{a} (0.01)	0.05^{ab} (0.02)	0.05^{a} (0.01)	0.02^{b} (0.00)	0.05^{b} (0.01)	0.08^{a} (0.00)	0.05^{b} (0.00)	0.04^{b} (0.01)	0.09^{a} (0.02)	0.08^{a} (0.03)	0.07^{a} (0.03)	0.08^{a} (0.02)
Total ketones			0.46^{c} (0.01)	0.74^{a} (0.02)	0.67^{ab} (0.09)	0.57^{bc} (0.03)	0.31^{b} (0.00)	0.50^{a} (0.01)	0.46^{a} (0.01)	0.46^{a} (0.05)	0.25^{c} (0.00)	0.59^{a} (0.02)	0.50^{b} (0.02)	0.46^{b} (0.02)
Total volatiles			23.11^{a} (0.70)	24.98^{a} (0.17)	24.35^{a} (0.28)	23.64^{a} (1.51)	15.56^{a} (0.10)	16.25^{a} (0.64)	15.40^{a} (2.48)	16.14^{a} (0.60)	21.11^{a} (0.61)	20.59^{a} (0.63)	20.30^{a} (0.71)	20.73^{a} (0.71)

Results are mean values of three replicates (SD), the means within each row for single cultivar, labelled by different letters, are significantly different (Tukey's test, $p<0.05$). Identification methods: identification by comparison with retention times and mass spectra of pure standards, by comparison with mass spectra from NIST05 library and by comparison with Kováts' retention indexes (KI) from literature (Vichi *et al*. 2003; Bianchi *et al*. 2007; Kandylis *et al.* 2011). tr.=traces; n.d.=not detected. *RT=room temperature.

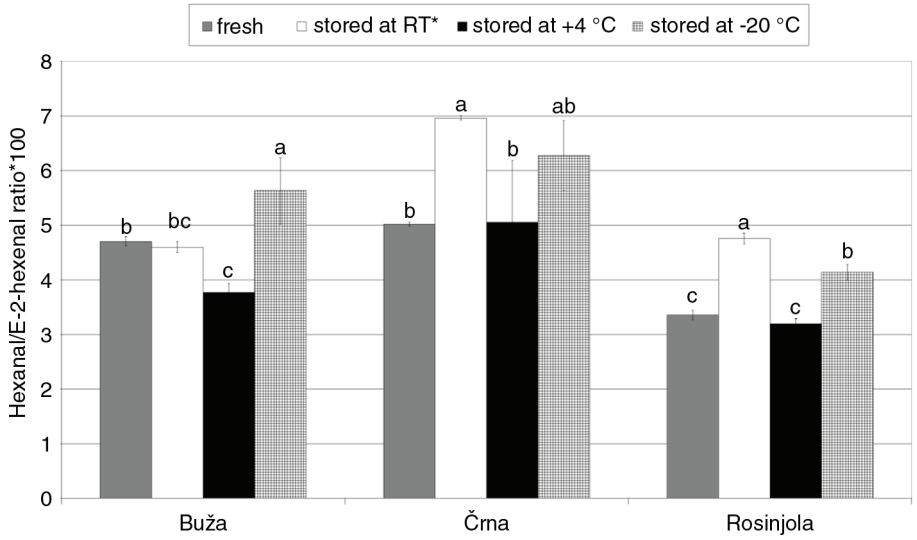

FIGURE 1. Hexanal/*E*-2-hexenal concentration ratios in Buža, Črna and Rosinjola virgin olive oils before (fresh) and after 12 months of storage at different temperatures. Results are mean values of three replicates ±SD; the means labelled by different letters for each cultivar are significantly different (Tukey's test, $p<0.05$). *RT=room temperature.

different temperatures were not detected. In the case of Rosinjola stored at RT a significant, although moderate, decrease of 12% compared to a fresh sample was detected. In this case, a decrease in total alcohols was mainly caused by a moderate decrease in C6 alcohol *E*-2-hexen-1-ol (Table 2). Angerosa (2000) considered that the reduction in the amount of C6 alcohols indicates diminishing of the sensory quality of olive oils.

The concentration of total esters, related tothe fruity odor of olive oils (Luna *et al*. 2006), remained unchanged after 12 months of storage in most of the studied cases with the exception of the Buža oil sample stored at −20 °C (decrease by 61% compared to initial concentration), and the Črna oil sample stored at RT (increase by 75% compared to initial concentration). The changes in the concentration of total esters were due to changes in the hexyl acetate concentration (Table 2). Taking into account the odor threshold found in the literature (Luna *et al*. 2006) (1.04 $mg \cdot kg^{-1}$), the hexyl acetate in our investigated samples has an odor activity value (OAV, ratio of concentration to odor threshold) lower than 1. Therefore, it can be assumed that the changes in hexyl acetate concentration should not considerably affect the aroma of Črna and Buža oils.

The concentration of total ketones, which are connected to fruity and etheric sensory attributes (Luna *et al*. 2006), was significantly increased after storage in all the studied cases, mainly due to an increase in pentan-3-on (Table 2). In Buža oils, an increase from 27% (storage at −20 °C) to 61% (storage at RT) was determined. In Črna oils, the increase ranged from 51% (storage at −20 °C) to 64% (storage at RT). The highest increase in the level of total ketone concentration compared to initial values was found in Rosinjola oils, from 84% (storage at −20 °C) to 136% (storage at RT). It is evident that the highest increase in total C5 ketone concentration, regardless of the cultivar origin of the oil samples, occurred at RT. Since pentan-3-on has a high odor threshold (70 $mg \cdot kg^{-1}$) found in the literature (Tena *et al*. 2007), it probably has no direct sensory contribution (OAV<1) to the aroma of the investigated olive oils, but could be considered a marker of oil freshness. Cavalli *et al*. (2004) also noticed an increase in C5 ketones in Cailletier, Blanquettier and Arbequines olive oil samples after 8 months of storage at ambient temperature in the dark and recommended these compounds as quality-freshness markers for VOOs.

4. CONCLUSIONS

After 12 months of storage in the dark at low temperatures VOO maintained better quality properties compared to oils stored at room temperature which indicates that refrigeration and freezing could slow down the oxidation rate of EVOO. A negligible but not statistically significant decrease in the total phenols was detected after 12 months of storage at all investigated temperatures. The VOO volatile profile shows quite good stability during storage time at all investigated temperatures. The concentration of total volatile compounds, total aldehydes, total alcohols and total esters in almost all stored samples was unchanged compared to fresh oil samples. However,

some changes in the behavior of individual volatile compounds among oils stored at different temperatures were detected. The concentration of total ketones increased after storage at all temperatures due to an increase in pentan-3-on, although these changes were less expressed at lower storage temperatures. Considering hexanal concentration and hexanal/*E*-2-hexenal ratio values as indicators of oxidation, it can be concluded that storage at low temperature, especially at +4 °C, is more appropriate to preserve the fresh oil volatile profile. Although storage at room temperature is common for use in households, storage at lower temperatures by olive oil producers and retailers could help to prolong the shelf life of EVOO. Furthermore, cold and frozen storage of VOO samples used for research or control purposes can be recommended, since changes in standard quality parameters, phenol content and volatile compound profiles are less noted at these temperatures.

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