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Variability of Phenolic and Volatile Compounds in Virgin Olive Oil from Leccino and Istarska Bjelica Cultivars in Relation to Their Fruit Mixtures

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Summary

Phenolic and volatile compounds are closely related to valuable gastronomic and nutritional properties, as well as oxidative stability of virgin olive oil. Since biochemical synthesis and transformation of these compounds during olive processing depend on the activity of endogenous enzymes, which are partially influenced by genetic factors, mixtures of different cultivars could have either a synergistic or antagonistic effect on phenolic and volatile compounds in the resulting oil. In this context, two specific cultivars from the Istrian peninsula, Leccino (L) and Istarska bjelica (B), were selected. Two monovarietal fruit samples (L100 and B100) and four mixtures in the following mass ratios: L/B=80:20, L/B=60:40, L/B=40:60 and L/B=20:80 were prepared. The mass fraction of total phenols was determined colourimetrically, while C6 and C5 volatiles from lipoxygenase pathway were determined by headspace solid-phase microextraction-gas chromatography. Mass fraction of total phenols in the oil samples from fruit mixtures changed linearly from (199.5±7.2) in Leccino to (642.0±61.7) mg/kg in Istarska bjelica, in a strict correlation with fruit mass ratio of the two cultivars. Leccino monovarietal samples had statistically higher values ($p \leq 0.05$) of C6 aldehydes ((15.32±1.69) *vs.* (10.91±0.62) mg/kg) and C6 alcohols ((2.96±0.98) *vs.* (0.17±0.05) mg/kg), but lower values of C5 compounds ((0.77±0.12) *vs.* (0.96±0.05) mg/kg) compared to Istarska bjelica samples. Volatiles having a direct contribution to the oil aroma (odour activity value >1.0) were 1-penten-3-one (84–201), *E*-2-hexenal (26–42), hexanal (1.8–2.4) and *Z*-2-penten-1-ol (1.3–2.6). A significant synergistic effect was observed for C6 aldehydes in the case of L/B=40:60 fruit mixture. The addition of Istarska bjelica to Leccino fruits caused a significant antagonistic effect on C6 alcohols, but no significant deviations from the expected values were found in the case of C6 esters and C5 compounds. Results suggest that fruit combinations of two chosen cultivars in olive processing offer interesting possibilities for targeted modulation of phenolic and volatile compounds in virgin olive oil, and consequently, their sensory and nutritional characteristics.

Key words: volatiles, phenols, olive oil, Istarska bjelica, Leccino, fruit mixtures

Introduction

Minor components, *i.e.* phenolic and volatile compounds, are closely related to valuable gastronomic and nutritional properties, as well as to oxidative stability of virgin olive oil (*1*). Factors influencing its biochemical synthesis and transformation during olive processing have

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been studied from many aspects in recent years. Activity of endogenous enzymes, which starts with crushing of fruits and continues during olive paste kneading (*2*), depends on the degree of tissue fragmentation, thermal energy released due to friction (*3,4*), oxygen availability and duration of single operation (*5*). Regarding phenolic fraction, its content and composition in virgin olive oil is related to β-glucosidase (which transforms hydrophilic phenolic glycosides into less hydrophilic aglycones) and esterase (which releases simple phenols from complex forms) (*6*), as well as to oxidative enzymes (polyphenol oxidase, peroxidase and lipoxygenase), which cause oxidative degradation of phenols (*7*). Generally, a higher temperature and fragmentation degree achieved by crushing contribute to higher solubility of phenolic compounds in the oil phase of olive paste. Higher kneading time commonly leads to a decrease of phenolic substances in oil, because of higher exposure to oxygen and activity of oxidative enzymes (*8*).

The biosynthesis of the main volatile compounds of virgin olive oil starts at the moment of cell disruption during fruit crushing, when a series of enzymes involved in lipoxygenase (LOX) pathway are released. The first substrates are phospholipids and glycerolipids in membrane structures (*9*) from which acyl hydrolase detaches polyunsaturated fatty acids. Linoleic and linolenic acids are then oxidised by lipoxygenase and cleaved by hydroperoxide lyase into C6 aldehydes, which can be later reduced to C6 alcohols by alcohol dehydrogenase and transformed to C6 esters by alcohol acyltransferase. It was generally assumed that milder crushing conditions (*i.e.* a lower increase of olive paste temperature) have a positive impact on the content of LOX volatiles in virgin olive oil (*2-4*). Volatiles are mostly produced at crushing, while during malaxation, their biosynthesis is slowed down and leads to higher values of C6 alcohols as well as C6 and C5 carbonyl compounds (*2*). However, longer malaxation time has a positive impact on volatile concentration in oil since it allows repartition of compounds between oil and water phases in olive paste (*10*). Lower temperature and sufficient oxygen availability during this operation are other parameters that contribute to higher concentration and more desirable composition of volatile compounds (*11*).

Besides processing factors, the activity of enzymes involved in the biosynthesis of phenolics and LOX volatiles is in great measure related to ripeness degree of healthy fruits and their genetic characteristics. It is well known that virgin olive oil obtained from different cultivars, cultivated under identical conditions, harvested at roughly equal ripeness degree, and processed in the same manner is characterised by more or less different composition and concentration of minor components (*2,12*). Fruits of each single cultivar probably bring their own specific enzymatic package, characterised by particular activity, sensibility or quantity of a single enzyme. Besides, differences among cultivars in a flesh/stone mass ratio, oil and water content or phenol and acid concentration contribute to the diversity of environment (*i.e.* olive paste) in which enzymes operate. Therefore, combination of fruits of different cultivars in olive processing could have either a synergistic or antagonistic effect on the synthesis and accumulation of minor compounds in the resulting oil. In fact, Angerosa and Basti (*13*) have shown that the volatile profile changes from qualitative and quantitative point of view when fruits of cultivar Coratina are combined with those of Frantoio or Koroneiki. On the other hand, when monovarietal oil samples are combined, the volatile profile can be easily predicted, since it is proportional to the mass ratio of the components in oil mixtures.

The aim of this research is to evaluate the effect of fruit combinations of two specific cultivars from the Istrian peninsula during olive processing on minor compounds in oil. Istarska bjelica (synonyms Istrska belica or Bianchera istriana) is an autochthonous cultivar characterised by late ripening and relatively high oil mass fraction in the fresh fruit (on average 21 %). Among cultivars introduced to Istria, Tuscan cultivar Leccino is the most widespread and, in the given pedoclimatic conditions, it ripens early and accumulates much lower oil mass fraction in fresh fruit (on average 12 %) compared to Istarska bjelica (*14-16*). In the local producing practice, both cultivars are harvested contemporaneously and often processed as fruit mixture, regardless of their different stages of ripeness.

Materials and Methods

Olive fruits and preparation of virgin olive oil samples

Olive fruits of two cultivars, Leccino (L) and Istarska bjelica (B), grown in the region of Istria (Croatia), were handpicked in the middle of October 2007. Maturity index (MI) of the fruits of each cultivar was determined applying the method described by Gutiérrez *et al*. (*17*), which is based on the evaluation of olive skin and pulp colour. Samples of fruit mixtures of two cultivars were prepared by combining fresh and healthy fruits in the following mass ratios: L/B=80:20, L/B=60:40, L/B=40:60 and L/B=20:80. The prepared mixtures of olive fruits, as well as two monovarietal fruit samples (L100 and B100), were processed in the laboratory within 24 h after the harvesting. The laboratory plant for oil extraction consisted of the hammer crusher, vertical olive paste mixers placed in the thermostated water bath and centrifuge (MC2 Ingenieria y Sistemas, Seville, Spain). Olive paste samples of each combination of varieties were divided into three portions of 600 g, which were malaxed at (26± 0.5) °C for 40, 50 and 60 min, respectively. Taking into account variations in malaxation duration in common production practice, the three portions were considered as independent repetitions of a single variety combination. After centrifugation at 3600 rpm for 70 s, the extracted oil was filtered through the filter paper and stored at room temperature in fully filled and sealed dark bottles until analyses.

Reagents and analytical materials

Caffeic acid (purity 99 %) and methanol were purchased from Panreac (Barcelona, Spain), while sodium carbonate decahydrate and Folin-Ciocalteu reagent were obtained from Merck KGaA (Darmstadt, Germany). Twelve standards of volatile compounds had a GC purity ≥95 %. *E*-2-hexenal, *E*-2-pentenal, pentan-3-one and *Z*-2-penten-

-1-ol were purchased from Sigma-Aldrich (Steinheim, Germany). Hexan-1-ol, 1-penten-3-one, *E*-2-hexen-1-ol, *E*-3-hexen-1-ol, hexanal, hexyl acetate, *Z*-3-hexenyl acetate and *Z*-3-hexen-1-ol were supplied from Fluka (Sigma--Aldrich, Buchs, Germany).

Determination of total phenols mass fraction

A mass of 2 g of oil samples was placed in a 20-mL screw cap test-tube and extracted twice with 5 mL of methanol/water (80:20, by volume). Each time the mixture was stirred for 5 min in a vortex apparatus (IKA, Staufen, Germany) and centrifuged at 3800 rpm for 4 min. Upper methanol layers were transferred into a 25-mL volumetric flask, which was then filled up with methanol/water (80:20, by volume). The determination of total phenolic content in the extracts was based on the procedure introduced by Gutfinger (*18*) using Folin-Ciocalteu reagent and caffeic acid as standard. The analyses were run in triplicate.

Analysis of volatile compounds

A fused silica fibre coated with highly crosslinked divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS), 1 cm length, 50/30 μm film thickness (Supelco, Bellefonte, PA, USA), was used for the headspace solid-phase microextraction (HS-SPME) and concentration of volatiles. The sample (4.0 g) was placed in a 10-mL vial containing a microstirring bar and sealed with 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 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 SPME liner (0.80 mm i.d.) in splitless mode for 3 min at 245 °C. 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.

Gas chromatography (GC) analyses were performed using a Varian 3350 gas chromatograph (Varian Inc., Harbour City, TA, USA) equipped with a flame ionization detector (FID) operated at 248 °C and capillary column Rtx-WAX (30 m×0.25 mm i.d.×0.25 μm film thickness; Restek). Initial oven temperature was 40 °C, increased after 8 min to 85 °C 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 pressure of 15 psi (10.3 kPa) at the column head. Volatiles were identified by comparing their retention times to those of the pure standards. Quantification was carried out using calibration curves of pure standards dissolved in freshly refined sunflower oil. The analyses were run in triplicate.

Statistical analysis

Differences among samples were tested by a one-way analysis of variance at 5 % significance level. The homogeneity of variance was tested by the Brown-Forsythe test. 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 (*19*).

Results and Discussion

In accordance with the available literature, the oil samples obtained from the two selected cultivars differ greatly in total phenolic content, which is on average two to four times higher in the oil from Istarska bjelica (*14,20-22*). This was also confirmed in this study, since monovarietal oil obtained from Istarska bjelica fruits had about three times higher values ((642.0±61.7) mg/kg) than the oil from Leccino fruits ((199.5±7.2) mg/kg) (Fig. 1). Certainly, besides genetic factors, the observed differences are also related to different fruit ripening stages of the two cultivars at the moment of processing. Namely, Leccino and Istarska bjelica have different dynamics of fruit ripening. Fresh olive fruits of Istarska bjelica used in this research had lower maturity index (0.7 – most of the fruits had yellowish green epidermis), compared to those of Leccino (2.8 – most of the fruits had light violet epidermis).

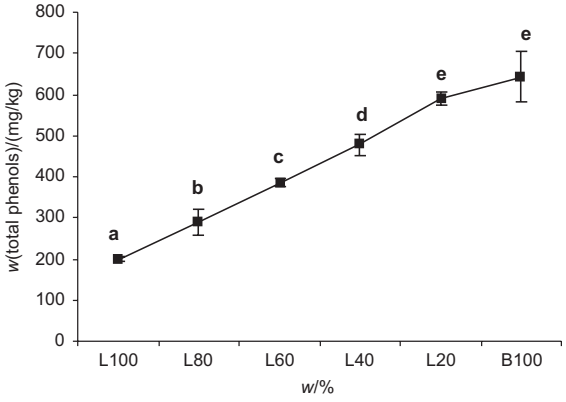

Fig. 1. Total phenols in the oil samples obtained from fruit mixtures of Leccino (L) and Istarska bjelica (B). Numbers next to the letter L or B represent the mass ratio of Leccino or Istarska bjelica fruits in fruit mixtures, respectively. Results are expressed as mean values of nine measurements (3 independent repetitions×triplicate analyses)±S.D.; the mean values labelled with different letters are significantly different (Tukey's test, $p<0.05$)

Nevertheless, in local production practice, both cultivars are often harvested contemporaneously and processed as fruit mixtures, regardless of their different stages of ripeness. The total phenolic content in the oil obtained from fruit mixtures changed linearly in strict correlation with the fruit mass ratio of the two cultivars. This suggests that no synergistic or antagonistic effects on the enzymatic activity were induced combining the fruits of these two cultivars with presumably different enzymatic patterns, water and oil content. Consequently, total phenolic mass fraction should be easily predicted in the oil obtained by processing fruit mixtures of Leccino and Istarska bjelica.

Contrary to the total phenolic content, Leccino monovarietal oil was richer in volatile components derived from lipoxygenase pathway ((19.15±2.18) mg/kg) than Istarska bjelica ((12.18±0.70) mg/kg) (Table 1). Hexanal and *E*-2-hexenal are the main C6 aldehydes that are produced at the initial part of this enzymatic pathway. Leccino monovarietal oil samples had significantly higher

Table 1. Mass fraction of volatile compounds from LOX pathway in oil samples obtained from fruit mixtures of Leccino (L) and Istarska bjelica (B)

Volatile compounds	*w*/(mg/kg)					
	L100	L80	L60	L40	L20	B100
hexanal	(0.17±0.03)ab	(0.15±0.01)a	(0.17±0.03)ab	(0.19±0.01)b	(0.18±0.01)b	(0.18±0.02)b
E-2-hexenal	(15.15±1.60)a	(14.95±0.63)a	(15.88±2.03)ab	(17.55±1.02)bc	(16.51±0.91)ac	(10.73±0.61)d
ΣC6 aldehydes	(15.32±1.69)a	(15.09±0.63)a	(16.06±2.06)ab	(17.73±1.03)bc	(16.69±0.92)ac	(10.91±0.62)d
hexan-1-ol	(0.20±0.06)a	(0.05±0.00)b	(0.05±0.01)b	(0.04±0.00)c	(0.03±0.01)c	(0.01±0.00)d
Z-3-hexen-1-ol	(0.37±0.25)a	(0.41±0.10)a	(0.39±0.19)a	(0.07±0.01)b	(0.08±0.01)b	(0.11±0.02)b
E-3-hexen-1-ol	tr.	0.09±0.01	n.d.	n.d.	n.d.	n.d.
E-2-hexen-1-ol	(2.38±0.72)a	(1.40±0.11)b	(0.08±0.02)c	(0.08±0.02)c	(0.06±0.01)c	(0.05±0.03)c
ΣC6 alcohols	(2.96±0.98)a	(1.94±0.14)b	(0.52±0.21)c	(0.19±0.03)c	(0.17±0.02)c	(0.17±0.05)c
hexyl acetate	tr.	tr.	n.d.	n.d.	n.d.	n.d.
Z-3-hexenyl acetate	(0.14±0.04)ab	(0.16±0.02)a	(0.13±0.02)b	(0.16±0.01)ab	(0.14±0.01)ab	(0.14±0.03)ab
ΣC6 esters	(0.14±0.04)a	(0.18±0.02)b	(0.13±0.02)a	(0.16±0.01)ab	(0.14±0.01)a	(0.14±0.03)a
1-penten-3-one	(0.09±0.02)a	(0.06±0.01)b	(0.11±0.02)a	(0.11±0.02)a	(0.11±0.01)a	(0.14±0.01)c
E-2-pentenal	(0.10±0.01)ac	(0.03±0.01)b	(0.09±0.01)a	(0.11±0.01)c	(0.11±0.01)c	(0.08±0.00)d
Z-2-penten-1-ol	(0.33±0.08)a	(0.47±0.03)bc	(0.40±0.05)b	(0.51±0.03)c	(0.53±0.03)c	(0.65±0.05)d
pentan-3-one	(0.25±0.07)a	(0.08±0.01)b	(0.06±0.02)b	(0.08±0.02)b	(0.07±0.02)b	(0.09±0.01)b
ΣC5 compounds	(0.77±0.12)a	(0.63±0.03)b	(0.66±0.09)b	(0.81±0.05)a	(0.82±0.07)a	(0.96±0.05)c
total volatiles	(19.15±2.18)a	(17.85±0.74)a	(17.37±2.36)a	(18.51±1.08)a	(17.83±0.99)a	(12.18±0.70)b

Results are mean values of nine measurements (3 independent repetitions×triplicate analyses)±S.D.; the mean values within each row labelled with different letters are significantly different (Tukey's test, $p<0.05$); tr.=traces; n.d.=not detected
Numbers next to the letter L or B represent the mass ratio of Leccino or Istarska bjelica fruits in fruit mixtures, respectively

values of the two C6 aldehydes compared to Istarska bjelica monovarietal oil samples, and this was consistent with the previously published data for these cultivars (*23*), although it must be stressed that differences in their fruit ripening stage at the moment of processing should not be neglected. Different values of C6 aldehydes could be due to different acyl hydrolase activity and consequently good or poor availability of free polyunsaturated fatty acids, as it was found by Sánchez-Ortiz *et al*. (*24*) for cultivars Picual and Arbequina, respectively. Nevertheless, by increasing the mass fraction of Istarska bjelica fruits in the mixtures with Leccino, no reduction of C6 aldehydes was observed. Moreover, a slight increase regarding Leccino monovarietal oil could be seen in samples with 40 % (L60) and 80 % (L20) of Istarska bjelica fruits, which became statistically significant in the case of 60 % (L40). The mass fraction of C6 alcohol representatives, which are products of catalytic action of alcohol dehydrogenase on C6 aldehydes, was also significantly higher in Leccino monovarietal oil samples, but its trend was opposite to that of C6 aldehydes. The addition of 60 and 80 % of Istarska bjelica to Leccino fruits caused a clear decrease of C6 alcohols, yielding values similar to Istarska bjelica monovarietal oil samples. In spite of apparently higher substrate availability (higher mass fraction of C6 aldehydes), the presence of Istarska bjelica in Leccino olive paste gave rise to antagonistic effect on C6 alcohol biosynthesis (values were lower than expected on the basis of the cultivar fruit mass ratio). In fact, the above discussed increase of C6 aldehyde mass fraction could be the consequence of their accumulation caused by the repression of C6 alcohol biosynthesis, rather than enhanced production. Similar synergistic phenomena in the case of C6 aldehydes, or antagonistic in the case of alcohols hexan-1-ol and *E*-2-hexen-1-ol, have been reported for fruit mixtures of Coratina and Koroneiki, or Coratina and Frantoio by Angerosa and Basti (*13*). The reason for repressed C6 alcohol biosynthesis could be a high mass fraction of phenolic compounds in Istarska bjelica fruits, and sensibility of alcohol dehydrogenase to their inhibitory action, since it is known that phenols are able to bind and inhibit various enzymes (*25*). Regarding the representatives of C6 esters, monovarietal oil samples of two cultivars contained similar values. The increase of Istarska bjelica mass fraction in Leccino olive paste did not cause significant changes, except in the case of 20 % addition (L80). This could mean that alcohol acetyl transferase from Istarska bjelica was more active, resulting in higher production when substrates (C6 alcohols) were available in abundance, such as in the case of L80 samples. As for representatives of C5 volatiles, products of an additional branch of the LOX pathway (2), significantly higher values can be observed in Istarska bjelica monovarietal oil samples. By increasing the mass fraction of Leccino in the mixtures with Istarska bjelica fruits, the mass fraction of C5 compounds in the resulting oil samples decreased. In the case of 60 and 80 % of Leccino, C5 compounds were even lower than in the oil samples produced from 100 % Leccino fruits.

Nevertheless, when considering the literature on odour thresholds of single volatile compounds (*12*,*26*), only four out of twelve analysed components had odour activity values (OAV) higher than 1.0, making direct con-

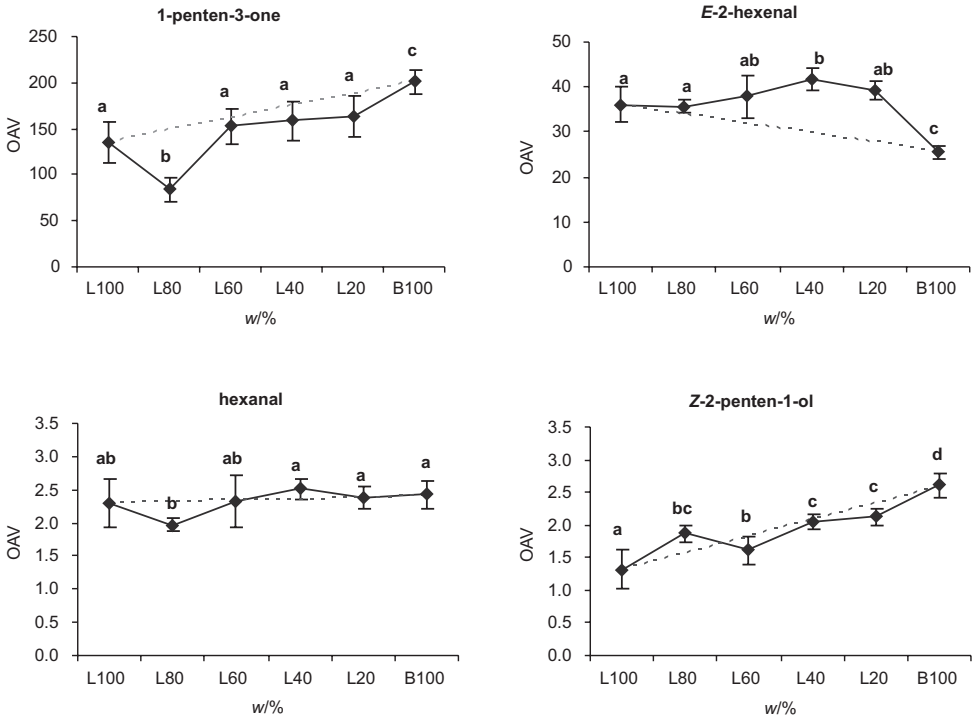

Fig. 2. Odour activity value (OAV) of compounds with direct contribution to the oil aroma in the oil samples obtained from fruit mixtures of Leccino (L) and Istarska bjelica (B). Numbers next to the letter L or B represent the mass ratio of Leccino or Istarska bjelica fruits in fruit mixtures, respectively. Results are expressed as mean values of nine measurements (3 independent repetitions×triplicate analyses)±S.D.; the mean values labelled with different letters are significantly different (Tukey's test, $p<0.05$). Dashed lines represent a linear trend between the values of monovarietal oil samples, calculated on the basis of fruit mass ratio. Literature threshold values (*26*): 0.42 mg/kg (*E*-2-hexenal), 0.0007 mg/kg (1-penten-3-one), 0.08 mg/kg (hexanal), 0.40 mg/kg (*Z*-2-penten-1-ol)

tribution to the pleasant aroma of oil samples. The odour activity values of 1-penten-3-one, *E*-2-hexenal, hexanal and *Z*-2-penten-1-ol, in relation to the varietal composition of fruit mixtures, are shown in Fig. 2. The highest contribution to the aroma of oil samples from both cultivars and their fruit mixtures was made by 1-penten-3-one, whose OAV ranged from 84 (L80) to around 201 (B100). This component is characterised by specific odour descriptors (pungent, mustard) considered positive for virgin olive oil aroma (*12*). Despite the highest odour activity values of monovarietal Istarska bjelica oil (OAV=201), no positive influence on Leccino samples was observed by increasing the mass fraction of Istarska bjelica fruits. The second most influencing compound was *E*-2-hexenal (odour descriptors: bitter almond, green-fruity (*12*)), with OAV ranging from 26 (B100) to around 40 (L20 and L40). The increase of odour activity value to the level of about 50 % in the samples with 20 and 40 % of Leccino fruits should have an evident impact on a stronger perception of green-fruity and almond odour notes regarding monovarietal Istarska bjelica oil. Hexanal (odour descriptors: green apple, grassy (*12*)) showed negligible variations of odour activity values (from 2.0 to 2.5) in relation to the fruit mixture composition. The compound with the most linear trend of odour activity values in the range from 1.3 (monovarietal Leccino oil) to 2.6 (monovarietal Istarska bjelica oil) was *Z*-2-penten-1-ol (odour descriptors: grassy, banana (*12*)). Consequently, a clear positive contribution of Istarska bjelica to the perception of grassy and banana odour notes in the oil obtained from the mixtures with Leccino fruits should be expected.

Conclusions

On the basis of the obtained results, it could be concluded that fruit combinations of the two chosen cultivars offer interesting possibilities for targeted modulation of minor compounds of virgin olive oil and, consequently, their sensory characteristics. While changes of the mass fraction of phenolic compounds could be managed simply, the volatile profile requires further research taking into account other characteristics of fruits, such as the ripening index, flesh/stone mass ratio or oil and water mass fraction.

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References

1. M. Servili, R. Selvaggini, S. Esposto, A. Taticchi, G. Montedoro, G. Morozzi, Health and sensory properties of virgin olive oil hydrophilic phenols: Agronomic and technological aspects of production that affect their occurrence in the oil, *J. Chromatogr. A*, *1054* (2004) 113–127.
2. F. Angerosa, M. Servili, R. Selvaggini, A. Taticchi, S. Esposto, G. Montedoro, Volatile compounds in virgin olive oil: Occurrence and their relationship with the quality, *J. Chromatogr. A*, *1054* (2004) 17–31.

3. A.M. Inarejos-García, G. Fregapane, M. Desamparados Salvador, Effect of crushing on olive paste and virgin olive oil minor components, *Eur. Food Res. Technol. 232* (2011) 441–451.
4. S.M. Preziuso, M.G. Di Serio, A. Biasone, R. Vito, M.R. Mucciarella, L. Di Giovacchino, Influence of olive crushing methods on the yields and oil characteristics, *Eur. J. Lipid Sci. Technol. 112* (2010) 1345–1355.
5. M. Servili, R. Selvaggini, A. Taticchi, S. Esposto, G. Montedoro, Air exposure time of olive pastes during the extraction process and phenolic and volatile composition of virgin olive oil, *J. Am. Oil Chem. Soc. 80* (2003) 685–695.
6. R. Briante, M. Patumi, S. Limongelli, F. Febbraio, C. Vaccaro, A. Di Salle *et al.*, Changes in phenolic and enzymatic activities content during fruit ripening in two Italian cultivars of *Olea europaea* L., *Plant Sci. 162* (2002) 791–798.
7. R. García-Rodríguez, C. Romero-Segura, C. Sanz, A. Sánchez-Ortiz, A.G. Pérez, Role of polyphenol oxidase and peroxidase in shaping the phenolic profile of virgin olive oil, *Food Res. Int. 44* (2011) 629–635.
8. L. Di Giovacchino, S. Sestili, D. Di Vincenzo, Influence of olive processing on virgin olive oil quality, *Eur. J. Lipid Sci. Technol. 104* (2002) 587–601.
9. J.J. Salas, M. Williams, J.L. Harwood, J. Sánchez, Lipoxygenase activity in olive (*Olea europaea*) fruit, *J. Am. Oil Chem. Soc. 76* (1999) 1163–1168.
10. A.M. Inarejos-García, A. Gómez-Rico, M. Desamparados Salvador, G. Fregapane, Influence of malaxation conditions on virgin olive oil yield, overall quality and composition, *Eur. Food Res. Technol. 228* (2009) 671–677.
11. A. Sánchez-Ortiz, C. Romero, A.G. Pérez, C. Sanz, Oxygen concentration affects volatile compound biosynthesis during virgin olive oil production, *J. Agric. Food Chem. 56* (2008) 4681-4685.
12. G. Luna, M.T. Morales, R. Aparicio, Characterisation of 39 varietal virgin olive oils by their volatile compositions, *Food Chem. 98* (2006) 243–252.
13. F. Angerosa, C. Basti, The volatile composition of samples from the blend of monovarietal olive oils and from the processing of mixtures of olive fruits, *Eur. J. Lipid Sci. Technol. 105* (2003) 327–332.
14. O. Koprivnjak, Analytical characterization of virgin olive oil from Pula area (Croatia), *PhD Thesis*, University of Udine, Udine, Italy (1996) (in Italian).
15. D. Bandelj Mavsar, E. Bešter, M. Bučar-Miklavčič, B. Butinar, D. Čalija, Ž. Kanjir *et al.*: *Factsheet on the Olive Variety Istrska Belica*, Phare Programme 00-0093 ECOS – Ouverture 1998–2001, Koper, Slovenia (2005).
16. D. Poljuha, B. Sladonja, K. Brkić Bubola, M. Radulović, K. Brščić, E. Šetić *et al.*, A multidisciplinary approach to the characterisation of autochthonous Istrian olive (*Olea europaea* L.) varieties, *Food Technol. Biotechnol. 46* (2008) 347–354.
17. F. Gutiérrez, B. Jímenez, A. Ruíz, M.A. Albi, Effect of olive ripeness on the oxidative stability of virgin olive oil extracted from the varieties Picual and Hojiblanca and on the different components involved, *J. Agric. Food Chem. 47* (1999) 121–127.
18. T. Gutfinger, Polyphenols in olive oils, *J. Am. Oil Chem. Soc. 58* (1981) 966–968.
19. STATISTICA (Data Analysis Software System), v. 9, StatSoft, Inc, Tulsa, OK, USA (2010) (*www.statsoft.com*).
20. G. Procida, L. Gabrielli Favretto, D. Vojnovic, M. Solinas, I. Zuzic, Olive oils of Istria. Chemical characterisation of the most common cultivars, *Ind. Aliment. 3* (1994) 308–312 (in Italian).
21. D. Škevin, D. Rade, D. Štrucelj, Ž. Mokrovčak, S. Neđeral, Đ. Benčić, The influence of variety and harvest time on the bitterness and phenolic compounds of olive oil, *Eur. J. Lipid Sci. Technol. 105* (2003) 536–541.
22. B. Butinar, M. Bučar-Miklavčič, M. Lipnik-Štangelj, Antioxidants in virgin olive oils produced from two olive cultivars of Slovene Istria, *Annales, Ser. Hist. Nat. 16* (2006) 201–208.
23. O. Koprivnjak, L.S. Conte, Đ. Benčić, N. Totis, Application of solid-phase microextraction of olive oil volatiles on varieties characterization, *Riv. Ital. Sost. Grasse, 80* (2003) 35–40.
24. A. Sánchez-Ortiz, A.G. Pérez, C. Sanz, Cultivar differences on nonesterified polyunsaturated fatty acid as a limiting factor for the biogenesis of virgin olive oil aroma, *J. Agric. Food Chem. 55* (2007) 7869–7873.
25. S. Quideau, D. Deffieux, C. Douat-Casassus, Plant polyphenols: Chemical properties, biological activities, and synthesis, *Angew. Chem. Int. Ed. 50* (2011) 586–621.
26. R. Aparicio, M.T. Morales, Characterization of olive ripeness by green aroma compounds of virgin olive oil, *J. Agric. Food Chem. 46* (1998) 1116–1122.