Influence of malaxation time on phenols and volatile compounds of virgin olive oil obtained from phenol enriched olive paste (Buža cv.)

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ABSTRACT
The malaxation of olive paste is an essential olive oil production step which allows not only satisfactory yields of oil extraction. During malaxation, changes of oil composition also occur because of the partition phenomena among oil, water and solid phase and the activity of fruit enzymes released during crushing. Quality and quantity of extracted oil can be influenced by varying the conditions of this operation (such as time, temperature, addition of technological coadjuvants). Different malaxation times (30, 45 and 60 min) were applied to olive paste of Buža cultivar which was previously enriched with the phenolic extract obtained from the freeze-dried olive pulp of Istarska Bjelica cultivar. Olive paste was phenol enriched at the level of 38% (w/w) in order to improve the phenolic content in resulting oils and to check the influence on volatile compounds. Phenols in olive pastes and corresponding olive oils were determined by RP HPLC with UV-DAD detection, whereas volatile compounds in oils were analyzed by SPME-GC-MS. An addition of phenolic extract to olive paste mostly affected the amounts of dialdehydic form of oleuropein aglycone and apigenin which increased by 473% and 90% in Buža paste, respectively. The major phenolic compound in olive paste was dialdehydic form of decarboxymethyloleuropein aglycone, as well as in the oil. By increasing the malaxation time, the decrease of free hydroxytirosol, tyrosol and verbascoside in enriched olive paste was observed. The similar was observed for oil samples, except for vanillin which increased (by 31%) along with the malaxation time. Among volatile compounds responsible for positive odour notes, the prolonged malaxation time had a significant negative effect (Tukey's test, $p<0.05$) on hexanal, hexan-1-ol and Z-3-hexenyl acetate. Longer malaxation time (60 min) of olive paste enriched with the phenolic extract showed more pronounced effect on phenols than on volatile compounds.

Keywords: Buža cultivar; malaxation time; olive oil; phenols; volatiles

INTRODUCTION

Volatile and phenolic compounds are largely responsible for the complex and desirable flavour of virgin olive oils (VOOs). Although the synthesis and biotransformation of these compounds mainly take place during the olive fruit tissue disruption in the crushing step of the VOO production process (Inarejos-García et al., 2011), these reactions also continue during the malaxation of olive paste (Clodoveo, 2012). During the malaxation step noticeable changes in VOO's chemical composition occur because of the partition phenomena of minor compounds between oil and water phases and the catalytic activities of endogenous enzymes, which are released in the moment of the crushing. Malaxation conditions, such as time and temperature, influence not only oil yield but also the volatile and phenol composition of final VOOs (Angerosa et al., 2001; Ranalli et al., 2003; Gomez-Rico et al., 2009).

Volatile compounds, responsible for the pleasant green and fruity odour notes of VOOs, are synthesised through the lipoxygenase pathway from free polyunsaturated fatty acids. Linoleic and linolenic fatty acid, detached by acyl hydrolase, are oxidized by lipoxygenase (LOX) and cleaved by hydroperoxide lyase (HPL) into C6 aldehydes, which can be later reduced to C6 alcohols by alcohol dehydrogenase (ADH) and transformed to C6 esters by alcohol acyl transferase (AAT) (Olías et al., 1993). Besides hydroperoxides formation, LOX can cleave them producing alkoxy radicals which lead to C5 volatile compounds formation that are contributors to green, sweet and pungent sensory perceptions (Angerosa et al., 2000).

During processing, considerable changes of the olive phenolic compounds take place due to the endogenous enzyme action. Sánchez-Ortiz et al. (2012b) tested the effect of a relatively high dose of synthetic phenol antioxidants, which are known as LOX inhibitors, on the synthesis of Arbequina and Picual VOO volatile compounds**.** In this study the impact of the increased level of phenolic compounds naturally present in olive fruits on the volatile compounds formation and the VOO phenol content was examined. Moreover, different malaxation times (30, 45 and 60 min) were applied to phenol enriched olive paste of Buža cultivar in order to improve the phenolic content in resulting oils and to check the influence on VOO volatile and phenolic compounds.

MATERIALS & METHODS

Olive fruits. Olive fruits of the cultivar Istarska Bjelica (maturity index, MI=0.7) and the cultivar Buža (MI=4.0) were handpicked during October and November of 2010. Fruits of Istarska Bjelica were used to obtain an aqueous solution of phenolic compounds intended for enrichment of Buža olive paste.

Preparation of the aqueous solution of phenolic compounds. The aqueous solution of phenolic compounds were prepared from the pulp of previously freeze-dried Istarska Bjelica fruits using methanol as the extraction solvent, according to the procedure described by Majetić Germek et al. (2013).

VOO samples preparation. Oil samples from Buža fruits were prepared using the laboratory plant (Abencor, MC2 Ingeneria y Sistemas, Spain) consisting of the hammer crusher, thermostated vertical olive paste mixers and the centrifuge. After fruit milling, an appropriate volume of the aqueous solution of phenolic compounds was added to the olive paste in order to increase the phenolic content by 30% (w:w) according to the colorimetric determination of the total phenols mass fraction. Control samples were obtained by the addition of the same volume of distilled water. Enriched olive pastes were malaxed for 30, 45 and 60 min at 25 ± 0.5 °C. Oil samples were extracted by centrifugation at 3600 rpm for 70 s and stored in the fully filled dark bottles at room temperature before being analysed. Two independent oil extraction procedures were performed for each oil sample.

Colorimetric determination of the total phenols mass fraction in olive paste and the aqueous solution. The total phenol mass fraction in both matrices was determined in order to define the amount of the aqueous solution to be added to olive paste. Total phenols were determined colorimetrically using Folin-Ciocalteu reagent as described in our previous work (Majetić Germek et al., 2013).

Determination of phenols composition of the olive paste and olive oil samples. Phenols were extracted from freeze-dried olive pastes according to the procedure described by Valenčič et al. (2010). Phenols extracted from the olive paste and olive oil samples were determined by RP HPLC with UV-DAD detection (Agilent Technologies 1100 series HPLC, Germany) according to a slightly modified method published by International Olive Council (2009). Tyrosol, apigenin and luteolin were used as calibration standards and syringic acid as an internal standard. For each sample four analyses were carried out.

Analysis of volatile compounds. The SPME sampling procedure described by Vichi et al. (2003) was used. Gas chromatograph (Shimatzu 2010, Japan) equipped with Supelco equity 5 capillary column 60 m × 0.25 mm i.d., 1 μm film tickness (Supelco, USA), and a quadrupole mass detector Shimadzu QP2010 (Japan) were used. A detailed description of analysis of volatile compounds is provided in our previous work (Majetić Germek et al., 2013). Volatile compounds were semiquantitated using the relative response factor of internal standard 4-methyl-2-pentanol and expressed as mg/kg of oil. Each sample was analysed in two parallel repetitions.

Statistical analysis. Differences among samples with different malaxation time were tested by 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 by Tukey's honest significant difference test ($p<0.5$). Statistical analyses were performed using the software package Statistica 10.

RESULTS & DISCUSSION

For this study, Buža cultivar was selected on the basis of its relatively low phenols content and high content of volatile compounds (Škevin et al., 2003; Brkić Bubola et al., 2012). Because of these characteristics Buža olive paste was considered suitable matrix for the enrichment with phenols in order to improve the phenolic content in resulting oils and to check the influence of malaxation time on phenolic and volatile compounds. For the olive paste enrichment, phenolic compound were extracted from the pulp of freeze-dried unripe fruits of Istarska Bjelica (MI=0.7), known as a cultivar with a high phenols content (Valenčič et al., 2010).

After enrichment of Buža olive paste, an increase of total phenols content compared to the control sample, determined by HPLC, was higher than intended (38% vs. 30%). This difference between the intended and achieved level of enrichment could be caused by a different measuring approaches of spectrophotometric and HPLC method (chromatographic separation and absorbance measurement of single phenols in the case of HPLC).

The phenol composition of enriched olive pastes malaxed for 30, 45 and 60 min is shown in Table 1. Among identified phenolic compounds the most abundant (20% of the total phenols mass fraction) was a dialdehydic form of decarboxymethyloleuropein aglycone (DMO-Agl-dA), probably a product of β-glucosidase and methylesterase activity on oleuropein during fruit crushing (El Riachy et al., 2011; Romero-Segura et al., 2012). Gómez-Rico et al. (2009) have reported that the majority of the oleuropein in olive fruits was transformed in its derivatives only a few minutes after crushing, among which DMO-Agl-dA was predominant. An addition of the phenolic extract to olive paste mostly affected the amounts of dialdehydic form of oleuropein aglycone (O-Agl-dA) and apigenin which increased by 473% and 90% in Buža paste, respectively. Also, in the enriched Buža olive paste (malaxation time 0 min) a statistically significant increase of DMO-Agl-dA, verbascoside, luteolin and luteolin-7-*O*-glucoside was observed. The increase of malaxation time up to 60 min has led to a decrease of free hdroxytirosol, tirosol and verbascoside in enriched olive paste. The mass fraction of flavonoids, such as luteolin-7-*O*-glucoside, luteolin and apigenin was slightly affected by the malaxation time, as also reported Gómez-Rico et al. (2009) for olive paste of cultivar Cornicabra. Also, a slight decrease, but not statistically significant, was observed for the total phenols mass fraction.

Table 1. Mass fraction (mg/kg)[a] of single phenolic compounds in Buža olive pastes enriched with a phenol extract (38%) during different malaxation times.

Phenolic compound		Malaxation time (min)			
(mg/kg)	Control[b]	0	30	45	60
Tyr-OH[c]	839 ± 54 a	816 ± 46 a	712 ± 36 b	658 ± 32 bc	620 ± 42 c
Tyr	214 ± 11 a	217 ± 26 a	202 ± 22 ab	174 ± 11 b	164 ± 18 b
DMO-Agl-dA	1916 ± 107 a	2853 ± 131 b	2897 ± 85 b	2754 ± 53 b	2783 ± 270 b
iso-oleuropein	204 ± 12 a	203 ± 10 a	200 ± 6 a	208 ± 10 a	199 ± 10 a
O-Agl-dA	78 ± 11 a	449 ± 30 b	349 ± 26 cd	425 ± 16 cb	383 ± 32 d
verbascoside	709 ± 61 a	946 ± 69 b	855 ± 26 cd	739 ± 25 ad	802 ± 98 dc
luteolin-7-*O*-glucoside	243 ± 6 a	329 ± 20 b	333 ± 24 b	324 ± 16 b	302 ± 42 b
luteolin	168 ± 7 a	219 ± 9 bc	228 ± 15 b	220 ± 15 bc	206 ± 20 c
apigenin	4 ± 0 a	8 ± 0 bc	8 ± 0 b	8 ± 1 bc	7 ± 1 c
total unidentified[d]	5219 ± 150 a	7157 ± 256 b	7684 ± 547 c	7948 ± 440 c	7567 ± 436 c
total phenols	9595 ± 339 a	13197 ± 458 b	13514 ±673 b	13459 ± 484 b	13034 ± 950 b

[a]Results are means of eight values (two independent repetitions of paste preparation × quadruplicate analyses) ± SD; means within each row marked with different letters are significantly different (Tukey's test, $p<0.05$).
[b]Control sample is Buža olive paste sample without addition of phenols extract, malaxed 0 min.
[c]Full names in Abbreviations.
[d]Sum of the mass fraction of all peaks of unidentified phenolic compounds on chromatogram.

An increase of phenols in Buža olive paste by 38% resulted in a higher increase of the total phenols in corresponding olive oils. A mass fraction of the total phenols in Buža olive oil obtained from enriched paste malaxed for 30 min was 82% higher than the control sample which was obtained without a phenol addition (data not shown). The composition of phenol compounds in enriched Buža olive oils malaxed for 30, 45 and 60 min is shown in Table 2. Oils obtained from the enriched olive paste

malaxed 45 and 60 min had a statistically significant lower mass fraction of total phenols than oils with the malaxation time 30 min. A negative effect of longer malaxation times on olive oil total phenols is also reported by Ranalli et al. (2003) and Stefanoudaki et al. (2011). During malaxation phenolic compounds partition between oil and water depends on the composition and relative amounts of the phases and relative polarities of phenols (Rodis et al., 2002). A longer malaxation time mostly negatively affected a mass fraction of the most identified phenolic compounds in Buža oils. A statistically significant decrease of O-Agl-A, L-Agl-dA and lignans was determinated with prolonged malaxation times. The reduction of the olive oil phenols during malaxation could be due to interactions with polysaccharides present in olive paste (Servili, Montedoro, 2002) and due to oxidation by polyphenol oxidase and peroxidase activity (García-Rodríguez et al., 2011). Unlike the other phenolic compounds, vanillin increased (by 31%) along with the malaxation time.

Table 2. Mass fraction (mg/kg)[a] of single phenolic compounds in Buža VOOs obtained from olive paste enriched with a phenol extract (38%) during different malaxation times.

Phenolic compound	Malaxation time (min)		
(mg/kg)	30	45	60
Tyr-OH[b]	10.7 ± 1.5 a	9.3 ±1.4 ab	9.0 ± 0,7 b
DMO-Agl-dA	182.8 ± 2.5 a	167.2 ± 7.3 b	170.9 ± 11.3 b
O-Agl-dA	24.5 ± 0.5 a	20.5 ± 2.5 b	22.2 ± 2.0 ab
O-Agl-A	62.9 ± 2.7 a	57.8 ± 0.9 b	51.5 ± 2.1 c
Tyr	7.3 ± 0.1 a	7.6 ± 0.1 b	7.3 ± 0.2 a
DML-Agl-dA	57.3 ± 1.2 a	56.2 ± 0.8 ab	54.4 ± 2.2 b
(DML-Agl-dA)ox	63.5 ± 1.3 a	63.1 ± 0.5 a	60.1 ± 2.4 b
L-Agl-dA	26.1 ± 1.1 a	23.5 ±1.2 b	22.0 ± 0.8 c
L-Agl-A	19.1 ± 0.6 a	18.9 ± 0.5 a	17.6 ± 0.8 b
luteolin	8.3 ± 0.2 a	7.4 ± 0.4 b	7.0 ± 0.9 b
methyl-luteolin	0.4 ± 0.0 a	0.4 ± 0.0 a	0.3± 0.0 b
apigenin	1.9 ± 0.1 a	1.7 ± 0.1 b	1.6 ± 0.1 b
vanillin	3.9 ± 0.4 a	5.1 ± 0.4 b	5.1 ± 0.7 b
vanillic+caffeic acid	2.2 ± 0.1 a	2.4 ± 0.2 b	2.3 ± 0.1 ab
p-coumaric acid	5.7 ± 0.2 a	5.2 ± 0.2 b	5.7 ± 0.3 a
lignans	31.8 ± 0.5 a	29.0 ± 1.1 b	27.7 ± 1.0 c
total unidentified[c]	248.3 ± 10.8 a	226.9 ± 13.2 b	219.7 ± 9.6 b
total phenols	756.8 ± 19.4 a	702.3 ± 26.7 b	684.6 ± 29.8 b

[a]Results are means of eight values (two independent repetitions of paste preparation × quadruplicate analyses) ± SD; means within each row marked with different letters are significantly different (Tukey's test, $p<0,05$).
[b]Full names in Abbreviations.
[c]Sum of the mass fraction of all peaks of unidentified phenolic compounds on chromatogram.

An addition of phenolic compounds to olive paste caused a statistically significant decrease of C6 aldehydes hexanal and *Z*-3-hexenal, and C6 alcohol *E*-2-hexen-1-ol (Figure 1). A reduction of these volatile compounds could be a consequence of a reduced activity of LOX, HPL and ADH by phenols added to olive paste (Sánchez-Ortiz et al., 2012a; Sánchez-Ortiz et al., 2012b). A reverse effect was observed on C6 ester *Z*-3-hexenyl acetate and C5 volatile compounds. It seems that AAT, an enzyme responsible for the synthesis of volatile esters, was unaffected by added phenols. An increase of all C5 compounds could be due to the induced homolytic activity of lipoxygenase by accumulation of 13-hydroperoxides in enriched Buža paste. Vancanneyet et al. (2001) suggested LOX involvement in hydroperoxide cleavage based on higher levels of C5 compounds in the leaves of HPL-deficient transgenic potato plants. These results suggest that some enzymes in the LOX pathway might be partially deactivated by added phenols.

A slight decrease of mass fraction of the most volatile compounds can be observed along with the malaxation time (Figure 1). Among these volatile compounds responsible for positive odour notes, the malaxation time had significant negative effect on hexanal, hexan-1-ol and *Z*-3-hexenyl acetate. Mass fractions of these volatiles were reduced by 12, 28 and 38%, respectively, in oil samples malaxed 60 min compared to 30 min.

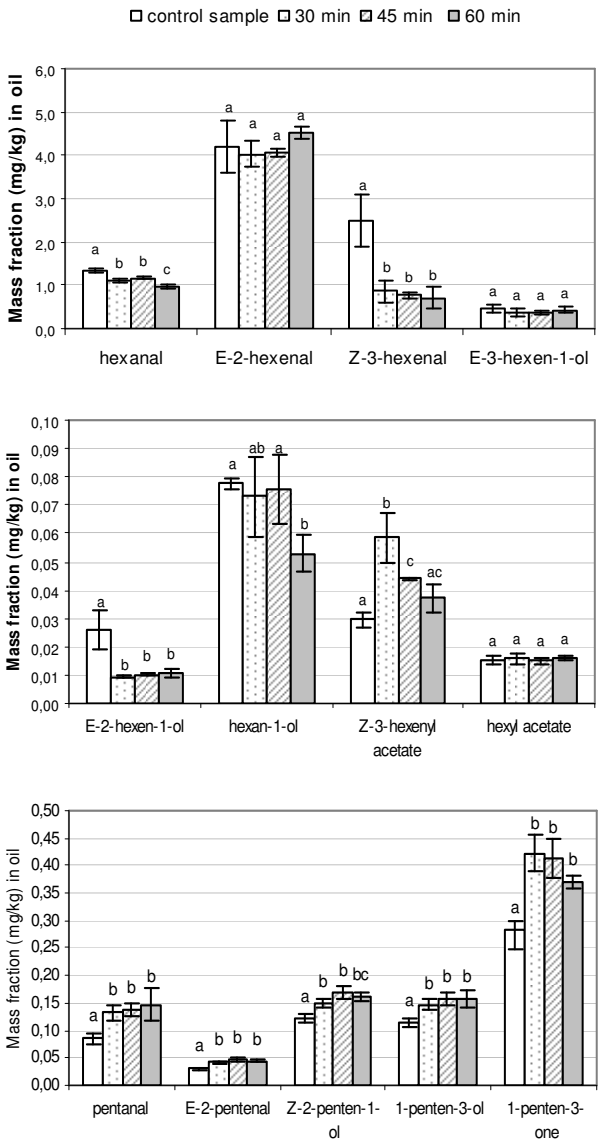

Figure 1. Mass fraction (mg/kg) of volatile compounds in Buža VOO samples obtained from olive pastes enriched with a phenol extract during different malaxation times. Control sample is Buža VOO sample obtained from paste without addition of phenols extract, malaxed 45 min. Results are means of four values (two independent repetitions of oil preparation × duplicate analyses) ± SD. Means within each volatile compound, marked with different letters, are significantly different (Tukey's test, $p < 0,05$).

A decrease of C6 esters by the malaxation time, especially of *Z*-3-hexenyl acetate, was reported by Angerosa et al. (2001) and Ranalli et al. (2003) and could be due to inactivation of AAT during malaxation. A slight positive effect of malaxation time, but not statistically significant, was observed for C5 volatile compounds what is in accordance with results of Angerosa et al., 2001.

Minor changes of total and single volatile compounds indicate that volatile compounds are influenced more by phenol enrichment of olive paste than by the malaxation time.

CONCLUSION

Phenol enrichment of olive paste caused decrease of C6 aldehydes and C6 alcohols in VOO, while C6 esters and C5 compounds increased. Longer malaxation time (60 min) of olive paste showed more pronounced effect on phenols than on volatile compounds.

Abbreviations: Tyr-OH - hidroxytirosol; Tyr - tyrosol; DMO-Agl-dA - dialdehydic form of decarboxymethyloleuropein aglycone; O-Agl-dA = dialdehydic form of oleuropein aglycone; O-Agl-A = aldehydic form of oleuropein aglycone; DML-Agl-dA = dialdehydic form of decarboxymethylligstroside aglycone; (DML-Agl-dA)ox = oxidized form of DML-Agl-dA; L-Agl-dA = dialdehydic form of ligstroside aglycone; L-Agl-A = aldehydic form of ligstroside aglycone.

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