LWT - Food Science and Technology 42 (2009) 50–55

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Bitterness, odor properties and volatile compounds of virgin olive oil with phospholipids addition

O. Koprivnjak [a,*], D. Škevin [b], S. Petričević [c], K. Brkić Bubola [d], Ž. Mokrovčak [b]

[a] Department of Food Technology & Control, School of Medicine, University of Rijeka, Braće Branchetta 20, 51000 Rijeka, Croatia
[b] Department of Food Technological Engineering, Faculty of Food Technology and Biotechnology, Pierottijeva 6, 10000 Zagreb, Croatia
[c] sms Food Development Centre, Kurtovići bb, 23231 Klis, Croatia
[d] Institute of Agriculture and Tourism, K. Huguesa 8, 52440 Poreč, Croatia

ARTICLE INFO

Article history:
Received 25 January 2008
Received in revised form 31 March 2008
Accepted 8 May 2008

Keywords:
Olive oil
Phospholipids
Bitterness
Volatiles
Odor properties

ABSTRACT

Bitter hydrophilic phenolic compounds contained in virgin olive oil (VOO) beneficially affect human health. However, consumers mostly do not tolerate oils with a pronounced bitter taste (bitterness index $K_{225} \geq 0.360$) and this could limit their consumption. The possibility of bitterness attenuation of quite bitter VOO ($K_{225} = 0.501$) by the addition of granular soy lecithin, as a source of phospholipids, up to the levels present in seed oils (2.5–30 g/kg), was evaluated by sensory difference tests. Statistically significant differences were found starting from 5 g/kg of added phospholipids ($p \leq 0.05$). Phospholipids addition caused a significant decrease of the total concentration of 20 volatiles determined by HS-SPME–GC analysis (slope 0.056; $r = -0.9962$), and the most influenced among them was *E*-2-hexenal (slope 0.048; $r = -0.9975$). Results of quantitative descriptive sensory analysis showed that the addition of phospholipids in a range from 5 to 10 g/kg slightly reduced olive fruity and green odor notes, significantly increased sweetness and decreased bitterness. The significant changes of overall sensory quality grading were not found between pure VOO and samples enriched with phospholipids. Thus, in the context of functional food formulation, addition of phospholipids could be proposed as a procedure useful for bitterness attenuation of VOO.

1. Introduction

Chemopreventive phytonutrients are naturally occurring ingredients of plant foods that beneficially affect human health. Many efforts in plant production and food processing are directed to enhance or preserve existing phytonutrients, as well as to create functional foods by their addition to the significant concentration levels. However, a lot of known phytonutrients, such as phenolic compounds, flavonoids, terpenes and glucosinolates, are bitter or astringent (Drewnowski & Gomez-Carneros, 2000). This imposes limitations in enrichment possibilities, because the consumers mostly do not tolerate pronounced bitter taste of food.

The main phenolic compounds responsible for the bitter taste of virgin olive oils are secoiridoids, hydrophilic aglycone derivatives of oleuropein and ligstroside. Among them, the major contribution has aldehydic form of oleuropein aglycone (Mateos, Cert, Perez-Camino, & Garcia, 2004), although high correlation coefficients between oil bitterness and the concentration of total phenols have also been reported (Morales & Tsimidou, 2000). Their health beneficial effects are related to the prevention of cardiovascular diseases, cancer and neuro-degenerative diseases (Servili et al., 2004; Visioli, Galli, Galli, & Caruso, 2002). These impacts are primarily attributed to their antioxidant activity *in vivo*, but some other pharmacological effects, such as inhibition of cyclo-oxygenases, have also been reported (Yang, Kong, & Zhang, 2007). Despite these positive aspects, consumers prefer virgin olive oils with low or moderate levels of bitterness, rejecting very bitter ones (Mateos et al., 2004).

In order to improve the acceptance of bitter food or drugs, different bitter-taste masking substances have been studied, e.g. sugars (Kreutzmann, Christensen, & Edelenbos, 2008), sweeteners, sodium ions, adenosine monophosphate, cyclodextrins (Binello, Cravotto, Nano, & Spagliardi, 2004), flavanones, hydroxybenzoic acid vanillylamides (Ley, Blings, Paetz, Krammer, & Bertram, 2006), lipoproteins and phosphatidic acid (Katsuragi et al., 1997). In most cases, bitterness-masking potential has been evaluated in aqueous solutions of different standard bitter substances, such as caffeine, quinine, propylthiouracil, and bitter peptides. Little information exists about bitter-taste masking substances applied to model systems or real food matrixes. As regards olive oil phenols, Pripp, Busch, and Vreeker (2004) studied the effect of sodium caseinate on bitterness of 65% oil in water emulsion. They have found that the

* Corresponding author. Fax: +385 51 212865.
E-mail address: kolivera@medri.hr (O. Koprivnjak).

0023-6438/$34.00
doi:10.1016/j.lwt.2008.05.002

addition of 1% of sodium caseinate significantly reduce the bitterness of emulsion containing phenols extracted from virgin olive oil, despite relatively weak binding of these phenols to proteins. According to our knowledge, the addition of bitterness-masking substances directly to virgin olive oils has not been reported yet. It must be noticed that no additives are permitted in virgin olive oils (Codex Alimentarius, 1981), so in case a bitterness-masking substance is used, the resulting olive oil is not be considered virgin, but it could be interesting as a particular functional food formula.

Katsuragi et al. (1997) have proposed phospholipids (PL), i.e. lecithin fractions with a high content of the phosphatidylinositol and phosphatidic acid, as a bitterness inhibitor for practical applications. They have demonstrated that these fractions sufficiently inhibit the bitterness of many bitter substances, when they are dissolved together in water solutions, incorporated to the granules or used as coating of the granules containing bitter substances. Phospholipids are one of the minor constituents of seed oils, in which they are usually present in concentration range from 10 to 20 g/kg (Bernardini, 1983). Virgin olive oils contain amounts of phospholipids that are 300–400 times lower than in seed oils (Koidis & Boskou, 2006). Thus, VOO could be a suitable matrix for addition of those possible bitterness inhibitors.

In this work we investigated the possibility of bitterness attenuation of extra virgin olive oil with pronounced bitter taste, by addition of granular soy lecithin as the source of PL. To select the appropriate concentration range of PL and to verify its influence on the perception of bitter taste, sensory difference tests were applied. Besides, the effect of the increased PL concentration on odor properties was evaluated by quantitative descriptive analysis and correlated to the concentration changes of volatile substances, determined by GC analysis of oil headspace volatile composition after solid-phase microextraction (SPME).

2. Materials and methods

2.1. Materials

A filtered extra VOO was purchased from a local Croatian olive oil producer. De-oiled soy lecithin in the form of granules around 2 mm in diameter, characterized by neutral taste and moderately expressed odor reminding dry soybeans, was supplied from Life Time Nutritional Specialities Inc. (Anaheim, USA). It contained 30 g/kg of phosphorus, 37 g/kg of choline, 22 g/kg of inositol, <1% of water and 97% of phospholipids. Methanol p.a. and *n*-hexane p.a. were purchased from Panreac (Barcelona, Spain). Superclean TM LC-18 SPE 1 mL tubes were supplied from Supelco Inc. (Bellefonte, USA). Twenty standards of aroma compounds had a GC purity $\geq$ 90%. Butyl acetate, *E*-2-hexenal, *E*-2-octenal, *E*-2-penten-1-ol, *E*-2-pentenal, ethyl-2-methylbutyrate, octanal, pentan-3-on, *Z*-2-penten-1-ol and *Z*-3-hexenyl acetate were purchased from Aldrich (Steinheim, Germany). 1-Hexanol, 1-penten-3-on, 3-methylbutan-1-al, *E*-2-hexen-1-ol, *E*-3-hexen-1-ol, hexanal, hexyl acetate, *Z*-2-hexen-1-ol and *Z*-3-hexen-1-ol were supplied from Fluka (Buchs, Germany), while 3-methylbutyl acetate from Merck (Darmstadt, Germany).

2.2. Sample preparation

The granular lecithin in amount of 50 g was added to 500 mL of VOO, previously warmed up to 40 °C. The mixture was stirred for 30 min with propeller blade laboratory mixer at 500 rpm. The oil containing dissolved and dispersed PL was separated from sediment and filtered over quantitative filter paper with medium wide pores, obtaining the primary solution of PL. The same treatment was applied to pure extra VOO (without PL) in order to obtain fully comparable samples. The oil samples with various concentration of PL were prepared by diluting the primary solution with proper portions of pure extra VOO.

2.3. Phospholipids quantification

The concentration of total PL in pure extra VOO and in the primary solution was determined as acetone insoluble matters at 0 °C by method of Lüde, described by Pardun (1964) and lately modified by List, Heakin, Evans, Black, and Mounts (1978). Oil sample (5 g) was mixed with 20 mL of acetone (in the water–ice bath at 0 °C) and filtered over a chilled quantitative filter paper with medium wide pores. The treatment was repeated four times and the filter paper with the sediment (containing acetone insoluble matters) was dried at 70 °C and weighed. Results are expressed as PL in grams per kg of the oil. The exact concentration of PL dissolved in the primary solution was calculated as a difference between the PL concentration in the primary solution and pure extra VOO.

2.4. Sensory analysis

Two panels, each composed of eight assessors trained to VOO sensory analysis, participated in three sensory test sessions, carrying out one session per day. Approximate minimal PL concentration (AMC) effective in VOO bitterness attenuation was determined applying simple ranking test on a set of four samples containing 0, 10, 20 and 30 g/kg of PL. Each assessor performed one analysis and, in total, 16 responses were obtained. AMC was defined as the lowest PL concentration at which significant difference of bitterness ($p \leq 0.05$) in relation to pure VOO was found.

Afterwards, efficiency of PL concentration lower than AMC value (i.e. in the middle of interval between AMC and the closest lower concentration from the ranking set) was verified using the triangle difference test, comparing this concentration with pure VOO. Sixteen assessors analyzed one or two series of samples, in order to obtain 24 responses. Assessors who analyzed two series had a 30-min break between series. The assessors were asked to identify the different sample and to specify if it was more or less bitter than remaining two. Responses were considered correct when both answers were exact. The same procedure was used to check the difference in bitterness between AMC and the above-described PL concentration lower than AMC. In both ranking and triangle test, oil samples (15 g) were presented to assessors in blue-colored glasses, coded with three-digit numbers, at 29 ± 1 °C, in random order. Apple, crisp bread and water were provided to rinse the mouth between tasting samples.

One panel of eight trained assessors performed the quantitative descriptive analysis and quality grading of five samples in one session (single determination), according to the method described in European Communities Regulation (1991). Different odor (olive fruity, ripe fruits, green grass or leaves) and taste attributes (bitter, pungent and sweet) were quantified using a six-point intensity ordinal rating scale from 0 (no perception), 1 (scarce), 2 (light), 3 (middle), 4 (strong) to 5 (extreme). For quality grading, a nine-point overall grading scale from 1 (lowest quality) to 9 (best quality) was applied. VOO with score equal to or higher than 6.5 is considered of extra quality. Samples were presented and coded as described for sensory tests.

2.5. Bitterness index determination

The bitterness index (K_{225}) was determined applying the method described by Gutiérrez Rosales, Perdiquero, Gutiérrez, and Olias (1992). The analyses were run in triplicate.

2.6. Volatile compounds analyses

Fused-silica fiber coated with highly crosslinked divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS), 1 cm length, 50/30 μm film thickness (Supelco, Bellefonte, USA) was used for the SPME and concentration of volatiles. The sample (3.5 g) was placed in a 10-ml vial containing a microstirring bar and sealed with 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 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 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. No carry-over effect was detected after that time.

GC analyses were performed using a Varian 3350 gas chromatograph equipped with a flame ionization detector operated at 248 °C and a 30 m × 0.25 mm i.d. × 0.25 μm film thickness capillary column Rtx-WAX (Restek, Bellefonte, USA). Initial oven temperature was kept 8 min at 40 °C, than raised to 85 °C at 2.5 °C/min and finally increased to 245 °C at 10 °C/min and kept 20 min. The carrier gas was helium at pressure of 15 psi at the column head. Volatiles were identified by comparing their retention times to those of the pure standards. Calibration graphs used for the quantifications were prepared by GC analysis of freshly refined sunflower oil solutions containing known amounts of the standards. Calibration curves (relative peak area vs. concentration of compound) and all quantifications were performed by the external standard method using Varian Star 4.51 software. All the analyses were run in triplicate.

2.7. Statistical analysis

The Friedman-type statistic for rank data and nonparametric analog to Fisher's LSD for rank sums were calculated and multiple comparison procedure was performed to determine the significance of differences between bitterness of samples evaluated by ranking test. The significance of differences in the case of the triangle test was determined by the comparison of results with critical number of correct responses (Meilgaard, Civille, & Carr, 1999). Differences among results obtained by quantitative descriptive analysis, as well as those related to concentrations of volatile compounds, were tested by one-way analysis of variance at 5% significance level. The homogeneity of variance was tested by Levene's test and the mean values were compared by the Tukey's honest significant difference test. Pearson linear correlation was applied to relate the concentration of volatile compounds to the concentration of added PL. All statistical analyses were performed using the software package Statistica 7.1 (StatSoft Inc., Tulsa, USA).

3. Results and discussion

3.1. Efficiency of PL in bitterness attenuation of VOO

Extra VOO used in the experiment was chosen on the basis of its strong bitter taste, evaluated by quantitative descriptive sensory analysis. This property was additionally checked by bitterness index determined at 225 nm that was 0.501 (Table 1). Since Gutiérrez Rosales et al. (1992) have reported that K_{225} value of the order $\geq$0.360 corresponds to quite bitter oils, which are rejected by many consumers, this result confirmed the suitability of the sample for the purpose of the study. For preliminary estimation of the possibility of bitterness attenuation, a pure VOO sample (0 g/kg of added PL) and samples with PL concentrations similar to those present in seed oils (10, 20 and 30 g/kg) were analyzed by simple ranking test. The results in Fig. 1 show that there were statistically significant differences between pure VOO and all samples enriched with PL, as well as between samples with 10 and 30 g/kg of PL. This confirms the effectiveness of PL dissolved in oil matrix as a possible bitter-masking substance. However, differences between 10 and 20 g/kg, as well as between 20 and 30 g/kg, were not statistically significant at the level of 95%, indicating that in this range the relation between two parameters (oil bitterness and PL concentration) was not linear for the chosen VOO. Nevertheless, the results of ranking test showed that the minimum PL concentration effective in bitterness attenuation of chosen VOO was lower than or equal to 10 g/kg. In order to select an appropriate concentration scale to study the influence of PL addition on the taste, odor and volatiles composition of VOO, the efficiency of concentration positioned in the middle of interval between 0 and 10 g/kg was verified by the triangle difference test. Two pairs of concentrations (0 vs. 5 g/kg and 5 vs. 10 g/kg) were tested and 24 responses were obtained for each pair. No statistically significant difference was found between 5 and 10 g/kg (assessors were correct at 10 out of 24 trials, $p \leq 0.40$), but it was between 0 and 5 g/kg of PL (21 correct answers out of 24 trials, $p \leq 0.001$). Assuming that values lower than 5 g/kg of PL could have impact not only to bitterness but as well to the other sensory characteristics of oil, a five-point concentration scale with 2.5 g/kg as the least distance in the range from 0 to 10 g/kg was chosen.

Fig. 2 shows sensory profiles of oil samples, obtained by quantitative descriptive analysis. Beside a strong bitter taste, pure VOO sample was characterized by strong pungency and scarce to light sweetness. It is evident that pungency was the taste property least influenced by PL addition, with no statistically significant differences of intensity between the samples. A significant decrease of

Table 1
Bitterness index (K_{225}) and overall sensory grading of VOO with phospholipids (PL) addition in range from 0.0 to 10.0 g/kg

PL concentration (g/kg)	K_{225}[a]	Overall sensory grading[b]
0.0	0.501 ± 0.007a	7.9 ± 0.2a
2.5	0.309 ± 0.002b	7.9 ± 0.2a
5.0	0.244 ± 0.002c	7.8 ± 0.2a
7.5	0.182 ± 0.003d	7.6 ± 0.3a
10.0	0.150 ± 0.002e	7.7 ± 0.2a

[a] Results are the means of three replications ± SD; the means within the column labelled by different letters are significantly different (Tukey's test, $p \leq 0.01$).
[b] Scale from 1 (lowest quality) to 9 (best quality), results are the means of values obtained by single determination from eight assessors ± SD; the means within the column labelled by different letters are significantly different (Tukey's test, $p \leq 0.05$).

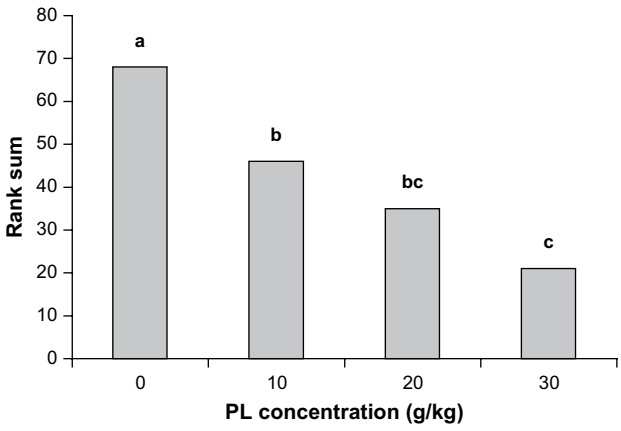

Fig. 1. Results of simple ranking test related to bitterness of oil samples containing 0, 10, 20 and 30 g/kg of added phospholipids (PL); data are rank sums of 16 responses; columns labelled by different letters are significantly different ($p \leq 0.05$).

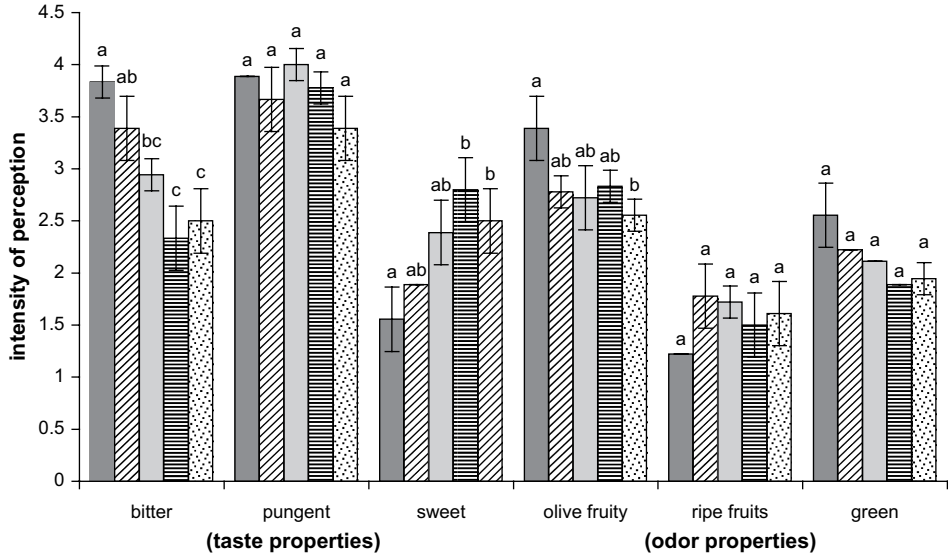

Fig. 2. Sensory profiles of oil samples enriched with 0.0 (■), 2.5 (▨), 5.0 (□), 7.5 (▤) and 10.0 (▩) g/kg of phospholipids. Intensity of perception was quantified using a six-point ordinal rating scale from 0 (no perception) to 5 (extreme); results are the means of values obtained by single determination from eight assessors ± SD; the means within each sensory attribute labelled by different letters are significantly different (Tukey's test, $p \leq 0.05$).

bitterness with respect to pure VOO was obtained starting from 5 g/kg of added PL. However, no significant differences were found among samples in the range from 5 to 10 g/kg, which is in accordance with the results of triangle tests. The opposite trend is evident for sweetness of oil. Despite neutral taste of used granular lecithin, addition of PL at levels of 7.5 and 10 g/kg caused significant differences with regard to pure VOO.

The K_{225} values determined on a methanol extract of oil by UV-spectroscopy (Table 1) also demonstrate a remarkable bitterness-masking potential of PL dissolved in VOO. This property could be ascribed to the bipolar character of PL, which are known as emulsifiers. Schwarz et al. (2000) have reported that some commercial emulsifiers added to the bulk oil (Tegocare 215 and Tegocare 450) form lamellar structures visible by transelectron microscopy. Polar groups of those molecules could entrap hydrophilic substances within the layers and similar behavior might be expected from PL. Since the main bitter-tasting substances in VOOs are hydrophilic phenols, it is most likely that PL in such a way hinders their contact with bitter-taste receptors located on taste buds.

3.2. Effect of PL on odor properties and volatile substances

As regards the odor properties, pure VOO was characterized by middle to strong "olive fruity", light to middle "green grass or leafs" and scarce "ripe fruits" odor intensity. In all samples enriched with PL, intensities of "olive fruity" as well as "green grass or leafs" odor notes showed a decreasing trend with respect to pure VOO. On the other hand, those samples had more pronounced "ripe fruits" notes that contribute to the richness of oil aroma. Although these changes were not statistically significant at the level of 95% (except for "olive fruity" in sample with 10 g/kg of PL), their consistency implies that PL could have some impact on the release of volatile compounds from oil. This was checked by the analysis of the oil headspace volatile composition after SPME. Twenty substances with known green, sweet or rancid (fatty, oxidized) odor descriptors (Kalua et al., 2007) were taken into consideration (Table 2). In order to obtain more information about the effect of PL addition, the concentration range of PL was extended up to 20 g/kg. The main compound in the headspace of pure VOO sample was *E*-2-hexenal, followed by 1-hexanol, *E*-2-hexen-1-ol and *Z*-3-hexen-1-ol (data not shown), the compounds that are characteristic for oils obtained from green or partly ripe olives (Kalua et al., 2007). Fig. 3A shows that PL addition caused a significant and almost linear decrease of the total concentration of 20 volatiles taken into consideration (slope 0.056; $r = -0.9962$) (Table 2), suggesting their retention due to interactions between PL and volatile compounds. This is to the highest extent linked to the decrease of the *E*-2-hexenal concentration that was characterized by a similar linear regression slope (0.048) and correlation coefficient ($r = -0.9975$) as of total

Table 2
Odor descriptors and results of linear regression analysis between volatile compound concentration (μg/kg) and phospholipids concentration (g/kg) in VOO samples

Volatile compound	Odor descriptors[a]	Slope	Correlation coefficient *r*
"Green" volatile substances			
1-Hexanol	Green, fruity, tomato	0.002	−0.0968
1-Penten-3-on	Green	0.000	−0.9098
Butyl acetate	Green, fruity	0.000	−0.3318
E-2-hexen-1-ol	Green, grass, leaves	0.002	−0.8249
E-2-hexenal	Green, apple, almond	0.048	−0.9975
E-2-penten-1-ol + *Z*-3-hexenyl acetate[b]	Green	0.001	−0.9298
E-2-pentenal	Green, apple, almond	0.000	−0.8579
E-3-hexen-1-ol	Green	0.000	−0.9116
Hexanal	Green	0.001	0.8036
Hexyl acetate	Green, fruity	0.000	−0.4807
Z-2-penten-1-ol	Green, banana, almond	0.000	−0.7739
Z-3-hexen-1-ol	Green	0.003	−0.9496
Total "other green" substances[c]		0.008	−0.9294
"Sweet-fruity" volatile substances			
3-Methylbutan-1-al	Malty, sweet	0.000	−0.0458
3-Methylbutyl acetate	Sweet, banana	0.000	0.9701
Ethyl-2-methylbutyrate	Fruity	0.000	−0.3633
Pentan-3-on	Sweet	0.000	−0.3755
Total "sweet-fruity" substances		0.000	0.1359
Other volatile substances			
Octanal	Rancid (fatty, oxidized)	0.000	−0.7642
Z-2-hexen-1-ol + *E*-2-octenal[b]		0.000	−0.8578
Total of 20 volatile substances		0.056	−0.9962

[a] Kalua et al. (2007).
[b] Substances that could not have been separated by GC analysis.
[c] Sum of "green" volatile substances excluding *E*-2-hexenal.

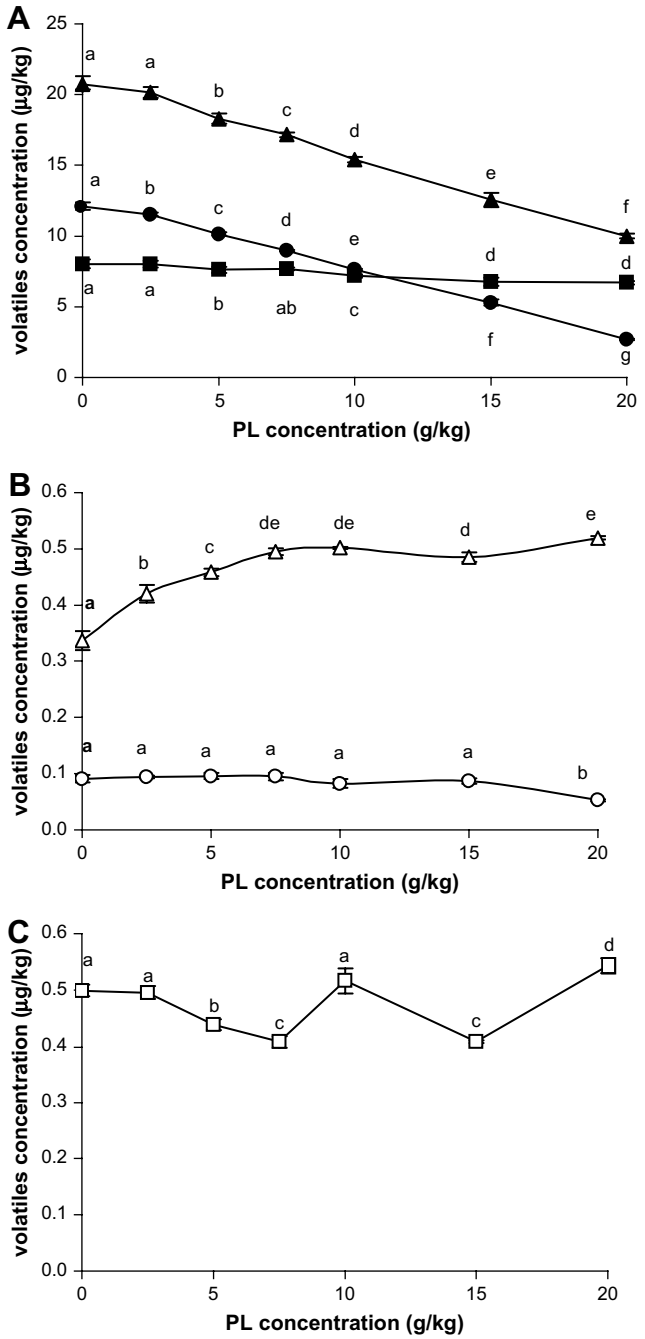

Fig. 3. Effect of phospholipids on concentration of total volatiles (▲), *E*-2-hexenal (●), other "green" compounds (■), hexanal (△), octanal (○) and "sweet-fruity" compounds (□) in oil headspace, determined by SPME–GC analysis. Results are the means of three replications ± SD; the means (marks) of the same curve labelled by different letters are significantly different (Tukey's test, $p \leq 0.05$).

volatiles. Total "other green" substances concentration, i.e. the sum of green substances excluding *E*-2-hexenal, decreased as well ($r = -0.9294$), although to a much less extent than *E*-2-hexenal (linear regression slope 0.008). Inside this group of compounds, only the concentration of hexanal, up to 7.5 g/kg of PL, showed a positive trend (Fig. 3B). Hexanal is known as oil oxidation marker that is produced by breakdown of linoleate 13-hydroperoxide (Morales, Rios, & Aparicio, 1997; Vichi, Pizzale, Conte, Buxaderas, & Lopez-Tamames, 2003). Since soybean lecithin naturally contains considerable amounts of linoleic acid, it could be a direct source of hexanal in oil samples enriched with PL. On the other hand, the concentration of octanal, the product of oleate 11-hydroperoxide breakdown, did not change significantly upon the PL addition. This suggests that used granular lecithin did not cause any prooxidant activity on oil matrix, composed predominantly of oleic acid. As regards "sweet-fruity" compounds (Fig. 3C), the total concentration of these substances showed a poor linear correlation with added PL ($r = 0.1359$). Among them, only 3-methylbutyl acetate (Table 2) had a positive linear trend ($r = 0.9701$).

As a whole, the results of SPME–GC analysis of the oil headspace volatile composition are in accordance with slightly reduced sensory perception of green odor notes in the samples with added PL. A likely reason for such PL influence could be its bipolar character. The polar moieties of its molecules can make bonds with functional groups of aldehydes, alcohols or ketones dissolved in VOO, reducing their volatility. Some accordance with this hypothesis could be found in the results of Tsoutsi, Konstantinou, Hela, and Albanis (2006). They have reported that increasing concentration of free fatty acids and sterols in olive oil spiked with organophosphorus insecticides reduces the release of those insecticides from oil matrix into the headspace.

Nevertheless, comparing overall sensory quality grading of oil samples (Table 1), that takes into account not only the intensity but also equilibrium of odor and taste characteristics, it is evident that the addition of PL had no undesirable impact. Differences among samples were not statistically significant and all oils had values higher than 6.5 that indicate the absence of any unacceptable sensory characteristics. It seems that a slight reduction of green odor notes was recompensed by higher intensities of ripe fruity notes and, thanks to the lower bitterness and higher sweetness, much agreeable taste of oils enriched with PL.

Despite promising results concerning bitterness attenuation and odor evaluation, it is noteworthy to remind that such oils cannot be labelled and marketed as VOO, but only as a kind of functional food. Besides, PL present in concentrations higher than naturally occurring in VOOs could influence the shelf life performance and this will be the objective of our further research.

Acknowledgement

The presented results are derived from the scientific projects "Bioactive and volatile compounds of virgin olive oil in processing and finishing" (062-0580696-0284) and "Stability of oil and bioactive compounds in oil during processing and storage" (058-0580696-0704) supported by Ministry of Science, Education and Sports of Republic of Croatia. We are grateful to Dubravka Štrucelj for valuable suggestions related to the topic and to the sensory assessors for their careful work during test sessions.

References

Bernardini, E. (1983). *Oilseeds, oils and fats, Vol. I.* Rome: Oil Publishing House. pp. 173–178.

Binello, A., Cravotto, G., Nano, G. M., & Spagliardi, P. (2004). Synthesis of chitosan–cyclodextrin adducts and evaluation of their bitter-masking properties. *Flavour and Fragrance Journal, 19*, 394–400.

Codex Alimentarius. (1981). *Standard for olive oils and olive pomace oils 33–1981 (Rev. 2-2003).* Rome: FAO–WHO.

Drewnowski, A., & Gomez-Carneros, C. (2000). Bitter taste, phytonutrients, and the consumer: a review. *American Journal of Clinical Nutrition, 72*, 1424–1435.

European Communities. (1991). Regulation no. 2568 on the characteristics of olive and olive-residue oil and the relevant methods of analysis. Annex XII: organoleptic assessment of virgin olive oil. *Official Journal of the European Communities, L24*, 49–74.

Gutiérrez Rosales, F., Perdiquero, S., Gutiérrez, R., & Olias, M. J. (1992). Evaluation of the bitter taste in virgin olive oil. *Journal of the American Oil Chemists Society, 69*, 394–395.

Kalua, C. M., Allen, M. S., Bedgood, D. R., Jr., Bishop, A. G., Prenzler, P. D., & Robards, K. (2007). Olive oil volatile compounds, flavour development and quality: a critical review. *Food Chemistry, 100*, 273–286.

Katsuragi, Y., Mitsui, Y., Umeda, T., Otsuji, K., Yamasawa, S., & Kurihara, K. (1997). Basic studies for the practical use of bitterness inhibitors: selective inhibition of bitterness by phospholipids. *Pharmaceutical Research, 14*, 720–724.

Koidis, A., & Boskou, D. (2006). The contents of proteins and phospholipids in cloudy (veiled) virgin olive oils. *European Journal of Lipid Science and Technology, 108*, 323–328.

Kreutzmann, S., Christensen, L. P., & Edelenbos, M. (2008). Investigation of bitterness in carrots (*Daucus carota* L.) based on quantitative chemical and sensory analyses. *LWT – Food Science and Technology, 41*, 193–205.

Ley, J. P., Blings, M., Paetz, S., Krammer, G. E., & Bertram, H.-J. (2006). New bitter-masking compounds: hydroxylated benzoic acid amides of aromatic amines as structural analogues of homoeriodictyol. *Journal of Agricultural and Food Chemistry, 54*, 8574–8579.

List, G. R., Heakin, A. J., Evans, C. D., Black, L. T., & Mounts, T. L. (1978). Factor for converting elemental phosphorous to acetone insolubles in crude soybean oil. *Journal of the American Oil Chemists Society, 55*, 521–522.

Mateos, R., Cert, A., Perez-Camino, M. C., & Garcia, J. M. (2004). Evaluation of virgin olive oil bitterness by quantification of secoiridoid derivatives. *Journal of the American Oil Chemists Society, 81*, 71–75.

Meilgaard, M., Civille, G. V., & Carr, B. T. (1999). *Sensory evaluation techniques* (3rd ed.). Boca Raton, FL: CRC Press. pp. 106–110.

Morales, M. T., Rios, J. J., & Aparicio, R. (1997). Changes in the volatile composition of virgin olive oil during oxidation: flavors and off-flavors. *Journal of Agricultural and Food Chemistry, 45*, 2666–2673.

Morales, M. T., & Tsimidou, M. (2000). The role of volatile compounds and polyphenols in olive oil sensory quality. In J. Harwood, & R. Aparicio (Eds.), *Handbook of olive oil: Analysis and properties* (pp. 393–458). Maryland: Aspen Publishers.

Pardun, H. (1964). Analytische Methoden zur Qualitätsbeurteilung von Soyalecithin. *Fette Seifen Anstrichmitte, 66*, 467–476.

Pripp, A. H., Busch, J., & Vreeker, R. (2004). Effect of viscosity, sodium caseinate and oil on bitterness perception of olive oil phenolics. *Food Quality and Preference, 15*, 375–382.

Schwarz, K., Bertelsen, G., Nissen, L. R., Gardner, P. T., Heinonen, M. I., Hopia, A., et al. (2000). Investigation of plant extracts for the protection of processed foods against lipid oxidation. Comparison of antioxidant assays based on radical scavenging, lipid oxidation and analysis of the principal antioxidant compounds. *European Food Research and Technology, 21*, 319–328.

Servili, M., Selvaggini, R., Esposto, S., Taticchi, A., Montedoro, G. F., & Morozzi, G. (2004). Health and sensory properties of virgin olive oil hydrophilic phenols: agronomic and technological aspects of production that affect their occurrence in the oil. *Journal of Chromatography A, 1054*, 113–127.

Tsoutsi, C., Konstantinou, I., Hela, D., & Albanis, T. (2006). Screening method for organophosphorus insecticides and their metabolites in olive oil samples based on headspace solid-phase microextraction coupled with gas chromatography. *Analytica Chimica Acta, 573–574*, 216–222.

Vichi, S., Pizzale, L., Conte, L. S., Buxaderas, S., & Lopez-Tamames, E. (2003). Solid-phase microextraction in the analysis of virgin olive oil volatile fraction: modifications induced by oxidation and suitable markers of oxidative status. *Journal of Agricultural and Food Chemistry, 51*, 6564–6571.

Visioli, F., Galli, C., Galli, G., & Caruso, D. (2002). Biological activities and metabolic fate of olive oil phenols. *European Journal of Lipid Science and Technology, 104*, 677–684.

Yang, D.-P., Kong, D.-X., & Zhang, H.-Y. (2007). Multiple pharmacological effects of olive oil phenols. *Food Chemistry, 104*, 1269–1271.