UDC 582.931:631.563:665.327.3
ISSN 1330-9862

original scientific paper

(FTB-1112)

Influence of Olive Fruit Storage in Bags on Oil Quality and Composition of Volatile Compounds

Olivera Koprivnjak[1], Lanfranco Conte[2] and Nicola Totis[2]*

[1]Institute for Agriculture and Tourism, C. Hugues 8, 52440 Poreč, Croatia

[2]Department of Food Sciences, University of Udine, via Marangoni 97, I-33100 Udine, Italy

Received: October 30, 2001
Accepted: February 21, 2002

Summary

The composition of olive oil volatile components depends on genetic factors, ripening grade of the fruit, fruit storage and processing conditions. Storage of olives in reticular or plastic bags is still a frequently used practice that has negative effects on oil quality, particularly on sensory characteristics. The changes of volatile compounds during this procedure were determined using headspace solid phase microextraction (HS-SPME). The method was optimised as regards sample conditioning and extraction time, and verified by testing the repeatability and linearity of the response. The main changes during fruit storage in bags are increase of methanol and ethanol concentration and decrease of 1-penten-3-one, *trans*-2-hexenal and *cis*-3-hexenyl acetate concentration. The changes in plastic bags are more evident and significant differences between the two types of storage are established.

Key words: fruit storage, olive oil, volatile components, SPME

Introduction

High-quality olive oil is characterized by a pleasant fruity flavour, moderate bitterness and piquantness that can primarily be attained by appropriate processing of healthy, fresh and optimally ripe fruit. A pleasant fragrance derives from the equilibrium of a number of volatile substances such as hydrocarbons, alcohols, aldehydes, ketones, esters and other compounds *(1)*.

These substances arise mainly in biochemical processes with fruit cell destruction during the processing where the most important phase is olive paste mixing (malaxion). The most studied process is the lipoxygenase pathway in which, starting from linoleic and linolenic acid, aliphatic C6 compounds and corresponding hexyl esters responsible for the »green« olive oil aroma are synthesized *(2,3)*. Since this is an enzymatic process in which hydroperoxide liase, alcohol dehydrogenase and alcohol acetyl transferase also participate, it is possible to influence the amount and composition of volatile compounds and sensory proprieties of the oils by varying and controlling the temperature and duration of this phase *(4–6)*.

More obvious changes of volatile components and sensory proprieties during the olive oil production process are the consequence of lengthy and inappropriate fruit storage and they are generally caused by the activity of microorganisms. There is little information on the substances responsible for unpleasant odours and their formation processes. Angerosa *et al. (7)* studied the appearance of a »fusty« defect and found that 3-methyl-butanol and 2-methyl-propanol increase with the increasing of the intensity of this defect. They assumed that these substances are intermediary products of enzymatic degradation of some amino acids. Morales *et al.*

* Corresponding author; Fax: ++385 (0) 52 431 659; E-mail: olivera@iptpo.hr

(8) studied the winey-vinegary defect in virgin olive oils and found that the substances with a high correlation to this sensory perception are acetic acid and ethyl acetate.

Storage of olives in reticular or plastic bags is common practice during olive fruit processing, but it has negative consequences on the sensory quality of olive oil. In this work, the changes of volatile compounds in olive oils caused by this procedure were studied. For volatile component extraction and concentration, the headspace solid phase microextraction (HS-SPME) technique was used. This technique has only recently been applied in the analysis of vegetable oils *(9,10)* and virgin olive oil *(11)*, although its suitability for flavour analysis had already been confirmed *(12)*.

Material and Methods

Samples

Olive fruits (Leccione cv.) of good quality and at beginning of ripening (maturity index was 1.3) were handpicked in Istria (Croatia) at the end of October. The olives were divided into lots of 12 kg. The reference sample was processed immediately after harvesting, while the other lots were stored in reticular and closed plastic bags for 5, 10 and 15 days at 18 ± 0.5 °C. The fruits were processed by pilot plant equipment composed of a hammer crusher, mixer and press with steel nets, under conditions described in a previous paper *(13)*. Two replicates were prepared of all samples.

Materials

Fused silica fibre coated with highly crosslinked divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS), 2 cm length, 50/30 μm film thickness (Supelco) was used for extraction and concentration of volatile compounds.

For method evaluation and GC-MS identity confirmation, the following compounds were used: methanol, purity >99 % (Riedel de Haën); ethanol, purity >99 % (Carlo Erba); *cis*-2-penten-1-ol, *cis*-3-hexen-1-ol, 2-methyl-1-butanol, 3-methyl-1-butanol, nonanol, pentanal, hexanal, heptanal, nonanal, decanal, *trans*-2-hexenal, 1-penten-3-one, *cis*-3-hexenyl acetate, 3-methyl-butyl acetate, acetic acid, hexanoic acid, heptanoic acid, purity from >95 to >99 % (Sigma-Aldrich-Fluka).

For calibration curves, refined olive oil purchased by Van den Bergh Italy (Inveruno, Milan) was used as oil medium.

Standard quality parameters and sensory analysis

Free acidity, peroxide value and standard absorbance values at 232 and 270 nm were determined according to the European Communities official methods *(14)*. Phenolic substances were extracted by the Montedoro and Cantarelli method *(15)*, while the total polyphenols content was determined according to Gutfinger *(16)*.

A panel of 5 trained assessors evaluated the sensory characteristics using a special profile sheet according to the European Communities official method *(14)*.

HS-SPME, GC and GC-MS analysis

According to the data published by Keszler *et al. (9)* and Jeleń *et al. (10)*, and comparison of two sample conditioning times (10 and 20 min) and three fibre exposure times (10, 20 and 40 min), the following procedure and working conditions for HS-SPME were chosen: olive oil (10 ± 0.001 g) was weighed into a 50 mL vial, then sealed with an aluminium cover and Teflon-lined septum, kept for 1 hour at room temperature and conditioned at 40 °C for 10 min. The SPME fibre was exposed to oil headspace for 20 min at 40 °C and then immediately transferred to the GC injection port at 250 °C for 3 min in splitless mode.

A Carlo Erba HRGC 5300 with FID detector was used for GC analysis. Compounds were separated on an HP-Innowax column (30 m × 0.32 mm, i.d. 0.5 μm; Hewlett Packard). Temperature program was: 4 min at 40 °C, from 40 to 70 °C at a rate of 10 °C/min, from 70 to 150 °C at 5 °C/min, from 150 to 250 °C at 10 °C/min and 10 min at 250 °C. The injector and flame ionisation detector temperatures were kept at 250 °C, while helium flow rate was 1.0 mL/min.

The same conditions were used for GC-MS analysis on a Varian 3400 gas chromatograph coupled to a Varian Saturn ion trap detector. The mass spectrometer scanned from *m/z* 30 to 500 at 1.0 s cycle time, the ion source was set at 170 °C and the spectra were obtained by electron impact (70 eV).

The constituents were identified by comparing retention times and MS spectra of pure standard substances. When standards were not available, tentative identification based on comparison of linear retention indices with data from the literature *(17,18)* and comparison of mass spectra with NIST90 and WILEY5 library spectra was performed.

Statistical analysis

The similarity between samples was tested by cluster analysis using Euclidean distances and Ward's method algorithms. Mathematical procedure was carried out using Statsoft Statistica software package *(19)* on a Pentium computer.

Results and Discussion

Standard physicochemical quality indicators of olive oil samples are shown in Table 1. Acidity, the consequence of hydrolytic enzymes activity in destroyed cells, increased markedly after 10 days in fruits from reticular bags, while in plastic bags this was evident only after 15 days of storage.

Increase in peroxide value and primary and secondary oxidation product indices (K232 and K270) was moderate and these values remained within the limits for *extra* quality.

The loss of polyphenolic substances was rapid in both cases, but more drastic in plastic bags. Sensory evaluation scores decreased below 6.5 points already after 5 days of storage, that is a limit for *extra* quality. This indicates that the changes of volatile components oc-

Table 1. Standard quality parameters, polyphenols and sensory score of olive oils obtained after fruit storage in bags

Oil sample	Type of storage – bags	Time of storage / day	Acidity *w* (oleic a.) /%	Peroxide value γ (O) / (mmol/kg)	K 232	K 270	*w* (polyphenols) /(mg/kg)	Sensory score
R-0	–	0	0.50 ± 0.01	3.86 ± 0.03	1.75 ± 0.02	0.11 ± 0.00	192.6 ± 5.6	7.0 ± 0.3
R-5	Reticular	5	0.83 ± 0.02	4.60 ± 0.02	1.70 ± 0.01	0.11 ± 0.00	104.6 ± 1.6	5.4 ± 0.6
R-10	Reticular	10	1.78 ± 0.04	6.12 ± 0.03	1.70 ± 0.02	0.14 ± 0.00	102.8 ± 1.1	4.6 ± 0.4
R-15	Reticular	15	4.88 ± 0.01	4.47 ± 0.07	1.83 ± 0.01	0.20 ± 0.00	66.0 ± 6.5	3.0 ± 0.3
P-5	Plastic	5	0.48 ± 0.01	4.52 ± 0.04	1.84 ± 0.01	0.12 ± 0.01	90.7 ± 2.4	5.1 ± 0.7
P-10	Plastic	10	0.49 ± 0.01	6.24 ± 0.08	1.82 ± 0.03	0.11 ± 0.00	37.3 ± 0.4	4.0 ± 0.3
P-15	Plastic	15	1.05 ± 0.02	5.63 ± 0.04	1.90 ± 0.01	0.14 ± 0.00	40.1 ± 1.6	3.0 ± 0.5

Table 2. Results of sensory evaluation of olive oils obtained after fruit storage in bags

Oil sample	Pleasant properties				Unpleasant properties				
	Fruity	Apple	Other ripe fruit	Green	Mouldy	Muddy sediment	Fusty	Rancid	Other*
R-0	1	1	–	3	–	–	–	–	–
R-5	1	–	–	1	–	1	2	–	–
R-10	1	–	–	–	2	2	2	–	–
R-15	–	–	1	–	2	1	3	–	–
P-5	–	–	–	–	–	2	2	–	2
P-10	–	–	–	–	–	2	3	1	2
P-15	–	–	–	–	–	2	2	3	–

Intensity of perception: 1 = scarce, 2 = mild, 3 = medium, 4 = strong, 5 = extreme
* fermented over-ripe fruit; fermented putrid fruit

curred more rapidly than the standard physicochemical parameters (Table 2).

To determine these changes, volatile components analysis using HS-SPME was carried out. The method was verified by testing the repeatability and linearity of the response.

In order to verify repeatability, a sample of virgin olive oil was analysed five times and twenty-four chromatographic peaks were taken into consideration. Repeatability was estimated by means of relative standard deviation (RSD). For peaks with an area of over 1 % it ranged from 0.5 to 8.4 % (average 3.3 %), while for those under 1 % from 2.1 to 9.3 % (average 5.4 %). The only peak with RSD over 10 % was peak 25 (acetic acid).

Linearity was checked by means of standard solution of volatile analytes in refined olive oil. The refined olive oil with pure GC headspace profile was used for preparing standard solutions in a concentration range from 0.1 to 1.0 mg/kg. Calibration curves were calculated for seven substances representing the common groups of virgin olive oil volatile compounds – aldehydes (*trans*-2-hexenal, pentanal), ketones (1-penten-3--one), alcohols (ethanol, *cis*-3-hexen-1-ol, 2-methyl-1--butanol) and esters (*cis*-3-hexenyl acetate). Linearity of calibration curves was characterised by r^2 values of 0.990 (ethanol, *cis*-3-hexen-1-ol) to 0.999 (pentanal). Data on the repeatability and linearity of response show that the method can be successfully applied for virgin olive oil analysis.

A typical GC chromatogram of oil from fruits stored in plastic bags is presented in Fig. 1. Details on peak identities are shown in Table 3. To facilitate the calculation, the peak areas were normalised with respect to β-ocimene that presented minimal variations related to duration and method of fruit storage. The values are given in Table 4.

Table 3. Chromatographic peaks selected for volatile pattern characterisation

Peak number	Retention time / min	Linear retention index	Component name
1	2.50	–	n.i.
2	2.80	–	n.i.
3	3.80	–	methanol[a]
4	4.61	–	ethanol[a]
5	5.62	–	n.i.
6	6.62	1041	1-penten-3-one[b]
7	8.14	1093	hexanal[b]
8	8.28	1101	n.i., fr. 41,43,31,57
9	9.17	1132	isoamyl acetate[b]
10	11.37	1217	2-methyl-1-butanol[b]
11	11.85	1231	*trans*-2-hexenal[b]
12	12.54	1260	β-ocimene[c]
13	12.94	1267	styrene[c]
14	13.69	1295	n.i., fr. 45,43,89,105
15	14.21	1317	n.i., fr. 69,41,79,135,150
16	14.53	1331	*cis*-3-hexenyl-acetate[b]
17	15.43	1363	hexanol[b]
18	16.34	1394	*cis*-3-hexen-1-ol[b]
19	16.94	1417	n.i., fr. 83,82,57,39
20	18.21	1461	acetic acid[b]

n.i. = not identified
a = tentatively identified by retention time of standards
b = identity of compounds confirmed by GC-MS analysis of standards
c = tentatively identified by mass spectra

Cluster analysis of these data resulted in the diagram presented in Fig. 2. The reference sample was

Table 4. Normalised peak areas from headspace gas chromatograms in respect to β-ocimene*

Compound	Oil samples						
	R-0	R-5	R-10	R-15	P-5	P-10	P-15
peak 1	0.05 ± 0.01	0.07 ± 0.02	0.33 ± 0.05	1.69 ± 0.51	0.68 ± 0.09	0.65 ± 0.01	0.95 ± 0.11
peak 2	0.15 ± 0.05	0.15 ± 0.04	0.35 ± 0.11	0.72 ± 0.10	0.11 ± 0.02	0.23 ± 0.01	1.68 ± 0.31
methanol	0.22 ± 0.02	0.18 ± 0.01	0.52 ± 0.05	0.86 ± 0.17	0.81 ± 0.06	1.33 ± 0.10	2.86 ± 0.45
ethanol	0.10 ± 0.01	0.23 ± 0.02	0.74 ± 0.04	1.33 ± 0.14	5.90 ± 0.51	6.40 ± 0.27	4.61 ± 0.38
peak 5	0.14 ± 0.03	0.27 ± 0.02	0.52 ± 0.02	1.07 ± 0.08	0.14 ± 0.02	0.12 ± 0.01	0.24 ± 0.03
1-penten-3-one	0.80 ± 0.06	0.53 ± 0.03	0.14 ± 0.00	0.20 ± 0.00	0.06 ± 0.01	0.05 ± 0.02	0.13 ± 0.01
hexanal	0.43 ± 0.03	0.39 ± 0.03	1.08 ± 0.03	1.58 ± 0.05	0.28 ± 0.03	0.14 ± 0.00	0.12 ± 0.02
peak 8	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.11 ± 0.01	0.12 ± 0.01	0.25 ± 0.02
isoamyl acetate	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.09 ± 0.01	0.13 ± 0.01	0.83 ± 0.08
2-methyl-1-butanol	0.31 ± 0.01	0.31 ± 0.02	0.38 ± 0.01	0.47 ± 0.02	0.60 ± 0.05	0.55 ± 0.02	0.79 ± 0.06
trans-2-hexenal	17.93 ± 1.03	14.69 ± 1.07	7.64 ± 0.28	1.90 ± 0.56	0.40 ± 0.05	0.29 ± 0.00	0.16 ± 0.01
β-ocimene	1.00	1.00	1.00	1.00	1.00	1.00	1.00
styrene	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.24 ± 0.02	0.33 ± 0.02	0.48 ± 0.06
peak 14	0.02 ± 0,00	0.08 ± 0.01	0.27 ± 0.01	0.40 ± 0.01	0.15 ± 0.01	0.17 ± 0.01	0.55 ± 0.03
peak 15	0.28 ± 0.02	0.27 ± 0.03	0.24 ± 0.01	0.26 ± 0.00	0.24 ± 0.02	0.24 ± 0.00	0.30 ± 0.01
cis-3-hexenyl-acetate	1.01 ± 0.03	0.64 ± 0.03	0.15 ± 0.01	0.18 ± 0.01	0.11 ± 0.00	0.06 ± 0.00	0.09 ± 0.00
hexanol	0.12 ± 0.01	0.25 ± 0.04	0.68 ± 0.05	1.45 ± 0.02	0.11 ± 0.01	0.11 ± 0.01	0.39 ± 0.02
cis-3-hexen-1-ol	1.19 ± 0.04	1.01 ± 0.03	0.92 ± 0.04	0.96 ± 0.02	0.18 ± 0.02	0.12 ± 0.00	0.15 ± 0.00
peak 19	0.48 ± 0.05	1.37 ± 0.09	2.59 ± 0.14	4.35 ± 0.09	0.12 ± 0.01	0.14 ± 0.00	0.41 ± 0.02
acetic acid	0.36 ± 0.03	0.28 ± 0.03	0.19 ± 0.01	0.08 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00

*mean of two replications

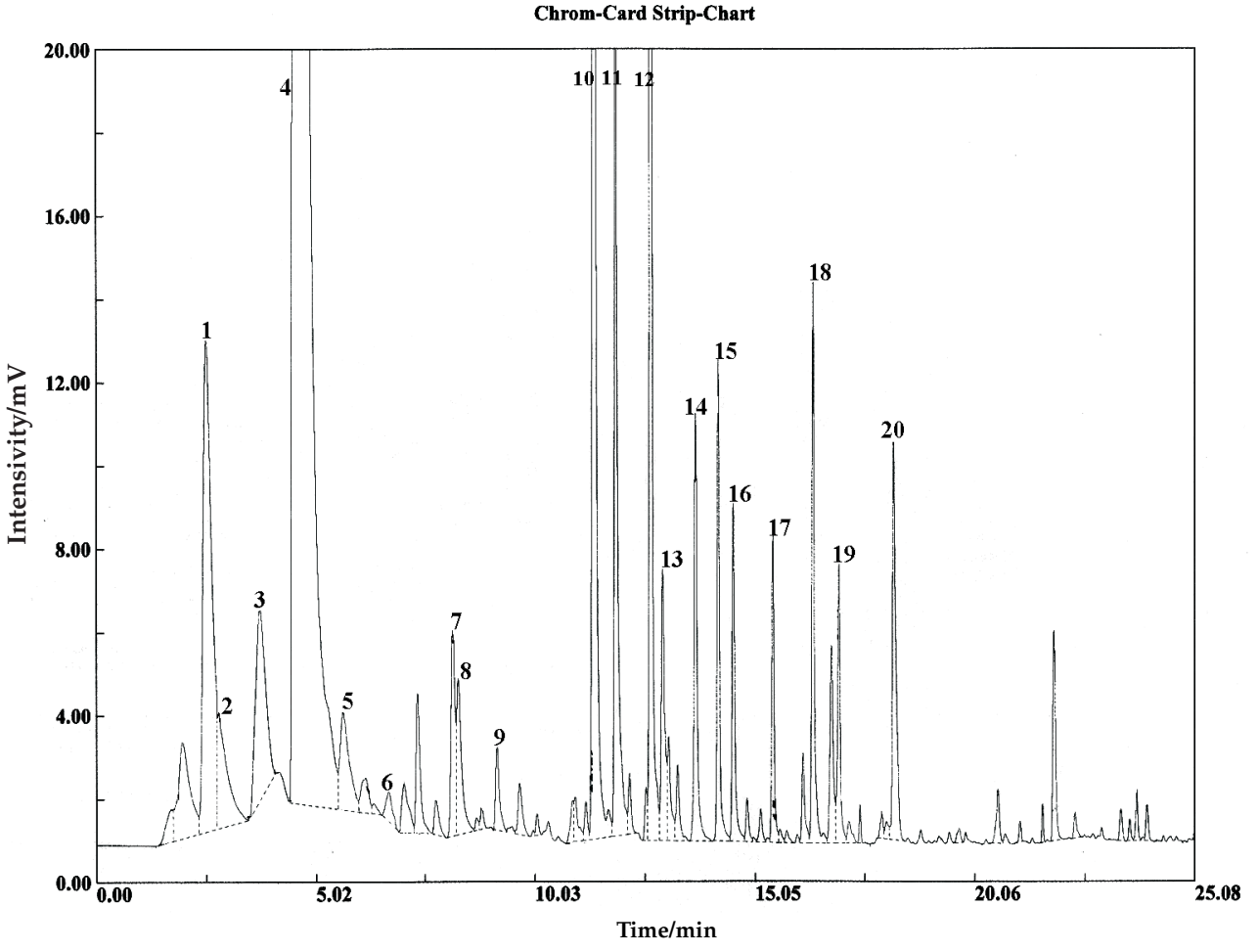

Fig. 1. Headspace gas chromatogram of P-5 oil sample. See Table 3 for each number's description.

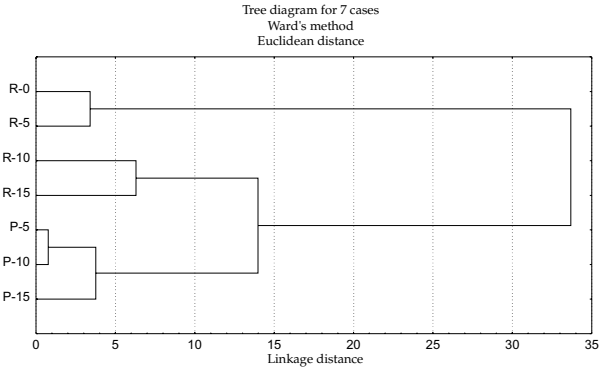

Fig. 2. Cluster analysis

characterized by high values of *trans*-2-hexenal (almond, fruity, green), *cis*-3-hexenyl acetate (banana, leaves), *cis*-3-hexenol (grass, banana), 1-penten-3-one (green, pungent) and hexanal (green apple). No odour defect was observed and general sensory description for this sample was »medium-strong intensity of green leaf-almond husk« (Table 2).

The most similar sample to the reference was that stored for 5 days in reticular bags. Its sensory description was »mild intensity of green odour notes, mild intensity of *fusty* defect and scarce intensity of *muddy sediment* defect«. Main changes of volatile components in this case were a moderate loss of *trans*-2-hexenal and a significant loss of 1-penten-3-one and *cis*-3-hexenyl acetate. At the same time, ethanol, substance 5, hexanal, hexanol, substance 19 and acetic acid increased. Among these substances, hexanal and hexanol have positive sensory impact, so the perceived odour defects could be the consequence of higher values of the rest of the components.

The changes of volatile compound composition that occurred in plastic bags are more emphasised. There are clear differences between oils from reticular and plastic bags (Table 4). These differences are attributable to lower values of hexanal, hexanol, *cis*-3-hexenol and substance 19, and higher values of methanol, ethanol and 2-methyl butanol (fusty) in samples from plastic bags. In addition, substance 8, isoamyl acetate and styrene are compounds present only in samples from plastic bags, whereas styrene probably derives from polymer material. High values of methanol and ethanol in plastic bags are most probably the consequence of fermentation activity and this can be correlated to the undesired »fermented over-ripe fruit« sensory description of these samples.

In the samples from reticular bags, besides mild intensity of *mouldy*, *muddy sediment* and *fusty* defect, a scarce presence of *fruity* and *green* odour notes was observed. That was not the case in samples from plastic bags where only defects were perceived. This is in accordance with a gradual loss of *trans*-2-hexenal in reticular bags and almost total loss in plastic bags.

Conclusions

Data concerning repeatability and linearity of response show that SPME can be successfully applied to virgin olive oil analysis. This method could be useful to distinguish between the oils obtained from fresh fruits and fruits stored in bags. The main changes during fruit storage in both types of bags are the increase of methanol and ethanol, and decrease of 1-penten-3-one, *trans*-2--hexenal and *cis*-3-hexenyl acetate. The changes in plastic bags are more emphasised and significant differences between the two types of storage are established.

Acknowledgement

This paper was done as part of scholarship offered from Italian Ministry of Foreign Affairs for academic year 2000/2001.

References

1. A. K. Kiristakis, *J. Am. Oil Chem. Soc. 75* (1998) 673–681.
2. J. M. Olias, A. G. Perez, J. J. Rios, L. C. Sanz, *J. Agric. Food Chem. 41* (1993) 2368–2373.
3. J. J. Salas, M. Williams, J. L. Harwood, J. Sanchez, *J. Am. Oil Chem. Soc. 76* (1999) 1163–1168.
4. F. Angerosa, R. Mostallino, C. Basti, R. Vito, *Food Chem. 72* (2001) 19–28.
5. J. J. Salas, J. Sanchez, *J. Agric. Food Chem. 47* (1999) 809–812.
6. M. T. Morales, R. Aparicio, *J. Am. Oil Chem. Soc. 76* (1999) 295–300.
7. F. Angerosa, L. Di Giacinto, M. Solinas, *Riv. Merceol. 29* (1990) 275–93.
8. M. T. Morales, G. Luna, R. Aparicio, *Eur. Food Res. Technol. 211* (2000) 222–228.
9. A. Keszler, K. Heberger, M. Gude, *Chromatographia, 48* (1998) 127–132.
10. H. H. Jeleń, M. Obuchowska, R. Zawirska-Wojtasiak, E. Wasowicz, *J. Agric. Food Chem. 48* (2000) 2360–2367.
11. G. Bentivenga, M. D'Auria, E. De Luca, A. De Bona, G. Mauriello, *Riv. Ital. Sostanze Grasse, 78* (2001) 157–162.
12. X. G. Yang, T. Peppard, *J. Agric. Food Chem. 42* (1994) 1925–1930.
13. O. Koprivnjak, G. Procida, Đ. Benčić, T. Zelinotti, *Food Technol. Biotechnol. 37* (1999) 209–214.
14. Commission Regulation (EEC) No. 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis, *Off. J. Eur. Commun. 34* (1991) 6–35.
15. G. Montedoro, C. Cantarelli, *Riv. Ital. Sostanze Grasse, 46* (1969) 115–124.
16. T. Gutfinger, *J. Am. Oil Chem. Soc. 58* (1981) 966–968.
17. H. Acree, H. Arn, http://www.nysaes.cornell.edu/flavornet/index.html., Cornell University (1997).
18. G. Dugo, *Perfumer & Flavorist, 19* (1994) 29–50.
19. Statsoft, Statistica – release 5, Tulsa, OK (1996).

Utjecaj skladištenja plodova masline u vrećama na kakvoću ulja i sastav hlapljivih sastojaka

Sažetak

Sastav hlapljivih sastojaka maslinovog ulja ovisi o genetičkim faktorima, stupnju zrelosti plodova i njihovu skladištenju te uvjetima preradbe. Još uvijek se često primjenjuje skladištenje maslina u mrežastim ili plastičnim vrećama, što negativno utječe na kakvoću ulja, osobito na senzorska svojstva. Promjene hlapljivih sastojaka tijekom ovoga postupka utvrđene su primjenom mikroekstrakcije na čvrstoj fazi u natprostoru (HS-SPME). Metoda je optimirana s obzirom na kondicioniranje uzorka i vrijeme ekstrakcije, te provjerena utvrđivanjem ponovljivosti i linearnosti odgovora. Glavne su promjene tijekom skladištenja plodova u vrećama povećanje koncentracije metanola i etanola, te smanjenje koncentracije 1-penten-3-ona, *trans*-2-heksenala te *cis*-3-heksenil acetata. Promjene u plastičnim vrećama više su izražene, a uočene su i bitne razlike između dvaju načina skladištenja.