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Changes in the volatile components of virgin olive oil during fruit storage in aqueous media

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Abstract

Storage of olives in sea water is a traditional way of fruit storage in olive oil production in Croatia that is still in frequent use. The changes of volatile compounds of olive oil, responsible for positive and negative odor properties that occur during this kind of fruit storage, were investigated. The influence of sea water was compared to brine and drinking water and with storage in the open air. The composition and content of volatile compounds were determined by dynamic headspace analysis with direct connection to gas chromatography and mass spectrometry. No significant differences were found among aqueous media. The olive oils from aqueous media had an essentially different composition and content of positive as well of negative volatile compounds in comparison to the reference sample and the samples stored in the air. The analytical method can give useful information about fruit storage before olive oil extraction.

1. Introduction

The quality of virgin olive oil is strictly correlated with the quality of olive fruits and both can be significantly decreased during lengthy and/or unsuitable fruit storage before an extraction process. A great number of different methods of olive fruit storage confirm the importance of the problem (Castellano, García, Morilla, Perdiguero & Gutierrez, 1993; García, Gutierrez, Castellano, Perdiguero, Morilla & Albi, 1996; Gutierrez, Perdiguero, García & Castellano, 1992; Kiristakis, Nanos, Polymenopoulos, Thomai & Sfakiotalus, 1998; Moussa, Metzidakis, Gerasopoulos & Kiritsakis, 1995).

Despite the advantages of controlled conditions of olive storage in terms of oil quality, traditional methods are still used because of their low costs. Dipping olives in sea water is one such practice often used in Croatia. Changes in basic physicochemical oil quality indicators during this procedure of olive preservation are minimal (Cantarelli, 1965; Koprivnjak, Procida, Benčić & Zelinotti, 1999; Petruccioli, Montedoro & Cantarelli, 1970) but undesired changes of sensory characteristics occur rapidly (Koprivnjak et al., 1999).

More detailed information about olive oil quality can be obtained by determining volatile components. In terms of pleasant organoleptic properties, the most interesting are aliphatic C_6 compounds and the corresponding hexyl esters responsible for the "green" aroma of olive oils (Angerosa, D'Alessandro, Basti & Vito, 1998; Angerosa, Di Giacinto & D'Alessandro, 1997; Guth & Grosch, 1993; Morales, Alonso, Rios & Aparicio, 1995; Morales, Aparicio & Calvente, 1996; Olías, Pérez, Rios & Sanz, 1993).

On the other hand, the appearance of some volatile substances can be related to the unpleasant odors. Angerosa, Di Giacinto and Solinas (1990) studied the changes in composition of volatile compounds which take place during storage of olives in piles or sacks, with the appearance of a "fusty" defect. During the oxidation of olive oils, Morales, Rios and Aparicio (1997) identified about 50 components that are probably responsible for the perception of rancid odor.

The aim of this work is to establish the changes of volatile compounds in olive oils that take place during the storage of olive fruits in sea water, brine and drinking water compared to the changes taking place during storage in wooden boxes at ambient conditions.

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The volatile components of headspace were sampled, applying the purge and trap technique with focusing of volatiles in a cryogenic trap. Since the sampling was performed at room temperature, this technique enables the acquisition of an aroma profile similar to the natural olfactory perception (Barcarolo, Casson & Tutta, 1992; Oguri, Onishi & Hanai, 1991).

2. Materials and methods

2.1. Samples

Olive fruits (*Olea europaea* cv. Bjelica) of good quality, grown in the Pula area (Croatia), were picked in the middle of November. The whole quantity of olives was divided into portions of 3 kg, which underwent methods of storage as shown in Table 1. The fruits were processed by pilot plant equipment, composed of a hammer crusher, a mixer and a press with steel nets. After crushing, the olive paste obtained was malaxed for 25 min at 30°C, than pressed at maximum pressure of 3.04×10^7 Pa. The oil was recovered from the liquid phase by centrifugation at 4000 rpm for 10 min. After filtration, the oil samples were stored in closed dark bottles at 0°C.

2.2. Sensory analysis

A panel of nine trained assessors carried out an evaluation of sensory characteristics according to the official method of the European Communities Commission (1991). The oil samples (15 ml each) were presented in covered blue glasses at 28±2°C. The cover was removed and the sample was smelled and tested by each panelist. The sensory characteristics were evaluated using a special profile sheet.

2.3. Headspace

Olive oil (about 7 g) was exactly weighed into a 10 ml vial and mixed with internal standard (ethyl propionate, 35.6 μg). The vials were sealed with an aluminium rubber septum and conditioned at 70°C for 15 min before the analysis. The stripping into a heated block (70°C) was carried out for 120 s with helium, at a rate of 8 ml/min. Volatile compounds were driven into a capillary tube that was inside a cryogenic trap (liquid nitrogen) maintained at −100°C, and connected in on-column mode to the capillary gas chromatograph. The connection to the analytical column was not direct, as a Y press fit was inserted and connected to a vapor exit valve. During the sampling step, helium was back-flushed through the analytical column with an outlet in the mentioned vapor exit device to avoid any contamination of the column.

2.4. GC–MS analysis

At the end of sampling (purging) time, the trap was heated to 240°C for 5 s and volatile compounds were desorbed and transferred to the analytical column. The electronic eight-port valve switched, so that helium flow came back to the original flow direction. The analytical column used was a capillary fused silica column, 50 m length×0.32 mm i.d., coated with PS 264, 3 μm film thickness. The capillary GC was coupled directly to a MD 800 mass spectrometer. Gas chromatographic conditions were the following: oven initial temperature of 40°C was held for 6 min, then raised by 5°C/min to 180°C and held for 5 min; subsequently the temperature was raised by 7°C/min to 200°C and held for 2 min, and finally raised by 7°C/min to 240°C with 10 min of final isotherm. The transfer line temperature was kept at 250°C. Mass spectrometer was scanning from m/z 29 to 300 at 0.5 s cycle

Table 1
Methods of storing and processing olives

No.	Sample name	Methods of storing and processing
1	Reference	Fruits were processed 24 h after harvesting by a device for processing small samples composed of a hammer crusher, a mixer and a press with steel nets
2	Air-10	Fruits were placed in shallow wooden boxes in a layer of 5 cm and kept in a dry and cool room for 10, 20 and 30 days and daily turned and then processed as described for sample 1
3	Air-20	
4	Air-30	
5	Sea-10	Fruits were put in 5 l jars with a wide neck and then filled up to the top with sea water. The samples were kept in a cool room for 10, 20 and 30 days and then processed as described for sample 1
6	Sea-20	
7	Sea-30	
8	Brine-10	Fruits were put in 5 l jars with a wide neck and then filled up to the top with 4% NaCl solution. The samples were kept in a cool room for 10, 20 and 30 days and then processed as described for sample 1
9	Brine-20	
10	Brine-30	
11	Water-10	Fruits were put in 5 l jars with a wide neck and then filled up to the top with drinking water. The samples were kept in a cool room for 10, 20 and 30 days and then processed as described for sample 1
12	Water-20	
13	Water-30	

time. The ion source was set at 200°C and the spectra were obtained by electron impact (70 eV). The tentative identification of compounds was carried out by a study of the MS spectra and comparison with members of the NBS library.

2.5. Statistical analysis

The differences between the methods of storage, based on volatile substances, were identified by using a cluster analysis (Euclidean distances and Ward's method algorithms) and an analysis of variance. The mathematical procedure was carried out using the Statsoft Statistica software package (Statsoft, 1996) on a Pentium computer.

3. Results and discussion

Our attention in this study was directed to those components that had already been identified as responsible for positive or negative odor properties. The list of these components with their sensory characteristics found in the literature is shown in Table 2.

3.1. Total positive and total negative volatile compounds

Fig. 1 presents the concentration ratio of total positive and total negative volatile compounds. Positive components in the reference sample were markedly prevalent but, during olive storage in the open air, this concentration decreased to 15% of initial value. The content of negative compounds increased twice during the first 20 days of storage, but positive volatile compounds still prevailed in all of these samples (concentration ratio over 3).

The dipping of olives in aqueous media caused a faster and more considerable loss of positive volatile substances in the oil samples. After 10 days, there remained only 10% of the initial concentration. At the same time, the negative components increased about 2.5 times and, consequently, they prevailed over positive volatile components, giving the concentration ratio under 1. Beside these quantitative changes, the composition of positive and negative fractions also varied, depending on the time and type of storage. The concentration changes of individual components are shown in Figs. 2 and 4 and are discussed below.

3.2. Individual positive components

The main components (92%) among positive volatile compounds of the reference sample were hexanal and 2-hexenal in ratio of 2:1. These substances are known as holders of green fruit, apple and almond odor notes that agree with the sensory characteristics of reference sample shown in Table 3.

Table 2
Odor description and retention time of some olive oil volatile compounds

Compound name	Retention time[a] (min)	Odour descriptors[b]
Positive compounds		
Methyl acetate	8.55	Green-nuts[c]
3-Methyl butanal	14.64	Sweet-fruity[c], cheesy-green[d]
2-Pentenal	16.44	Green-apple[c], woody-bitter-oily[d]
2-Pentenol	20.67	Banana[c]
Hexanal	22.11	Green apple[c], green fruit[f], fatty-oily-grassy[e]
2-Hexenal	24.65	Fruity-almonds[c], fragrant-green-leafy[e]
1-Hexanol	25.25	Fruity-aromatic-soft[d], fruity-banana[f], oxidised green bean[e]
Isoamyl acetate	25.47	Banana[c]
Negative compounds		
Ethyl acetate	12.59	Sweet-aromatic[c], vinery[g]
Isobutanol	13.31	Ethyl acetate like-green[c], fusty[h]
1-Penten-3-ol	16.21	Wet earth-vegetable water[c], onion-toasted[d]
Pentanal	16.94	Oxidised-fishy[e]
3-Methyl-1-butanol	18.85	Yeast[c], fusty[h]
2-Methyl-1-butanol	19.05	Fish oil-vegetable water[c]
Other components		
Methanol	4.25	
Ethanol	5.80	
Acetone	6.88	
Isobutanal	9.67	
2,3-Butanedione	11.16	

[a] Retention time of experimental conditions described in this paper.
[b] Properties found in literature as listed below.
[c] Morales et al., 1995.
[d] Flores, Grimm, Toldra and Spanier, 1997.
[e] Kochhar, 1993.
[f] Morales et al., 1996.
[g] Odello, Giomo, Versini and Zironi, 1997.
[h] Angerosa et al., 1990.

The main components of positive substances in the samples obtained from the olives stored in the open air were still hexanal and 2-hexenal. However, after 10 days of this kind of storage almost 90% of hexanal was already lost, while the concentration of 2-hexenal doubled. As a consequence, their ratio declined and ranged from 1:8 to 1:2 during 30 days of storage. Among other positive substances, the increase of 1-hexanol was the most stressed, while 3-methylbutanal, 2-pentenol and 2-pentenal increased only slightly after 10 days of storage. These changes lead to a conclusion that there is an intensified enzymatic activity in olive fruits stored for a short time in the air, probably induced by a gradual disintegration of cell structure due to the water loss through the epidermis. This primarily refers to alcohol dehydrogenase that catalyzes oxidation of hexanal and to 1-hexanol hydroperoxide lyase and *trans*-isomerase that catalyze decomposition of linolenic acid hydroperoxide to 2-hexenal (Olías et al., 1993).

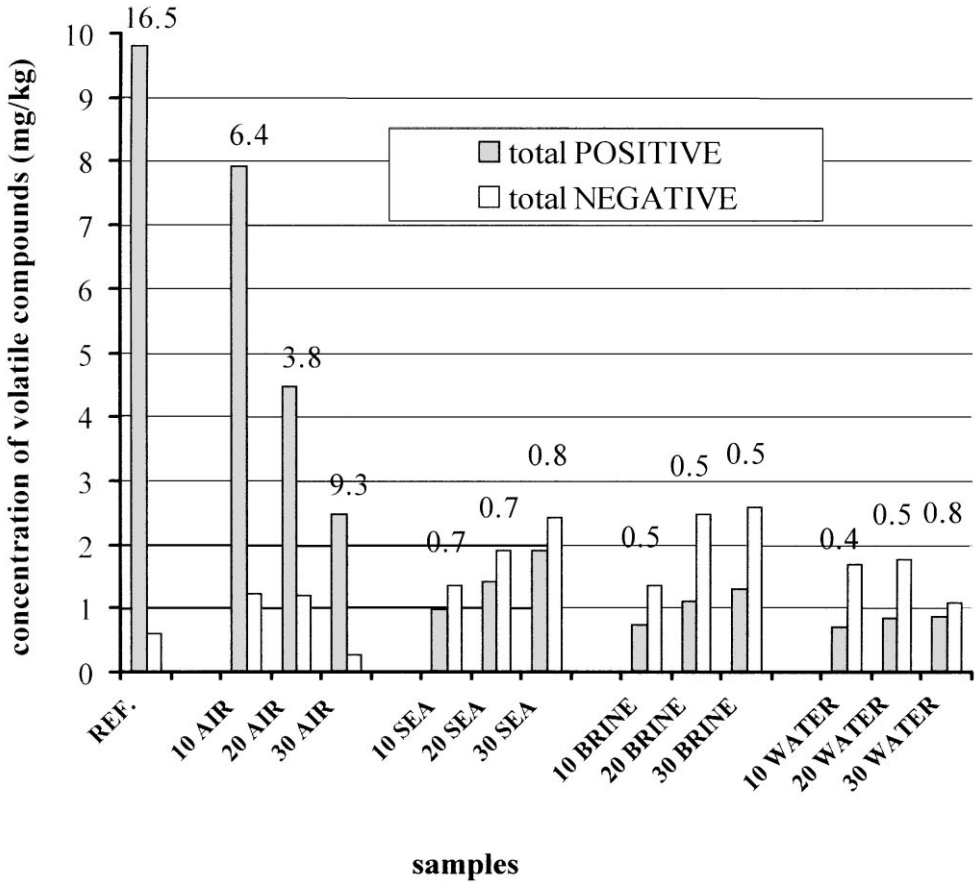

Fig. 1. Concentration ratio of total positive and total negative compounds in olive oils obtained from different methods of fruit storage.

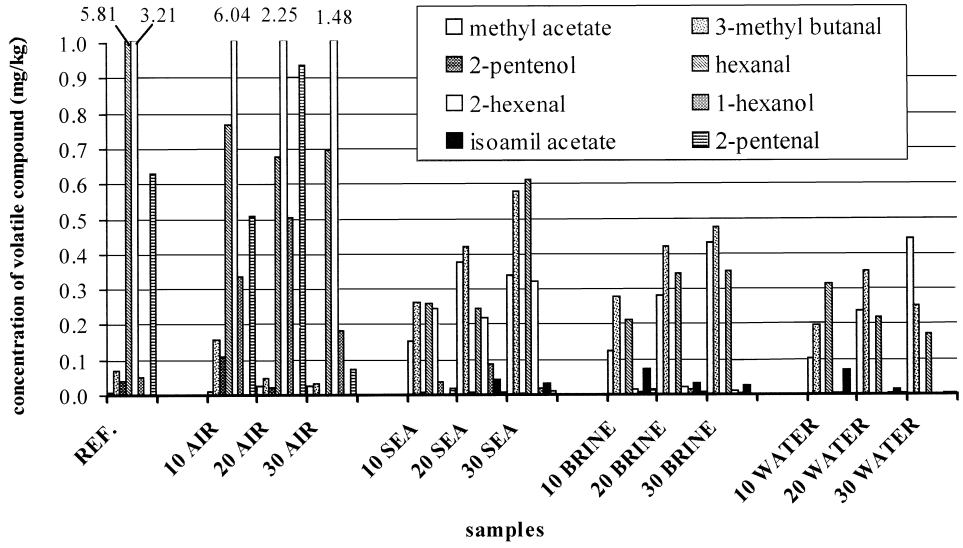

Fig. 2. Concentrations of individual positive compounds in olive oils obtained from different methods of fruit storage.

The sensory analysis of the samples stored in the air did not correspond to such quantitative and qualitative changes of positive volatile compounds. The sample extracted after 30 days of storage was characterized by better odor properties than the samples extracted after 10 and 20 days.

Explicit changes of positive compound composition in regard to the reference sample appeared during the

storage of olives in aqueous media. This refers primarily to the considerable increase of methyl acetate (about 30 times), and 3-methylbutanal (about 5 times). The concentration of hexanal decreased to the level of 5%, while 2-hexenal was retained only in the samples stored in sea water, at the level of about 8% of the initial value. The mentioned changes proceeded at very low concentrations of total positive compounds (range from 0.7 to 1.9 mg/kg) and only a few panelists noticed some scarce fruity odor in the samples stored in aqueous media.

The similarity of samples, regarding the composition of positive compounds, was tested by cluster analysis using Ward's method. Fig. 3 shows the result of this test. All the samples from the aqueous media created a group separated from the reference sample and from the samples stored in air. The sample most similar to the reference one was that obtained from olives stored in the open air for 10 days. Three aqueous media were not significantly different in composition of positive volatile compounds (analysis of variance, $P < 0.05$). The only exception was 2-hexenal whose concentrations were significantly higher in the samples from the sea water (Duncan test, $P < 0.05$).

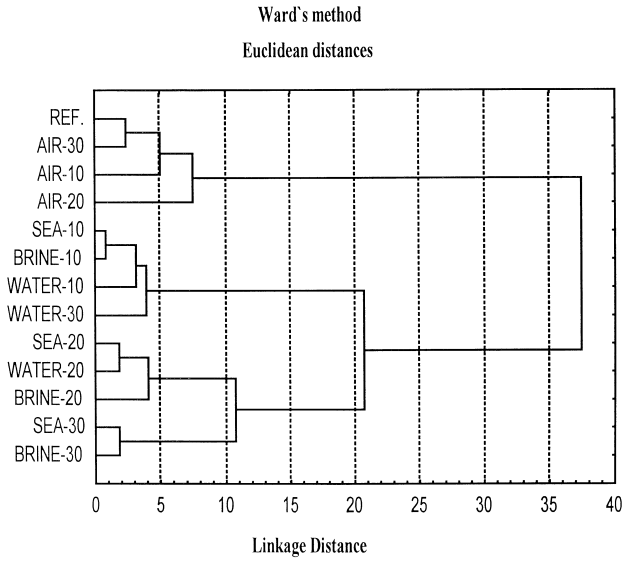

Fig. 3. Clustering of olive oil samples using the positive volatile dataset.

3.3. Individual negative components

The main components in the reference sample were 1-penten-3-ol (wet earth, vegetable water) and pentanal (oxidized, fishy) at concentrations below 0.3 mg/kg. Since no unpleasant odours were detected during the sensory analysis (Table 3), we suppose that these concentrations were under threshold value.

In the samples stored in air, 1-penten-3-ol and pentenal also prevailed among negative components. Their presence can be connected to the defects "mouldy" and "rancid" noted by some of panellists. Morales et al. (1996) consider that 1-penten-3-ol, as well as 2-methyl-1-butanol, are related to the appearance of the defect called "vegetable water". Contrary to expectations, the increasing intensity of this defect in our samples corresponded to the decrease of concentration of the mentioned as well as total negative compounds (sample "air, 30 days").

During the storage of olives in the aqueous media, the changes of individual negative components proceeded in an essentially different way. Ethyl acetate, responsible for the "vinery" defect, increased constantly in the samples from the sea water and brine up to a 35 times higher concentration than in the reference sample. The values of isobutanol ("ethyl acetate-like") increased about 10 times, even after 10 days in each of the three aqueous media. Despite this, the "vinery" defect was detected only in one sample (sea, 10 days) where the concentration sum of ethyl acetate and isobutanol was the smallest.

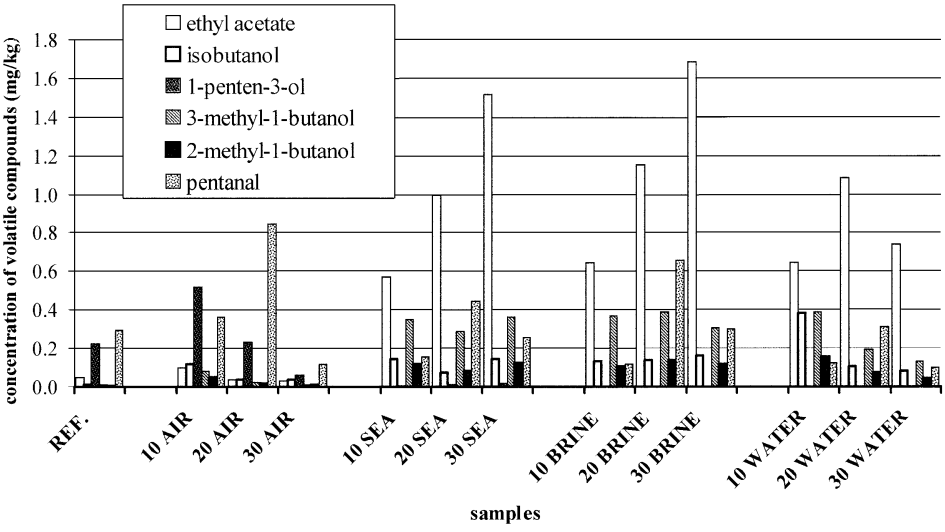

Fig. 4. Concentrations of individual negative compounds in olive oils obtained from different methods of fruit storage.

Table 3
Results of sensory evaluation[a]

Samples	Pleasent properties				Unpleasent properties				
	Fruity	Apple	Other ripe fruit	Green	Vinery	Mouldy	Rancid	Vegetable water	Brine
Reference	3	1	–	2	–	–	–	–	–
Air 10	1	–	1	1	*[b]	*	*	1	–
Air 20	–	–	–	–	–	–	1	2	–
Air30	2	–	–	1	–	*	–	2	–
Sea 10	*	–	–	*	1	*	–	1	1
Sea 20	–	–	–	–	–	1	–	2	2
Sea 30	*	–	–	–	–	–	–	–	2
Brine 10	*	–	–	–	–	–	–	–	2
Brine 20	–	–	–	–	–	–	–	–	2
Brine 30	–	–	–	–	–	–	–	–	2
Water 10	*	–	–	–	–	–	–	1	–
Water 20	–	–	–	–	–	–	–	2	–
Water 30	*	–	–	–	–	–	–	1.5	–

[a] Intensity of perception: 1 = scarce, 2 = mild, 3 = medium, 4 = strong, 5 = extreme.
[b] * = sensory property observed by less than 50% of panelists.

During 30 days of storage, the concentrations of 3-methyl-1-butanol increased in sea water and brine up to 30 times and ranged from 0.29 to 0.39 mg/kg. These concentrations are over the threshold value for 3-methyl-1-butanol, that is 0.2 mg/kg according to Guth and Grosch (1993). Angerosa et al. (1990) link this substance to the appearance of the "fusty" defect, but this unpleasant odor was not identified by the sensory analysis in any of samples.

As indicated, 1-penten-3-ol and 2-methyl-1-butanol may contribute to the appearance of the "vegetable water" defect. While 1-penten-3-ol disappeared in almost all of the samples from the aqueous media, 2-methyl-1-butanol reached concentrations of 0.1 mg/kg, that were 30 times higher than in the reference sample. Despite this, the "vegetable water" defect was not found in all of the samples, but only in those from the sea and drinking water. This could mean that 2-methyl-1-butanol at the stated concentration is not the only substance responsible for the "vegetable water" defect.

Cluster analysis, based on negative compounds (Fig. 5), showed similarity between the reference and the samples stored in the air. The samples from the three aqueous media formed a separate cluster with three groups in which the similarity of samples mainly depends on the time of storage, although there were no significant differences between the aqueous media in the composition of negative volatile compounds (analysis of variance, $P < 0.05$).

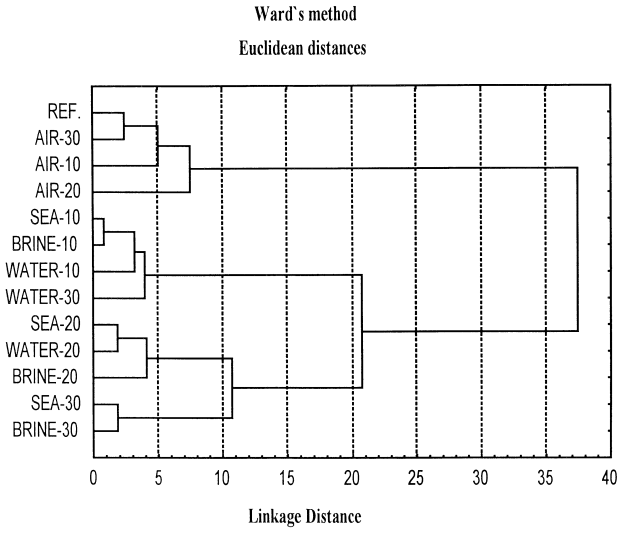

Fig. 5. Clustering of olive oil samples using the negative volatile dataset.

3.4. Other volatile compounds

In addition to the mentioned substances, important changes of concentrations of other volatile compounds were observed (Figs. 6 and 7). The presence of methanol in oil is probably the consequence of pectin degradation in olive fruits induced by activation of pectolytic enzymes. In the samples stored in air, the concentration of methanol increased constantly up to 9.3 mg/kg after 30 days, when it was 7 times higher than in the reference sample. Similar values of methanol were detected in the samples stored in the aqueous media.

In the case of ethanol, there were clear differences between the samples stored in the air and those stored in aqueous media. The concentration of ethanol increased about 2 times in the air while in water solutions these values were from 8 to 15 times higher than in the reference sample. During the storage of olives, the aqueous media became suitable culture media for microorganisms, because of sugar crossing from fruit to water. It is known that, during the first fermentation phase in table olive processing, growth of yeast and Gram-negative bacilli takes place. Their metabolism, in addition to other substances, gives

rise to ethyl alcohol, and this can explain its higher concentration in the oil samples from aqueous media.

The cluster analysis, based on all the considered substances (Fig. 8), showed a clear difference between the samples stored in air and those stored in the aqueous media. The samples from wooden boxes formed a cluster with the reference sample, but as the time of storage increased, the similarity decreased. Analysis of variance ($P < 0.01$) detached 12 of 20 components that had a significant influence on clustering. The most important among them were: ethanol, 3-methyl-1-butanol, 2-hexenal, 2-pentenal and ethyl acetate.

It can be generally concluded that storage of olives in aqueous media gives rise to essentially altered composition and content of volatile substances in olive oils. The concordance between the analytical data and the sensory evaluation was not as expected and this emphasizes the importance of synergism and antagonism processes

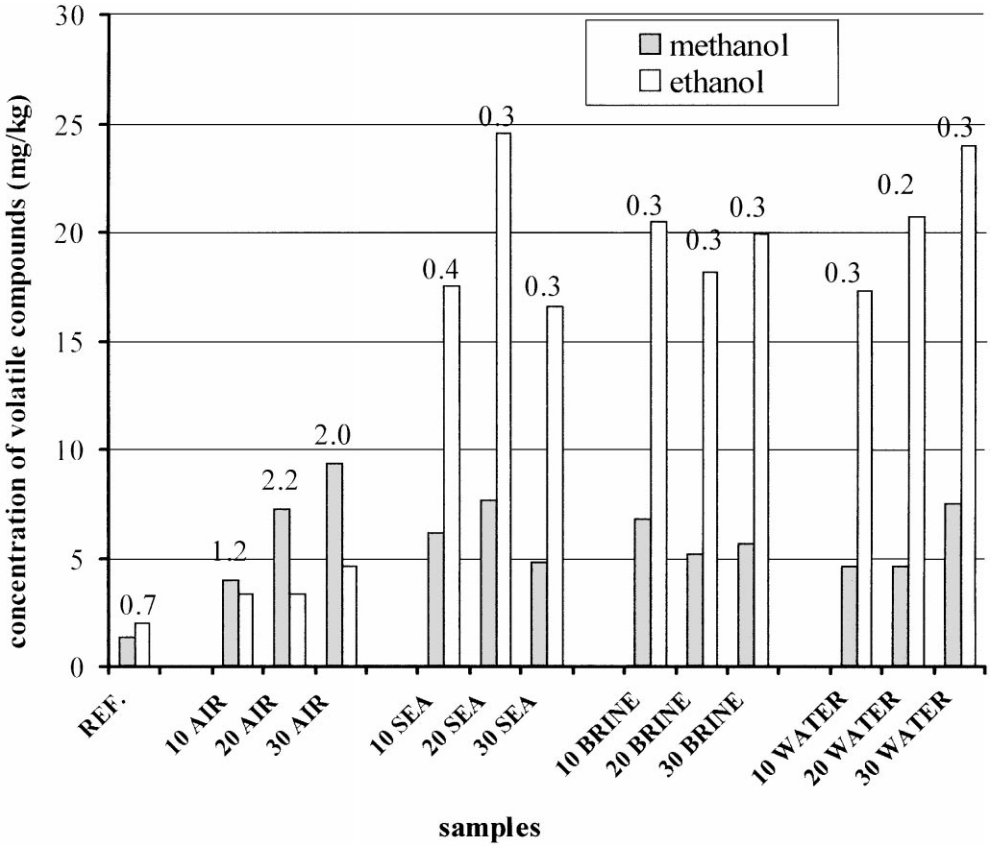

Fig. 6. Concentration ratio of methanol and ethanol in olive oils obtained from different methods of fruit storage.

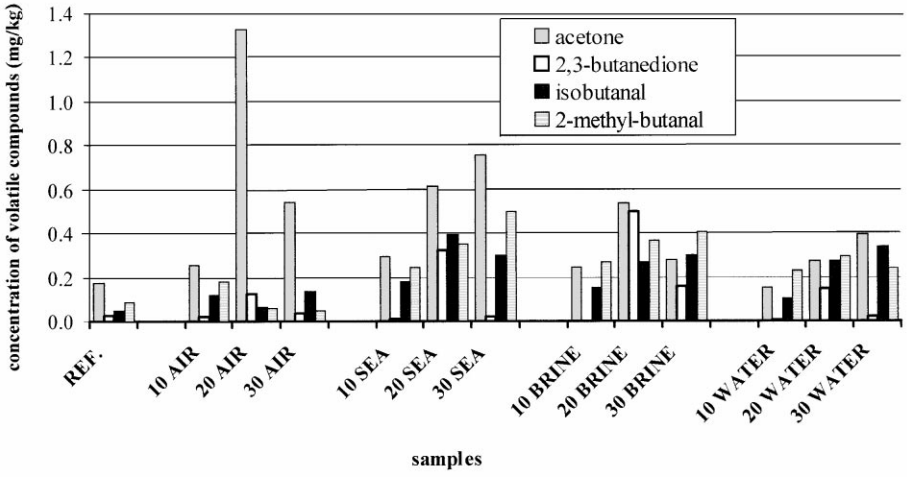

Fig. 7. Concentrations of other volatile compounds in olive oils obtained from different methods of fruit storage.

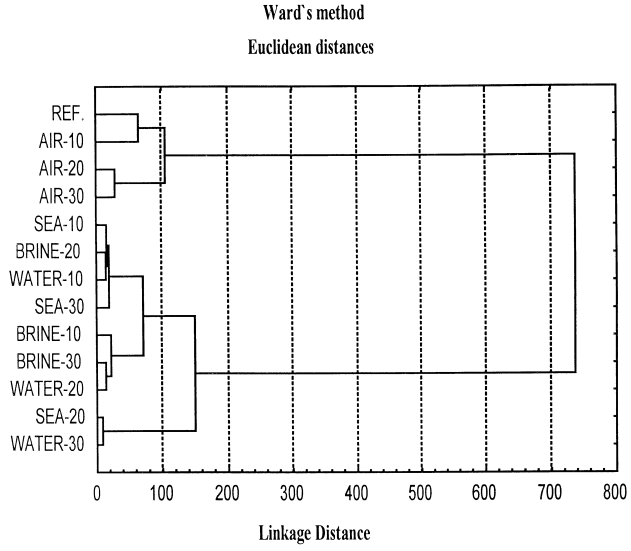

Fig. 8. Clustering of olive oil samples using total volatile dataset.

in the final perception of odor properties of olive oils. Nevertheless, the mentioned analytical method can give useful information that implicates the procedure of fruit storage applied before olive oil extraction.

Acknowledgements

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