Food Chemistry 116 (2009) 651–656

Contents lists available at ScienceDirect

Food Chemistry

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

Volatile phenols in virgin olive oils: Influence of olive variety on their formation during fruits storage

Stefania Vichi [a,*], Agustí Romero [b], Joan Gallardo-Chacón [a], Joan Tous [b], Elvira López-Tamames [a], Susana Buxaderas [a]

[a] Departament de Nutrició i Bromatologia, Xarxa de Referència en Tecnología dels Aliments (XaRTA), Facultat de Farmàcia, Universitat de Barcelona. Avda Joan XXIII, s/n, E-08028 Barcelona, Spain
[b] Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Mas de Bover Crta. De Reus – El Morell 43120 Constantí (Tarragona), Spain

ARTICLE INFO

Article history:
Received 18 November 2008
Received in revised form 25 February 2009
Accepted 26 February 2009

Keywords:
Virgin olive oil
Volatile phenols
Solid phase microextraction
Off-flavor
Storage
Microbiological degradation

ABSTRACT

The potential significance as odorants and markers of olive fruits degradation has been recently pointed out for volatile phenols in virgin olive oil (VOO) and related to the appearance of VOO sensory defects. The few studies carried out in order to elucidate the factors affecting their formation in olive fruits or VOOs, indicated that they could be considered as analytical indices of olive fruits degradation during storage, likely reflecting the microbiological activity. In the present study, the effect of the olive variety ('Arbequina', 'Arbosana' and 'Leccino') on the production of volatile phenols during twelve days of storage in closed plastic bags was evaluated. The different resistance of each variety to the microbiological attach was observed during olive fruit storage, and it was reflected by the evolution of guaiacol, 4-ethylphenol and 4-ethylguaiacol, and related to free acidity values. On the contrary, a scarce dependence on the microbial growth or varietal factors was observed for 4-vinyl derivatives, which appeared more directly related to the time of olives storage. The evolution of volatile phenols found certain correspondence in the sensory characteristics of the resulting VOOs, while the rest of VOO chemical quality indices did not show major variations during fruits storage.

1. Introduction

The presence of some volatile phenol has been reported in the volatile fraction of virgin olive oils (VOOs) with off-flavor (Brenes, Romero, García, Hidalgo, & Ruiz Jiménez, 2004; Jiménez, Aguilera, Beltrán, & Uceda, 2006; Morales, Luna, & Aparicio, 2005; Reiners & Grosch, 1998; Sánchez Saez, Herce Garraleta, & Balea Otero, 1991), but only recently the potential significance as odorants and markers of olive fruits degradation has been pointed out in virgin olive oil (VOO) for volatile phenols comprising methyl, ethyl and vinyl derivatives of phenol and guaiacol. High amounts of these phenols were found in olive oils with strong fusty, musty and muddy defects, and their concentration in VOOs were significantly correlated to the time of olives storage and in accordance with sensory evaluation, indicating that they could be considered as analytical indices of olive fruits degradation during storage, likely reflecting the microbiological activity (Vichi, Romero, Gallardo-Chacón, Tous, López-Tamames, & Buxaderas, 2009; Vichi, Romero, Tous, López-Tamames, & Buxaderas, 2008).

Very few studies have been carried out in order to elucidate the factors affecting the formation of volatile phenols in olive fruits or VOOs. The concentration of 4-ethylphenol was observed to be significantly higher in oils from ground-picked olives than in those from hand-picked fruits (Jiménez et al., 2006), indicating that the microbiological state of olives could influence the production of volatile phenols. Moreover, the degree of limited aerobiosis during olives storage at low temperature demonstrated a higher influence on the increase of some volatile phenols, such as guaiacol, *m*-cresol, and 4-ethyl derivatives, which were more abundant in olives stored in plastic bags than in olives stored in open boxes, while no major variations were observed in the evolutions of *o*-, *p*-cresol and 4-vinyl derivatives according to the type of storage (Vichi et al., 2009). However, the concentrations of volatile phenols in oils obtained after storing healthy olives at low temperature were much lower than those of strongly defective olive oils (Vichi et al., 2009), suggesting that in addition to aerobiosis conditions and time of storage, there are many other possible factors influencing the production of volatile phenols.

* Corresponding author. Tel.: +34 93 4024508; fax: +34 93 4035931.
E-mail address: stefaniavichi@ub.edu (S. Vichi).

0308-8146/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2009.02.086

In the present study, the influence of the olive variety on the production of volatile phenols during olive fruits storage was evaluated together with the microbiological profile of olive surface, VOOs sensory analysis and quality indices.

2. Materials and methods

2.1. Reagents and materials

The SPME fiber used as divinylbenzene/carboxen/polydimethylsiloxane 50/30 μm, 2 cm long (DVB/CAR/PDMS) from Supelco (Bellefonte, PA, USA). *o*-, *p*-cresol, 4-ethylphenol, 2,3-dimethylphenol, 4-vinylphenol (solution 10% w/w in propylene glycol), guaiacol, 4-ethylguaiacol and vinylguaiacol came from Sigma–Aldrich (St. Louis, MO, USA). Chloroform, acetic acid, ethanol, diethyl ether, cyclooctane of spectrophotometric grade, potassium iodide, sodium thiosulfate and sodium hydroxide were from Panreac (Barcelona, Spain).

Mac Conkey agar, MRS agar, yeast extract, casein peptone and Sharpe agar were supplied by Oxoid (Basingstoke, Hampshire, England). Sabouraud-chloramphenicol agar medium was from Sharlau (Barcelona, Spain). Sodium chloride, mannitol, cycloheximide and nisin were purchased by Sigma-Aldrich (St. Louis, MO, USA).

2.2. Olive fruits storage and oil extraction

Three stocks of 'Arbequina', 'Arbosana' and 'Leccino' olives harvested in 2007/2008 at IRTA-Mas de Bover (Constantí, Spain) and with ripeness index of 3.5, 3, and 4, respectively, according to the "Estación de Olivicultura de Jaén" (Uceda & Hermoso, 2001), were stored at a temperature of 17 ± 2 °C. Storage of olives was carried out in plastic bags each containing 10 kg of fruits, during 12 days. One bag for each variety was open every 3–5 days and all the contained olives were processes by a pilot extraction plant Abencor (Comercial Abengoa S.A., Sevilla, Spain) equipped with a hammer crusher, a paste beater and a pulp centrifuge. The virgin olive oils obtained were then decanted, transferred into dark glass bottles and stored at dark at 4 °C until the analyzes.

2.3. Virgin olive oils quality indices and sensory analysis

Peroxide value, free acidity, coefficients of specific extinction at 232 and 270 nm (K_{232} and K_{270}) of VOO samples obtained from the olives conservation assay were determined in duplicate according to EC regulation UE 796/2002. The sensory analysis of the same samples was carried out according to Regulations UE 796/2002 by the Official Tasting Panel of Virgin Olive Oils of Catalonia, which relies on IOOC and ISO 17025 accreditation. Global sensory punctuation, intensity of sensory defects and fruity attribute were assessed and expressed as median of the panelists' scores.

2.4. HS-SPME analysis of volatile phenols

SPME analyzes of virgin olive oil were carried out as described elsewhere (Vichi et al., 2008). Briefly, 2 g of sample was placed into a 10 ml vial fitted with a silicone septum. This was then immersed in a silicone oil bath at 60 °C, and the oil was maintained under magnetic stirring (700 rpm). After 10 min of sample conditioning, a DVB/CAR/PDMS fiber was exposed to the sample headspace for 30 min and immediately desorbed in the gas chromatograph injector. Extraction of each sample was performed in duplicate.

2.5. GC–MS analysis

Identification of compounds was performed by gas chromatography coupled to quadrupolar mass selective spectrometry using an Agilent 5973 Network detector (Agilent Technologies, Palo Alto, CA, USA). Analytes were separated on a Supelcowax-10 (Supelco, Bellefonte, PA, USA) 30 m × 0.25 mm ID, 0.25 mm film thickness. Column temperature was held at 50 °C for 10 min, increased to 240 °C at 8 °C/min. The injector temperature was 265 °C and the time of desorption of the fiber into the injection port was fixed at 10 min. A cleaning step of further 20 min of desorption was required after each analysis. Helium was the carrier gas, at a linear velocity of 38 cm/s. The temperature of the ion source was 175 °C and the transfer line, 280 °C. Positive electron ionization mass spectra (EIMS) were recorded at 70 eV ionization energy, 2 scan/s.

GC–MS analysis in the complete scanning mode (SCAN) in the 40–300 m/z range was performed to allow the identification of compounds in olive and oil samples, by comparison of their mass spectra and retention times with those of standard compounds. *m*-cresol was identified by comparison of mass spectrum and retention index with those available in mass spectrum library, Wiley 6th and in literature, respectively.

Quantitative assessment of volatile phenols was carried out in the selected ion monitoring mode (SIM), by analyzing the following ions: m/z 109, 124 (guaiacol), 107, 108 (*o*-, *m*-, *p*-cresol); 137, 152 (4-ethylguaiacol), 107, 122 (4-ethylphenol, 2,3-dimethylphenol), 135, 150 (4-vinylguaiacol), 91, 120 (4-vinylphenol). Base peak ions were used for quantification of compounds.

Response factors of volatile phenols were calculated by a calibration curve performed by analyzing deodorized sunflower oil with different concentrations of volatile phenols. Standard solutions were prepared in the range 0.01–10 mg/kg and analyzed in duplicate under the same conditions described for samples. Internal standard (2,3-dimethylphenol) concentration was maintained at 5 mg/kg.

2.6. Microbiological profile of olives

The viable cell number in olives surface was determined as follows: a suspension of 50 g of olives in 100 ml of sterile water with 0.9% NaCl was serially diluted in 0.9% NaCl after sonication, and 100 μl of appropriate dilutions were plated in triplicate. Fungi were evaluated on Sabouraud-chloramfenicol agar; lactic acid bacteria on MRS agar supplemented with 100 mg/l cycloheximide (MRS-C); acetic acid bacteria on MYP agar (2.5% mannitol, 0.5% yeast extract, 0.3%, peptone, 2% agar) supplemented with 100 mg/l cycloheximide and 50 mg/l nisin (MYP-CN) and enteric bacteria on Mac Conkey agar. The plates were incubated at 30 °C during 3–5 days and viable counts were expressed as log cfu/g olives.

2.7. Statistics

Data were analyzed using the package "Statgraphics Plus 5.1". In order to assess significant differences between evolutions of each volatile phenol according to the olive variety, factorial ANOVA and Fisher's LSD (least significant differences) were applied on the basis of the mean of two replicates and the standard deviation of the analytical method previously calculated for each compound.

Moreover, the correlation between the amounts of each phenol and the time of olives storage was assessed by simple regression applying the best fitting model (exponential). The degree and significance of the correlation were expressed by regression coefficient (r) and p values, respectively.

3. Results and discussion

3.1. Virgin olive oils quality indices

Quality indices of VOOs obtained from olives of three varieties stored under the same conditions are reported in Table 1. Impor-

Table 1
Quality indices (mean of two replicates) of virgin olive oils extracted after different periods of olives storage in plastic bags at 17 °C.

	Variety	Days of storage			
		0	5	8	12
Free acidity (g oleic acid/100 g oil)	AE[a]	0.3	0.5	0.7	**1.2**[d]
	AO[b]	0.3	0.4	0.4	**1.1**[d]
	L[c]	0.1	0.2	0.3	0.3
Peroxide value (meq O_2/kg)	AE	4.4	4.3	4.1	4.3
	AO	4.9	5.1	4.4	6.9
	L	4.0	5.3	4.3	6.2
K232	AE	1.60	1.18	1.27	1.75
	AO	1.51	1.68	1.40	1.55
	L	1.66	1.40	1.44	1.62
K270	AE	0.10	0.07	0.07	0.11
	AO	0.15	0.10	0.08	0.12
	L	0.08	0.06	0.07	0.08
Oxidative stability[e] (h)	AE	7.3	n.a.	3.6	3.7
	AO	15.3	5.3	11.8	6.9
	L	10.6	6.5	6.1	6.5
Score of classifying defect	AE	0	**2.9**[d]	**2.9**[d]	**4.8**[f]
	AO	0	0	**0.5**[d]	**2.0**[d]
	L	0	0	**2.0**[d]	**3.4**[d]
Score of fruity attribute	AE	3.8	2.0	0.8	**0**[d]
	AO	5.3	4.5	3.2	2.9
	L	4.2	3.8	2.8	1.9

n.a.: Not available.
[a] 'Arbequina' olives.
[b] 'Arbosana' olives.
[c] 'Leccino' olives.
[d] Classified as virgin olive oil according to EU regulations 796/2002 and 640/2008.
[e] Oxidative stability determined by Rancimat test (h).
[f] Classified as lampant olive oil according to EU regulations 796/2002 and 640/2008.

tant increases of free acidity were registered in oils from 'Arbequina' and 'Arbosana' fruits in particular at the later period of storage, while only slight variations were observed for 'Leccino' oils. After twelve days of olives storage, free acidity of 'Arbequina' and 'Arbosana' oils exceeded the limits fixed for EVOO by EU regulations 796/2002 and 640/2008 (in force from October 2008). Quality indices reflecting the oxidative status of the oils did not present marked increases during olive fruit storage, as can be observed in Table 1, while oxidative stability rapidly decreased during the first 5 days of fruits storage. Regarding the sensory parameters provided for VOO classification (EU regulation 796/2002), 'Arbequina' oils were downgraded from EVOO to VOO and lampant commercial categories after 5 and 8 days of olives storage, respectively. 'Arbosana' and 'Leccino' oils lost the EVOO category after 8 days of storage for the appearance of sensory defects, but kept the VOO classification, according to EU regulations 796/2002 and 640/2008 during the entire assay (Table 1). Sensory indices were decisive parameters for classifying the oils on the basis of their quality.

3.2. Microbiological profile of olives surface

As the production of volatile phenols is likely to be attributed to the microbiological activity, different resistance of olives to the attack of microorganisms depending on the variety may influence their formation. To confirm this supposition, the microbiological profile of stored olives was monitored at each sampling point (Fig. 1). Although a low initial contamination (below 10 cfu/g) was observed for the three varieties tested, a higher microbial proliferation took place in 'Arbequina' olives during storage. In particular, the growth of acetic and enteric bacteria was observed only in stored 'Arbequina' olives. Fungi showed a similar growth in 'Arbequina' and 'Arbosana' olives, while in 'Leccino' olives only reached relevant concentration after 12 days of storage. No proliferation of lactic bacteria was observed in any variety.

The extent of microbiological growth was reflected by the free acidity values in 'Arbequina' and 'Arbosana' varieties and by the sensory parameters of the resulting VOOs in the three olive varieties (Table 1), while did not seem to affect the rest of oil's quality indices, under the trial conditions.

3.3. Influence of the olives variety on the formation of volatile phenols during storage

The influence of the variety on the evolution of volatile phenols during olives storage was studied in olives from 'Arbequina', 'Arbosana' and 'Leccino' kept in plastic bags at 17 °C. Excepting *o*-cresol measured in 'Arbosana' oils, volatile phenols formation showed a significant positive correlation to the time of storage (Fig. 2).

During the time of olives storage, the evolutions of cresols, but in particular of guaiacol and 4-ethyl derivatives, appeared to be strongly dependant on the olive variety (Fig. 2), while the increases of 4-vinyl derivatives during fruits storage showed not significant or weak differences in function of this factor (Fig. 2). Regarding cresol isomers, the olive variety showed a significant but quantitatively poor effect on the production of *o*- and *p*-cresol, while had an important influence on the formation of *m*-cresol. The highest amounts of *m*-cresol were registered in oils from 'Arbosana' olives. 'Arbequina' oils showed the most abundant production of guaiacol, 4-ethyl phenol and 4-ethylguaiacol, as well as slightly higher concentrations of *o*- and *p*-cresol (Fig. 2).

These results can be associated with the microbiological profiles observed for each olive variety. In fact, the higher production of guaiacol, 4-ethyl phenol and 4-ethylguaiacol in stored 'Arbequina' fruits corresponded to a larger microbial proliferation in this olive

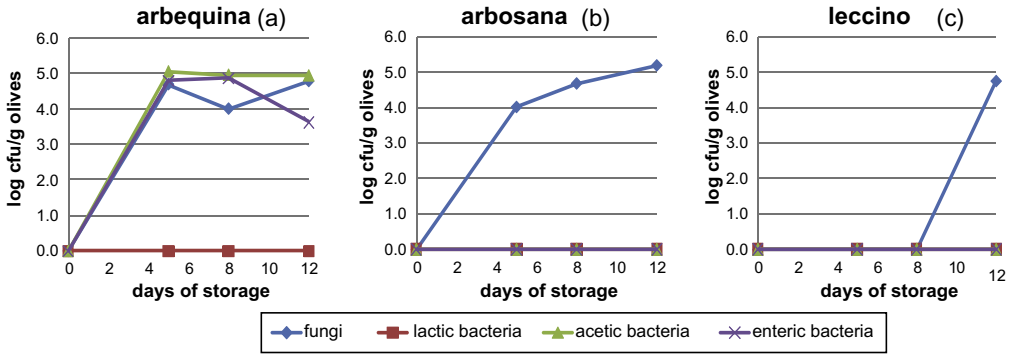

Fig. 1. Microbiological profile of the olives' surface during their storage in closed plastic bags at a temperature of 17±2 °C: (a) 'Arbequina' olives, (b) 'Arbosana' olives, and (c) 'Leccino' olives. Values are means of three replicates.

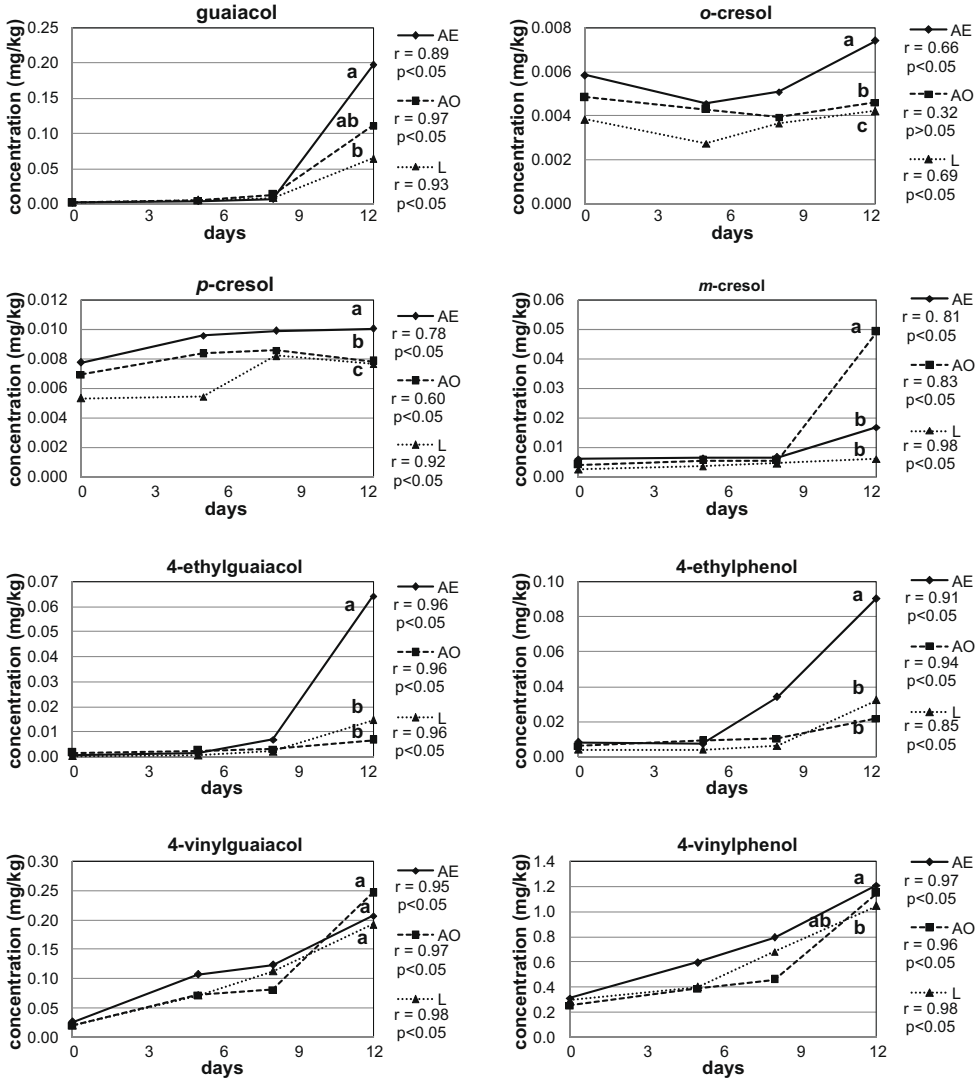

Fig. 2. Evolution of volatile phenols (mg/kg, means of two replicates) in virgin olive oils during storage of olives of 'Arbequina' (AE), 'Arbosana' (AO) and 'Leccino' (L) variety. Different letters in the graphics indicate significantly different evolutions of volatile phenols according to the olive variety, calculated by factorial ANOVA on the basis of the standard deviations previously calculated for the method. The degree of correlation (coefficient of regression, r) and the significance of the correlation (p) between the amounts each volatile phenol and the storage time, assessed by simple regression applying the best fitting model (exponential), are also reported.

variety (Fig. 1). In particular, these results seem to confirm that the formation of these volatile phenols could be related to a conspicuous development of enteric bacteria, as hypothesized in our previous studies (Vichi et al., 2009). Indeed, the capacities of these microorganisms to *o*-demethylate, dehydroxylate, decarboxylate, and reduce ferulic and coumaric acid, with the concurrent production of not substituted phenols and their methyl and ethyl derivatives, have been demonstrated (Grbić-Galić, 1986).

The different resistance of each olive variety to the microorganisms attach could determine important differences in the formation of guaiacol, 4-ethylphenol and 4-ethylguaiacol during olive fruits storage, and their consequent concentration in virgin olive oils. On the contrary, the production of other phenols like 4-vinyl derivatives seems to be scarcely dependent on the olives' microbiological deterioration and other fruit varietal characteristics and appears to be more directly related with the time of olives storage (Fig. 2). These compounds are thought to be formed by decarboxylation of cinnamic acids principally by yeasts (Chatonnet, Dubourdieu, Boidron, & Lavigne, 1993; Chatonnet, Dubourdieu, Boidron, & Pons, 1992; Vanbeneden, Gils, Delvaux, & Delvaux, 2008) and a large number of bacteria (Cavin, Andioc, Etievant, & Divies, 1993; Chatonnet et al., 1992; Lindsay & Priest, 1975; van Beek & Priest, 2000). Nevertheless, their production may be the result of other fruits' physiological processes during storage. Although no data are available about *Olea Europaea* fruits, the ability to quantitatively decarboxylate hidroxycinnamic acids to the correspondent styrene derivatives was already proved for some plant cell cultures (Takemoto & Achiwa, 1999).

3.4. Sensory evaluation of virgin olive oils

The intensity of sensory defects was evaluated during olives storage in virgin olive oils obtained from three distinct varieties (Fig. 3). Winey, fusty, musty and rancid defects were detected in the VOOs obtained from the assay. The highest intensities of these defective notes were registered for oils from stored 'Arbequina' olives, followed by 'Leccino' samples. A possible masking effect on fusty note detected in 'Arbequina' oils could have occurred due to the fast rising of the rancid, musty and winey note at the final stages of storage (Fig. 3). 'Arbosana' was the only variety in which the fusty note was absent along the storage of olive fruits. The oils from this variety, showing the lowest levels of ethyl derivatives,

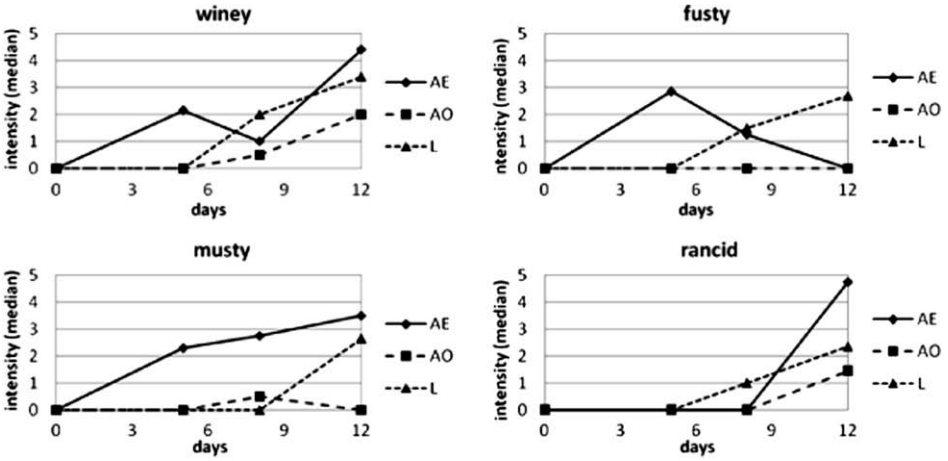

Fig. 3. Evolution of sensory defects in oils from 'Arbequina' (AE), 'Arbosana' (AO) and 'Leccino' (L) olives during storage. The median of the panelists' scores in oils versus the time of olives storage is represented in the graphics.

presented the lowest intensities of sensory defects, together with the highest scores of fruity attribute (Table 1) during the olive fruits storage.

The appearance and the higher intensity of musty, fusty and winey notes in 'Arbequina' oils (Fig. 3) corresponded to the highest amounts of guaiacol and 4-ethyl derivatives registered in these samples during olives storage (Fig. 2), in turn related to the extent of the microbial growth (Fig. 1). These volatile phenols can be then associated with the conditions of olives storage that favor the appearance of sensory defects such as musty, fusty and winey, confirming the previously reported results (Vichi et al., 2009). Cresol isomers could not be directly related to the organoleptic deterioration of oils. In fact, their evolution in oils was comparable (even if significantly different), for the three olive varieties (Fig. 2), and when their production was clearly higher, like for *m*-cresol in 'Arbosana' olives, it was not in agreement with the decrease of sensory quality (Table 1 and Fig. 2).

Quite high intensities of rancid defect were detected in VOOs obtained at the end of the storage period from olives of the three varieties, in particular from 'Arbequina' fruits (Fig. 3). These rancid note intensities were not supported by the chemical indices related to the oil's oxidative status (Table 1). At certain concentrations of 4-vinylphenol, its varnish-like descriptor already reported in wines (Bayonove, Baumes, Crouzet, & Günata, 2000; Boutou & Chatonnet, 2007; Ribereau-Gayon, Glories, Maujean, & Dubourdieu, 2004), could be confused with the varnish/paint note associated with lipid oxidation (Hamilton, Kalu, Prisk, Padley, & Pierce, 1997), thus inducing the perception of the rancid defect. It must be considered that 'rancid' is the only descriptor related to this perception that the sensory assessors can use under the UE 760/2002 Regulation.

In conclusion, the different resistance of each variety to the microbiological attach was observed during olive fruit storage, and it was reflected by the evolution of particular volatile phenols, such as guaiacol, 4-ethylphenol and 4-ethylguaiacol. On the contrary, a scarce dependence on the microbial growth or varietal factors was observed for 4-vinyl derivatives, which appeared more directly related to the time of olives storage. The former compounds could be considered suitable analytical indices of olive fruits microbiological degradation during storage, while the latter could be potential markers of the time of olives' storage. Among VOO quality indices provided by EU Regulations, only free acidity and sensory parameters reflected the different degree of olive fruits microbiological deterioration. The promising role of volatile phenols as analytical indicators of VOO quality was evidenced by certain correspondence between their evolution and sensory characteristics of the resulting VOOs. In fact, the VOOs from the variety showing the highest amounts of guaiacol and 4-ethyl derivatives developed the highest intensities of fermentative sensory defects. This also confirms our previous results, which suggested the relation between these phenols and the conditions of olives storage that favor the appearance of musty, fusty and winey defects in VOO. Regarding 4-vinyl derivatives, they were proposed to be involved in the perception of the rancid note.

Acknowledgements

This study was supported by the Generalitat de Catalunya, project 2005SGR00156; by the Ministerio de Ciencia y Tecnología (MCYT), project AGL2008-03392/ALI, and by the Ministerio de Educación y Ciencia (MEC), Juan de la Cierva program. The authors are grateful to the Official Tasting Panel of Virgin Olive Oils of Catalonia for the sensory evaluation of samples.

References

Bayonove, C., Baumes, R., Crouzet, J., & Günata, Z. (2000). Aromas. In C. Flanzy (Ed.), *Enología: Fundamentos científicos y tecnológicos* (pp. 137–176). Madrid: AMV Ediciones.

Boutou, S., & Chatonnet, P. (2007). Rapid headspace solid-phase microextraction/gas chromatographic/mass spectrometric assay for the quantitative determination of some of the main odorants causing off-flavors in wine. *Journal of Chromatography A, 1141*, 1–9.

Brenes, M., Romero, C., García, A., Hidalgo, F. J., & Ruiz Jiménez, M. V. (2004). Phenolic compounds in olive oils intended for refining: Formation of 4-ethylphenol during olive paste storage. *Journal of Agricultural and Food Chemistry, 52*, 8177–8181.

Cavin, J., Andioc, P., Etievant, P., & Divies, C. (1993). Ability of wine lactic bacteria to metabolize phenol carboxylic acids. *American Journal of Enology and Viticulture, 1*, 76–80.

Chatonnet, P., Dubourdieu, D., Boidron, J. N., & Lavigne, V. (1993). Synthesis of volatile phenols by *Saccharomyces cerevisiae* in wines. *Journal of the Science of Food and Agriculture, 62*, 191–202.

Chatonnet, P., Dubourdieu, D., Boidron, J. N., & Pons, M. (1992). The origin of ethylphenols in wines. *Journal of the Science of Food and Agriculture, 60*, 165–178.

European Commission. (2002). Official Journal of European Communities, May 6, Commission Regulation (EC) No 796/2002 of 6 May 2002 amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-pomace oil and on the relevant methods of analysis and the additional notes in the Annex to Council Regulation (EEC) No 2658/87 on the tariff and statistical nomenclature and on the Common Customs Tariff.

European Commission, Official Journal of European Communities. July 4, Commission Regulation (EC) No 640/2008 of 4 July 2008 amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-pomace oil and on the relevant methods of analysis.

Grbić-Galić, D. (1986). *o*-Demethylation, dehydroxylation, ring reduction and cleavage of aromatic substrates by *Enterobacteriaceae* under anaerobic conditions. *Journal of Applied Bacteriology, 61*, 491–497.

Hamilton, R. J., Kalu, C., Prisk, E., Padley, F. B., & Pierce, H. (1997). Chemistry of free radicals in lipids. *Food Chemistry, 60*, 193–199.

Jiménez, A., Aguilera, M. P., Beltrán, G., & Uceda, M. (2006). Application of solid-phase microextraction to virgin olive oil quality control. *Journal of Chromatography A, 1121*, 140–144.

Lindsay, R. F., & Priest, F. G. (1975). Decarboxylation of substituted cinnamic acids by enterobacteria: The influence on beer flavor. *Journal of Applied Bacteriology, 39*, 181–187.

Morales, M. T., Luna, G., & Aparicio, R. (2005). Comparative study of virgin olive oil sensory defects. *Food Chemistry, 91*, 293–301.

Reiners, J., & Grosch, W. (1998). Odorants of virgin olive oils with different flavor profiles. *Journal of Agricultural and Food Chemistry, 46*, 2754–2763.

Ribereau-Gayon, P., Glories, Y., Maujean, A., & Dubourdieu, D. (2004). La natura chimica, l'origine e le conseguenze dei principali difetti organolettici. *Trattato di enologia II. Chimica del vino stabilizzazione, Trattamenti* (pp. 225–272). Bologna: Edagricole.

Sánchez Saez, J. J., Herce Garraleta, M. D., & Balea Otero, T. (1991). Identification of cinnamic acid ethyl ester and 4-vinylphenol in off-flavor olive oils. *Analytica Chimica Acta, 247*, 295–297.

Takemoto, M., & Achiwa, K. (1999). Synthesis of styrenes through the decarboxylation of trans-cinnamic acids by plant cell cultures. *Tetrahedron Letters, 40*, 6595–6598.

Uceda, M., & Hermoso, M. (2001). La calidad del aceite de oliva. In D. Barranco, R. Fernández-Escobar, & L. Rallo (Eds.), *El cultivo del olivo* (pp. 589–614). Madrid, Spain: MundiPrensa.

van Beek, S., & Priest, F. G. (2000). Decarboxylation of substituted cinnamic acids by lactic acid bacteria isolated during malt whisky fermentation. *Applied and Environmental Microbiology, 66*, 5322–5328.

Vanbeneden, N., Gils, F., Delvaux, F., & Delvaux, F. R. (2008). Formation of 4-vinyl and 4-ethyl derivatives from hydroxycinnamic acids: Occurrence of volatile phenolic flavor compounds in beer and distribution of Pad1-activity among brewing yeasts. *Food Chemistry, 107*, 221–230.

Vichi, S., Romero, A., Gallardo-Chacón, J., Tous, J., López-Tamames, E., & Buxaderas, S. (2009). Influence of olives' storage conditions on the formation of volatile phenols and their role in off-odor formation in the Oil. *Journal of Agricultural and Food Chemistry, 57*, 1449–1455.

Vichi, S., Romero, A., Tous, J., López-Tamames, E., & Buxaderas, S. (2008). Determination of volatile phenols in virgin olive oils and their sensory significance. *Journal of Chromatography A, 1211*, 1–7.