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Influence of Olives' Storage Conditions on the Formation of Volatile Phenols and Their Role in Off-Odor Formation in the Oil

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Volatile phenols are spoilage compounds of many foods, which have also been detected in the volatile fraction of defective virgin olive oils (VOOs). However, their formation in olive fruits or VOOs, as well as the factors affecting their production, has yet to be elucidated. In the present study, the evolution of volatile phenols was monitored for the first time in VOOs obtained from olives stored during different periods under two different conditions of limited aerobiosis. Moreover, their odor activity values (OAVs) in VOO samples were calculated as a first assessment of their sensory importance, and the microbiological profile of the olives' surface was evaluated at each sampling point in order to clarify the possible causes of volatile phenols formation. Although volatile phenols seem to acquire their sensory significance at advanced stages of olives' alteration, they 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.

KEYWORDS: Virgin olive oil; volatile phenols; solid phase microextraction; quality markers; off-flavor; olive fruit degradation; olives' storage

INTRODUCTION

Volatile phenols are potent odorants considered as spoilage compounds in many foods (*1*−*6*). The presence of some volatile phenols has been also reported in the volatile fraction of virgin olive oils (VOOs) with off-flavor (*7*−*10*). Recently, a series of nine volatile phenols comprising methyl, ethyl, and vinyl derivatives of phenol and guaiacol were assessed in extra virgin olive oils (EVOOs) and defective olive oils, pointing out their potential contribution to the perception of sensory defects and their relation with a poor oil quality (*11*). High amounts of volatile phenols were found in olive oils with strong fusty, musty, and muddy defects (*9, 11*) as well as in stored olive paste (*12*), indicating that their presence in olive oil has likely a microbiological origin. These defects are in fact well-known to be caused by microbial proliferation during olives' or oil storage at unsuitable conditions (*9, 13*). Nevertheless, no studies have been carried out to investigate the formation of volatile phenols in olives or virgin olive oils, and the factors affecting their production have yet to be elucidated.

The aim of the present work was to study the evolution of volatile phenols in virgin olive oils during olive storage under different conditions and to evaluate their possible role as olive oils' quality markers, reflecting the olive fruits' deterioration. For this scope, volatile phenols were monitored for the first time in virgin olive oils obtained from olives stored during different periods at relatively low temperature (5−8 °C) and at two conditions of limited aerobiosis: in plastic bags and in open boxes. Moreover, the microbiological profile of the olives' surface was evaluated at each sampling point in order to clarify the possible causes of volatile phenols formation.

MATERIALS AND METHODS

Reagents and Materials. The SPME fiber used was divinylbenzene/carboxen/polydimethylsiloxane, 50/30 μm, 2 cm long (DVB/CAR/PDMS) from Supelco (Bellefonte, PA). 2,3-Dimethylphenol, phenol, *o*-, *p*-cresol, 4-ethylphenol, 4-vinylphenol (solution 10% w/w in propylene glycol), guaiacol, 4-ethylguaiacol, and 4-vinylguaiacol came from Sigma-Aldrich (St. Louis, MO).

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, Cetrimide agar, yeast extract, casein peptone, and Sharpe agar were supplied by Oxoid (Basingstoke,

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Table 1. Quality Indices of Olive Oil Extracted after Different Periods of Olives' Storage in Plastic Bags and in Open Boxes

	free acidity (g oleic acid/kg oil)		peroxide value (mequiv O_2/kg)		K_{232}		K_{270}		sensory score		score of classifying defect		score of fruity attribute	
days	bag[a]	box[b]	bag	box	bag	box	bag	box	bag	box	bag	box	bag	box
0	0.51 ± 0.01	0.51 ± 0.01	7.3 ± 0.9	7.3 ± 0.9	1.91 ± 0.03	1.91 ± 0.03	0.11 ± 0.01	0.11 ± 0.01	7.6	7.6			4.8	4.8
3	0.32 ± 0.04	0.41 ± 0.04	5.5 ± 1.1	6.5 ± 1.1	1.52 ± 0.01	1.50 ± 0.01	0.10 ± 0.00	0.10 ± 0.00	7.0	6.4			4.3	4.5
6	0.80 ± 0.04	0.54 ± 0.04	5.5 ± 1.0	5.5 ± 1.1	1.80 ± 0.01	1.73 ± 0.01	0.09 ± 0.00	0.10 ± 0.00	5.6	6.2			3.6	4.0
9	**1.20 ± 0.04**[c]	0.82 ± 0.04	n.a.	n.a.	1.89 ± 0.01	1.84 ± 0.01	0.11 ± 0.00	0.11 ± 0.00	4.9	5.3	**1.5**[c]		3.1	3.4
16	**1.91 ± 0.01**[c]	**1.55 ± 0.01**[c]	8.8 ± 0.9	6.7 ± 0.9	1.91 ± 0.03	1.83 ± 0.03	0.14 ± 0.01	0.13 ± 0.01	3.8	3.8	**4.3**[d]	**2.5**[c]	0.5	**0**[d]
21	**3.09 ± 0.01**[d]	**2.47 ± 0.04**[d]	6.8 ± 1.1	7.9 ± 1.0	1.93 ± 0.03	1.82 ± 0.03	0.16 ± 0.01	0.13 ± 0.01	3.8	3.8	**5.4**[d]	**5.1**[d]	**0**[d]	**0**[d]

[a] Oils from olives stored in plastic bags. [b] Oils from olives stored in open boxes. [c] Classified as virgin olive oil according to EU regulations. [d] Classified as lampant olive oil according to EU regulations. Limits of quality indices according to EU Regulation 796/2002 (*14*) and 640/2008 (*15*): free acidity: EVOO ≤ 0.8, VOO ≤ 2.0; median of sensory defect: EVOO = 0, VOO ≤ 3.5; median of the fruity attribute: EVOO > 0, VOO > 0; n.a.: not available values.

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).

Olive Fruits' Storage and Oil Extraction. Olives of the Arbequina variety handpicked in December 2007 in Reus (Spain) were stored in a room at a temperature of 5 ± 3 °C by night and 8 ± 3 °C by day, with a relative humidity of 70%. Storage of olives was carried out in plastic bags and in open boxes, each containing 10 kg of fruit, during 21 days. Every 3−9 days the olives of an entire bag and an entire box were processed 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 in the dark at 4 °C until the analyses.

Virgin Olive Oils' Quality Indices. The peroxide value, free acidity, and coefficients of specific extinction at 232 and 270 nm (K_{232} and K_{270}) of VOO samples obtained from the olive conservation assay were determined in analytical duplicate according to EC regulation UE 796/2002 (*14*). The sensory analysis of the same samples was carried out according to Regulations UE 796/2002 (*14*) and UE 640/2008 (*15*) (in force from October 2008) by the Official Tasting Panel of Virgin Olive Oils of Catalonia, which relies on IOOC and ISO 17025 accreditation. Global sensory punctuation, the intensity of sensory defects, and fruity attributes were assessed and expressed as a median of the panelists' scores.

HS-SPME Analysis of Volatile Phenols. Two grams of oil spiked with 2,3-dimethylphenol (internal standard) was weighed into a 10 mL vial fitted with a silicone septum and placed into a silicon oil bath at 60 °C, where the oil was maintained under magnetic stirring (700 rpm). After 10 min of sample conditioning, a DVB/CAR/PDMS fiber was exposed during 30 min to the sample headspace and immediately desorbed in the gas chromatograph injector. Each extraction was performed in duplicate.

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). Analytes were separated on a Supelcowax-10 (Supelco, Bellefonte, PA), 30 m × 0.25 mm i.d., 0.25 μm film thickness. Column temperature was held at 50 °C for 10 min and increased to 240 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 that of the transfer line was 280 °C. Positive electron ionization mass spectra (EIMS) were recorded at 70 eV of ionization energy and 2 scan/s.

GC-MS analysis in the complete scanning mode (SCAN) in the 40−300 *m*/*z* range allowed 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 the mass spectrum and retention index with those available in a mass spectrum library, Wiley's sixth, and in the 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), 77, 94 (phenol), 107, 108 (*o*-, *m*-, and *p*-cresol); 137, 152 (4-ethylguaiacol), 107, 122 (4-ethylphenol and 2,3-dimethylphenol), 135, 150 (4-vinylguaiacol), 91, and 120 (4-vinylphenol). Base peak ions (underlined) 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. The internal standard (2,3-dimethylphenol) concentration in samples was maintained at 5 mg/kg.

Microbiological Profile of Olives. To determine the viable-culturable cell number on olives' surface, a suspension of 50 g of olives was prepared in 100 mL of sterile water with 0.9% NaCl. After 5 min in the ultrasound bath, the suspension was serially diluted in 0.9% NaCl, 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); enteric bacteria on Mac Conkey agar, and *Pseudomonas* on Cetrimide agar supplemented with 100 mg/L cycloheximide (Cetrimide-C). The plates were incubated at 30 °C during 3−5 days, and viable counts were expressed as log cfu/g olive.

Statistical Analysis. Data were analyzed using the package "Statgraphics Plus 5.1". Simple regression was applied to relate the concentration of volatile phenols in oils to the time of olive storage.

RESULTS AND DISCUSSION

Virgin Olive Oils' Quality Indices and Sensory Profiles. VOOs quality indices and sensory evaluation at different periods of olive storage in both plastic bags and in open boxes are reported in **Table 1**. The footnotes report the limits of each parameter established for the distinct categories of VOO by the EU regulations. Free acidity progressively increased during the olives' storage, according to previously reported results (*16*−*18*), in particular in oils from olives stored in bags. As well, K_{270} showed a slight augment at the end of the storage period, while the initial peroxide value and K_{232} were maintained almost constant during the olives' storage period. Neither peroxide values nor specific extinctions at 232 and 270 nm exceeded the limits fixed for the EVOO category during the 21 days of olives' storage. In this respect, the scarce effect of low-temperature olive fruits storage on some olive oil analytical quality indices has been already reported (*16*, *19*). On the other hand, VOOs sensory punctuation was directly related to the time of olive storage and showed comparable decreases for olives stored in bags and boxes. The intensity of sensory defects was higher in oils from olives stored in bags, although they maintained a slight fruity note for a longer period (**Table 1**). Free acidity, but in particular sensory evaluation, was the index which reflected better the loss of oils' quality during the olives' storage and determined the downgrading of oils from EVOO to VOO and lampant commercial categories, according to EU regulations 796/2002 (*14*)

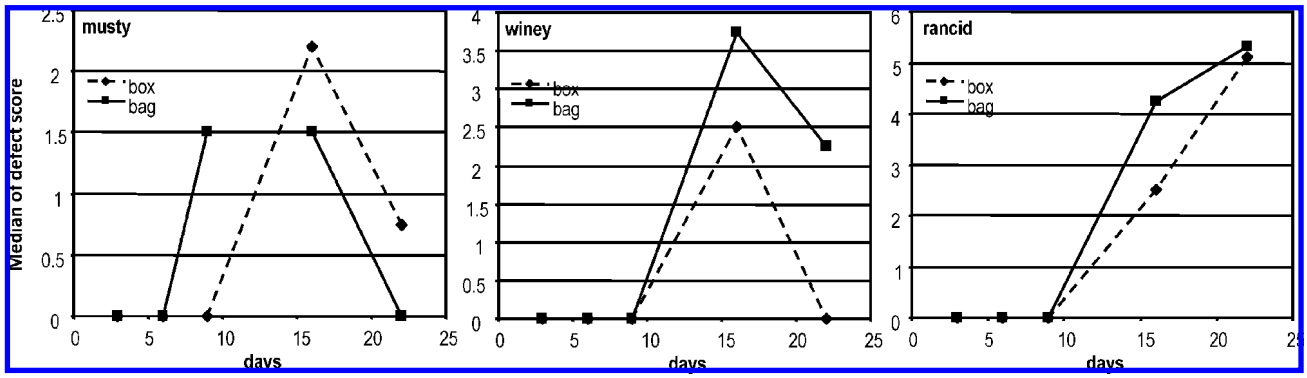

Figure 1. Evolution of sensory defects in oils from olives stored in plastic bags and in open boxes. The median of the defects intensity in oils versus the time of olives' storage is represented in the graphics.

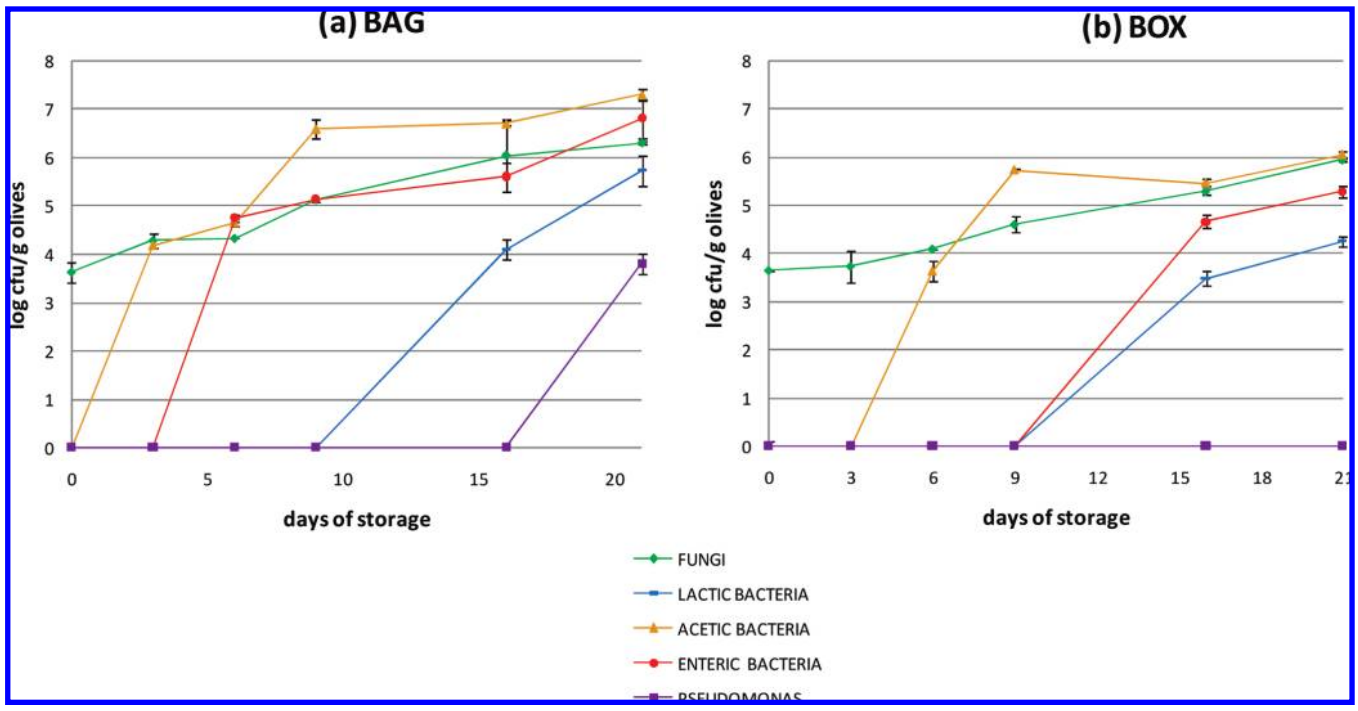

Figure 2. Microbiological profile of the olives' surface during their storage under different conditions: (**a**) in plastic bags; (**b**) in open boxes.

and 640/2008 (*15*). On the basis of these parameters, VOOs obtained from olives stored in bags lost the extra quality category after 9 days, and oils from olives stored in both bags and boxes became of lampant category after 16 days (**Table 1**).

In **Figure 1** the intensity of the sensory defects detected in oil samples is shown. As expected, oils from olives stored in plastic bags presented higher intensities of sensory defects than oils from fruits stored in open boxes. Musty, winey, and rancid notes were the off flavors detected after 16 days of olives storage, with the exception of mustiness, which was detected in oils from olives stored in bags after 9 days. The punctuations of musty and winey notes decreased after the fast increase of the rancid note, indicating that a masking effect could have occurred (**Figure 1**). Finally, it is noteworthy that the fusty note, typical of oils obtained from olives stored in sacks or piles (*13*), was not detected at the conditions applied in the study.

Microbial Analysis of Olives. The formation of volatile phenols has been related with microbial proliferation in several foods and agricultural emissions (*1*−*4*, *20*). With the aim to contribute to clarify the possible causes of volatile phenols formation during the storage of olives, the changes in the microbiological profile on the surface of stored fruits used for this study were monitored at each sampling point. **Figure 2** illustrates the evolution of microorganisms during storage of olive in plastic bags or in open boxes. Proliferation of lactic, acetic, and enteric bacteria, fungi, and *Pseudomonas* was evaluated. At the initial time, only fungi presented a concentration above 10^3 cfu per gram of olives, while the concentration of the rest of the monitored microorganisms was lower than 10 cfu/g. Their behavior during storage was very similar under the two storage conditions, independently from aerobiosis conditions. On the contrary, the proliferation of the rest of microorganisms was faster in olives stored in plastic bags, in agreement with the analytical and sensory quality of the oils (**Table 1** and **Figure 1**). Surprisingly, acetic bacteria showed a slightly faster growth in bags storage, at the conditions which theoretically had a higher degree of anoxia (**Figure 2a**) However, this difference was mainly observed at the initial stage of storage, when residual amounts of oxygen were still present in the bags. Lactic bacteria showed a higher proliferation in olives stored in bags, but only after 21 days of storage. Among the evaluated microorganisms, the main differences were observed in the evolution of enteric bacteria, whose concentration was near to 10^5 cfu/g after six days of storage of olives in plastic bags, while it remained below 10^1 cfu/g until 16 days of olive storage in

Table 2. Concentration (Mean of Two Analytical Replicates) of Volatile Phenols (μg/Kg) in VOOs Obtained from Olives Stored in Plastic Bags and Open Boxes during Different Periods

		conc after the following no. of days of storage					
compd	storage	0	3	6	9	16	21
guaiacol	bag[a]	2.3 ± 0.7	14.4 ± 1.0	15.3 ± 1.2	34.3 ± 0.8	70.7 ± 3.8	109.6 ± 7.1
	box[b]		6.0 ± 0.2	3.0 ± 0.7	5.3 ± 1.7	13.7 ± 1.3	21.8 ± 1.0
phenol	bag	56.4 ± 2.6	109.9 ± 6.3	110.4 ± 6.7	119.6 ± 0.7	119.8 ± 19.8	99.7 ± 1.3
	box		66.2 ± 1.7	55.7 ± 7.1	68.8 ± 10.6	76.1 ± 2.0	70.4 ± 9.4
o-cresol	bag	5.1 ± 1.0	11.2 ± 1.4	12.4 ± 1.4	10.8 ± 0.1	11.9 ± 2.3	30.4 ± 1.6
	box		5.0 ± 0.1	5.3 ± 1.3	5.5 ± 0.3	7.4 ± 0.6	34.9 ± 1.3
p-cresol	bag	7.9 ± 1.8	21.1 ± 2.7	24.7 ± 1.0	27.2 ± 3.5	34.0 ± 0.2	36.0 ± 4.5
	box		13.8 ± 1.4	14.3 ± 1.5	14.8 ± 1.3	24.9 ± 3.7	29.7 ± 2.3
m-cresol	bag	6.2 ± 1.1	7.6 ± 0.9	10.0 ± 0.4	11.7 ± 0.4	17.8 ± 0.8	35.0 ± 1.4
	box		6.4 ± 2.6	6.4 ± 0.4	7.9 ± 1.0	11.4 ± 2.4	16.8 ± 0.8
4-ethylguaiacol	bag	1.3 ± 0.5	2.0 ± 0.2	2.3 ± 0.1	6.2 ± 0.6	9.8 ± 0.6	14.8 ± 1.9
	box		2.0 ± 0.2	1.5 ± 0.4	1.8 ± 0.4	4.1 ± 0.4	6.8 ± 0.2
4-ethylphenol	bag	8.4 ± 1.2	19.9 ± 0.5	18.6 ± 1.9	23.8 ± 0.2	39.4 ± 1.8	76.3 ± 7.8
	box		12.5 ± 1.2	10.9 ± 0.4	13.9 ± 2.3	22.8 ± 2.3	35.3 ± 2.8
4-vinylguaiacol	bag	26.7 ± 3.4	51.1 ± 6.0	82.9 ± 3.1	149.6 ± 1.9	427.4 ± 78.7	780.1 ± 117.3
	box		85.8 ± 7.9	47.9 ± 7.5	74.0 ± 13.3	321.1 ± 75.8	683.1 ± 79.4
4-vinylphenol	bag	319.6 ± 8.5	530.3 ± 5.1	878.2 ± 35.9	1038.7 ± 3.5	2798.5 ± 42.2	5305.3 ± 824.5
	box		990.4 ± 27.7	520.1 ± 75.0	669.5 ± 127.4	2309.4 ± 186.9	4301.2 ± 80.3

[a] Oils from olives stored in plastic bags. [b] Oils from olives stored in open boxes.

open boxes. Likewise, a growth of *Pseudomonas* was noticed only in olives stored 21 days in bags. The type of microorganisms found in the present study was quite in accordance with those reported by Angerosa et al. (*21*) to grow under other conditions of olives' storage.

Evolution of Volatile Phenols during Olives' Storage and Influence of the Storage Conditions. Table 2 reports the concentration of nine volatile phenols in virgin olive oils obtained from Arbequina olives stored during different periods in plastic bags and in open boxes. Although the possible differences among distinct batches of olives could not be evaluated in this study, some characteristic trends of formation of volatile phenols in the extracted oils could be clearly evidenced. Phenol and 4-vinyl derivatives, in particular 4-vinylphenol, were the most abundant volatile phenols along the whole period of storage. Moreover, the amounts of phenol derivatives were always higher than those of the correspondent guaiacol derivatives during the whole period of olives storage, except for guaiacol in olives stored in bags, which exceeded the concentration of phenol after 21 days (**Table 2**). The results of the concentration of volatile phenols obtained in oils extracted from stored olives, compared with those of strongly defective olive oils (*11*), suggest that the conditions tested in the study are not the most favorable for the formation of volatile phenols, in particular for 4-ethyl derivatives. In addition to aerobiosis conditions and time of storage, there are many other possible factors influencing the production of volatile phenols, such as the initial quality and microbiological state of olives, the temperature of storage, and likely the resistance of each olive variety to microbiological attack. The influence of these variables on the production of volatile phenols should be investigated in further studies.

On the basis of the concentrations of volatile phenols in virgin olive oil samples reported in **Table 2**, the trend of formation of these compounds during olives storage was evaluated by considering the percent of increase ($I_{\%} = (C_0/C - 1) \times 100$) of each volatile phenol versus time, for each type of olive storage (**Figure 3**). This allowed appreciating which compounds were more influenced by the time and the conditions of olive storage. Simple regressions were carried out to correlate the percent of increase of each compound to the time of storage. Except for *p*-cresol, which showed a linear correlation with time, the best fittings were obtained by applying an exponential model, which is shown in **Figure 3** for each phenol. The same figure reports the regression coefficient (r) and the significance of the correlation (p) between the percent of increase of phenols and the time of storage. As expected, the increase of the monitored phenols was significantly correlated with the time of olives' storage under both conditions. The only exception was phenol, which did not show any significant increase during the storage and could not be associated with the degradation of olives. On the contrary, guaiacol in olives stored in plastic bags was the compound with the highest increase after 21 days of storage (more than 50 fold its initial concentration), followed by 4-ethyl derivatives in bags storage and 4-vinyl derivatives for both the storage conditions (between 10 and 30 fold their initial concentration).

A clearly different behavior between olives stored in bags or boxes was observed for guaiacol, *m*-cresol, 4-ethylguaiacol, and 4-ethylphenol (**Figure 3**), which presented higher percent increases in oils from olives stored in bags. On the contrary, no major differences were observed in the evolutions of *o*- and *p*-cresol and 4-vinyl derivatives due to the type of container used during olives' storage. The evolution of these compounds seem to be scarcely dependent on the degree of anoxia and more directly related to the time of olives' storage under conditions of limited aerobiosis. 4-Vinylguaiacol and 4-vinylphenol are thought to be formed by decarboxylation of ferulic and coumaric acid, respectively (*1, 2, 20*). Both of these phenolic acids are largely present in the olive fruit and oil (*22*). The ability to decarboxylate these phenolic acids has been reported for a large number of microorganisms, such as yeasts (*3*), lactic bacteria (*23, 24*), acetic bacteria (*1*), and enteric bacteria (*25*). All these classes of microorganisms have been detected in the present study and observed to increase in olive fruits subjected to storage. In particular, as no major differences were observed in the evolution of 4-vinyl derivatives in oils from olives stored in bags or boxes (**Figure 3**), yeasts or lactic or acetic bacteria could be responsible for their formation because they were the microorganisms showing the most similar growth under both conditions (**Figure 2**). The reduction step leading to 4-ethyl derivatives is much less frequent and has been reported as particularly effective in some yeast of *Dekkera*, *Pichia*, and *Candida* species (*26, 27*), lactic acid bacteria (*23, 24, 28*), and

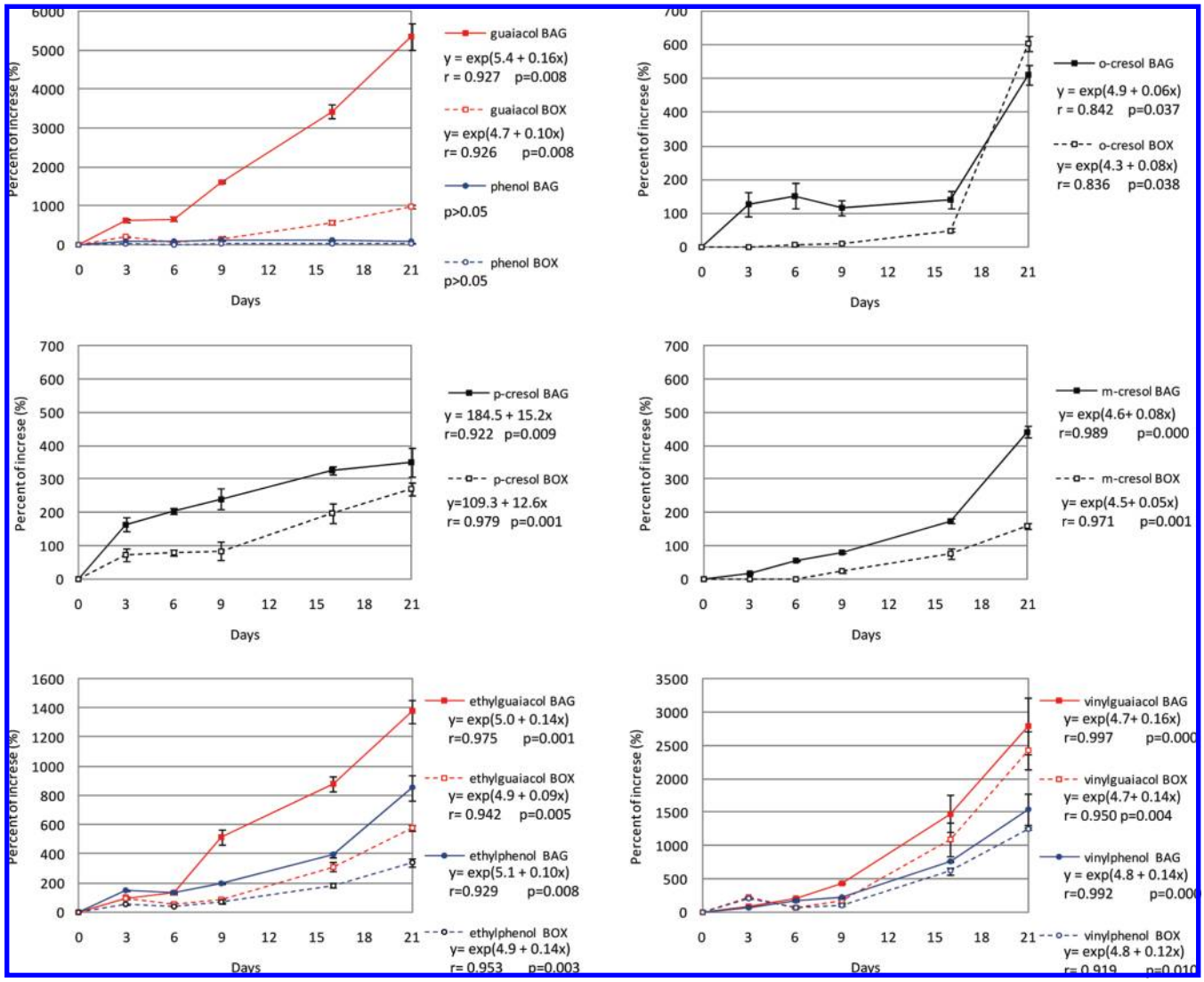

Figure 3. Formation of volatile phenols in virgin olive oils during storage of olives in plastic bags (**a**) and in open boxes (**b**). Data are expressed as percent of increase with respect to the initial amounts $I_{\%} = (C_0/C - 1) \times 100$. The model of regression, the degree of correlation (coefficient of regression, *r*), and the significance of the correlation (*p*) are reported for each compound.

enteric bacteria (*29*). The latter have been demonstrated to possess *o*-demethylation, dehydroxylation, decarboxylation, and reduction activity under anaerobic conditions, with the concurrent production of not substituted phenols and their methyl and ethyl derivatives. Enteric bacteria were also proved to produce guaiacol from vanillic acid by decarboxylation (*30*). The proliferation of enteric bacteria was the main difference observed in the evolution of the microbiological profile of olives stored in bags and boxes (**Figure 2**), and it could be thus hypothesized to possibly be responsible for the differences found in the evolution of compounds such as guaiacol and 4-ethyl derivatives in the correspondent VOOs (**Figure 3**).

As previously commented, the microbial growth observed in olives stored in bags was only slightly higher than that in olives stored in boxes, and basically differed in the proliferation of enteric bacteria (**Figure 2**). In particular, the concentration of the most abundant microorganisms, such as fungi and acetic and enteric bacteria after 16 days of olives storage in boxes (each around 10^5-10^6 cfu/g), was comparable to their concentration in bags storage after 6−9 days (**Figure 2**). Certain correspondence can be found between this microbiological profile and the evolution of volatile phenols, showing the highest dependence on the kind of olives' storage: guaiacol, *m*-cresol, and ethyl derivatives. In fact, the evolution of these phenols in box storage reached after 16 days the concentrations (**Table 2**) and the percents of increase (**Figure 3**) observed in bag storage after 6−9 days. This relation between the microbiological profile of stored olives and the oil's characteristics can also be found by observing the quality indices more influenced by the kind of storage, such as the free acidity and the score of the sensory defect (**Table 1**). These indices determined the downgrading of oils from EVOO to VOO after 9 and 16 days of bag and box storage of olives, respectively. Likewise, a musty defect was perceived in oils after 16 days of box storage and after 9 days of bag storage (**Figure 1**).

OAVs of Volatile Phenols in Virgin Olive Oils from Stored Olives. A first assessment of the potential importance of volatile phenols to the aroma of oils from stored olives was made by determining their odor activity values (OAVs) in oil samples. OAVs were calculated as the ratio of concentration and odor detection threshold (ODTs). ODTs had been previously calculated in refined sunflower oil as the lowest concentration of compound perceived by 50% of a 12 experienced assessors panel by a three-alternative forced-choice (3-AFC) procedure (*11*). Only guaiacol, *o*-, *p*-cresol (ODT of *m*-cresol was not available), and 4-vinyl derivatives reached OAVs ≥ 1 along

Table 3. Odor Activity Values (OAVs) of Volatile Phenols in Virgin Olive Oils Obtained from Olives Stored in Plastic Bags and Open Boxes for Different Periods[a]

			odor activity values (OAVs) after the following no. of days of storage					
compd	ODT[b] (μg/Kg)	storage	0	3	6	9	16	21
guaiacol	10	bag[c]		1	2	3	7	11
		box[d]					1	2
phenol	100	bag		1	1	1	1	1
		box						
o-cresol	25	bag						1
		box						1
p-cresol	25	bag			1	1	1	1
		box					1	1
4-ethylguaiacol	50	bag						
		box						
4-ethylphenol	200	bag						
		box						
4-vinylguaiacol	200	bag					2	4
		box					2	3
4-vinylphenol	400	bag		1	2	3	7	13
		box		2	1	2	6	11

[a] Only OAVs ≥ 1 are reported in the table. The ODT of *m*-cresol was not available. [b] Odor detection threshold in refined sunflower oil (*11*). [c] Oils from olives stored in plastic bags. [d] Oils from olives stored in open boxes.

the storage. 4-Vinyl derivatives were the phenols with the major odor activity in virgin olive oils obtained from stored fruits, together with guaiacol, which reached higher sensory impact in oils obtained from olives stored in bags (**Table 3**). *o*- and *p*-Cresol isomers reached concentrations equal or slightly above their ODT in oils from olives stored during long time in both plastic bags and open boxes. Although concentrations of 4-ethylguaiacol and 4-ethylphenol notably increased storing olives in bags (**Table 2**), their amount remained below the ODT (**Table 3**).

Due to the relatively low sensory impact showed by volatile phenols at the condition applied in this study, their OAVs could not always be correlated with the sensory defects detected by the panel (**Figure 1**). However, the absence of a fusty defect in samples with concentration of 4-ethylphenols below their ODTs seems to be in agreement with a possible relation between the formation of these compounds and the conditions which favor the appearance of the fusty defect, as suggested by the high sensory impact of 4-ethylphenols in the strongly fusty reference oil (*11*). On the contrary, the results of OAVs (**Table 3**) do not preclude a possible relation between mustiness (**Figure 1**) and guaiacol amounts in oil, as proposed by Morales et al. (*9*). In addition, the high intensity of the rancid note at the end of storage was not justified by the chemical indices observed (**Table 1**), and it reached similar values for VOOs from bag and box storage of fruits (**Figure 1**). The high increase of the rancid note corresponded to a noteworthy increase of 4-vinylphenol OAV (**Table 3**). These observations lead us to hypothesize that the "varnish" note, characteristic of 4-vinylphenol (*31*−*33*), could contribute to the perception of the rancid defect, which can include this descriptor.

Although volatile phenols seem to acquire their sensory significance at advanced stages of olives alteration, the results of this study indicate that they could be considered as analytical indices of olive fruits degradation during storage, likely reflecting the microbiological activity.

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