Postprint of Food Research International, Volume 125, November 2019, 108568
DOI: https://doi.org/10.1016/j.foodres.2019.108568

Changes in volatile composition during the processing and storage of black ripe olives

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Running title: Volatile composition of black ripe olives

Abstract

The present study revealed the effects of each step of black ripe olive processing (preservation, darkening, packing + sterilization) and storage on the volatile composition of two olive cultivars (Manzanilla and Hojiblanca). The preservation step enriched the volatile profile of the olives, mainly in ethyl acetate, methyl acetate, and ethanol. The darkening step produced the total or partial elimination of 55-65% of the volatiles identified before this step. Around 70% of the volatiles in the final products corresponded to compounds that were formed or increased significantly as a result of the sterilization treatment at 121 ºC. Although differences in the volatile compositions and contents between Manzanilla and Hojiblanca were found, the dominant volatiles in both cultivars were benzaldehyde, dimethyl sulfide and ethyl acetate. Storage for 8 months had little influence on their volatile profiles, although the stability of individual volatiles in Manzanilla was better than that in the Hojiblanca cultivar.

Keywords: black ripe olives, processing, storage, volatile composition, Manzanilla cultivar, Hojiblanca cultivar

Highlights

- The changes in volatiles during the processing of black ripe olives were studied.
- The preservation step increased the total amount and number of volatiles.
- The darkening step produced considerable losses in the volatile composition.
- The sterilization treatment led to a noticeable enrichment in volatile compounds.
- Storage for 8 months had little effect on the volatile profile of black ripe olives.

1. Introduction

Black ripe olives, which are also known as Californian-style black olives or olives darkened by oxidation, are one of the most important types of table olives commercialized worldwide. This processing style accounts for 40-50% of all table olives exported from Spain, the world's main producer and exporter of table olives (ASEMESA, 2019). Once in the production plant, the olives, mostly in the green and cherry stages of ripening, are directly processed or (more commonly) preserved before oxidation (Sánchez, García, & Rejano, 2006). Preservation is usually done in brine or an acidified solution (de Castro, García, Romero, Brenes, & Garrido, 2007). Once the stored fruits are sorted and graded, they are treated with one to several lye solutions (1-4% NaOH, w/v). Between lye treatments, the fruits are suspended in water through which air is bubbled. During this operation, the fruits darken progressively. After darkening, the olives are washed several times with water to remove most of the residual lye and lower the pH in the flesh to around 8 units, and then placed in 0.1% (w/v) of ferrous gluconate or ferrous lactate to maintain their black color. Finally, the olives are packed with a brine containing 2-4% NaCl and 10-40 mg L^{-1} of iron (added as ferrous gluconate or ferrous lactate) to prevent deterioration of their black color, and heat sterilized (generally at 121-126 ºC). To ensure microbiological stability, a minimum value for accumulated lethality of $15F_0$ should be reached (IOOC, 2004). Volatile compounds give rise to the aroma of food products and, therefore, are important factors in consumer perception and acceptance of foods. It is well known that processing technology greatly influences the volatile composition of olives and,

consequently, the flavor of the final product (Sabatini & Marsilio, 2008). In recent years, studies on the volatile composition of table olives have been relatively numerous (Bleve et al., 2015; Cano-Lamadrid et al., 2015; Cortés-Delgado et al., 2016; De Angelis et al., 2015; de Castro, Sánchez, Cortés-Delgado, López-López, & Montaño, 2019; de Castro et al., 2018; López-López, Sánchez, Cortés-Delgado, de Castro, & Montaño, 2018; Martorana et al., 2017; Randazzo et al., 2017; Sánchez et al., 2017, 2018; Selli, Kelebek, Kesen, & Sonmezdag, 2018). However, although optimal processing conditions and microbial spoilage have been extensively studied in black ripe olives, literature concerning volatile composition is almost nonexistent for this product. In fact, to the best of our knowledge, only one publication on this subject has been found to date (Sansone-Land, Takeoka, & Shoemaker, 2014). In that publication, the authors found clear differences among the volatile compositions of black ripe olives produced in different countries, which could be attributed to a number of factors, including olive cultivar, location of growth, processing method, storage conditions after processing, and age of the samples (that is, the length of storage after packing). As a consequence, it is not surprising that differences in aroma and flavor characteristics among black ripe olives from different countries (Lee, Kitsawad, Sigal, Flynn, & Guinard, 2012) and cultivars (López-López, Sánchez, Montaño, Cortés-Delgado, & Garrido-Fernández, 2019) have been found.

The technique of solid-phase microextraction (SPME) followed by gas chromatography-mass spectrometry (GC-MS) is frequently used in the field of food analysis for a variety of purposes, including aroma profiling, determination of contaminants, chemical fingerprinting, metabolomics investigations, and determination

of nutraceutical values, among various other applications (Reyes-Garcés et al., 2018). In the case of table olives, SPME-GC-MS has been successfully applied to study the volatile composition of unfermented "Campo Real" table olives (Navarro, De Lorenzo, & Pérez, 2004), Greek-style table olives (Bleve et al., 2015; De Angelis et al., 2015; Randazzo et al., 2017), "alcaparras" table olives (Malheiro, de Pinho, Casal, Bento, & Pereira, 2011), and Spanish-style table olives (Cano-Lamadrid et al., 2015; Cortés-Delgado et al., 2016; de Castro et al., 2018, 2019; López-López et al., 2018; Martorana et al., 2017; Sánchez et al., 2017, 2018).

In this work we have used SPME-GC-MS to study the changes in volatile components during the typical Californian-style processing with the aim of determining the origin of each volatile compound in the final product and its stability during storage. The study was carried out with olives of the Hojiblanca and Manzanilla cultivars grown in the same location and processed using the same processing method. In that way, the effect of the olive cultivar on its volatile composition was also disclosed.

2. Materials and methods

2.1. Black ripe olive processing

Olives (Manzanilla and Hojiblanca cultivars) were grown at Lora de Estepa (Seville province, Spain) and harvested at their mature-green stage. Fifteen kg of each cultivar were preserved in 25 L PVC vessels for 3 months with an acidified solution containing 2.4% (v/v) acetic acid before processing. Then the olives were subjected to darkening

following the habitual procedure for the elaboration of black ripe olives. Briefly, olives were treated in a horizontal stainless steel cylindrical container (0.4m diameter, 0.7m length) with a lye solution of 3% (w/v) NaOH, which progressively penetrated the flesh until the alkali reached the pit. Next, the lye was removed and the olives were washed (2 water washings, each of 24 h duration) until the pH reached 8.0. During the washing treatment, additions of 3N HCl and CO_2 injections into the containers were carried out to neutralize the excess NaOH. During the lye treatment and washing, air was injected through the bottom of the container. Then, a 0.1% (w/v) ferrous gluconate solution was added to fix the black color. The darkened olives were packed in "A314" glass bottles (145 g of olives plus 170 mL of brine capacity) and covered with a 3.5% (w/v) NaCl solution containing 0.02% (w/v) ferrous gluconate as cover brine, and the bottles were subjected to sterilization for 17 min at 121 °C in a computer-controlled retort (Steriflow, SAS, Paris, France). This sterilization process was previously calculated to achieve the recommended value of accumulated lethality ($15F_0$). The sterilized packed olives were stored at room temperature.

2.2. Sampling

After 3 months of preservation in 2.4% acetic acid, samples of the preservation liquids from the two vessels were taken for analyses of physico-chemical and microbiological characteristics. Analyses of volatile compounds in olive pulp were performed before preservation (fresh green olives), after 3 months of preservation, after the darkening step, after packing plus sterilization, and after 8 months of storage. The initial sampling

of the packed product was performed after 15 days (and not immediately after sterilization treatment) to ensure that the required equilibrium inside the bottles had been reached.

2.3. Physicochemical and microbiological analyses

The pH, titratable acidity, and combined acidity were measured following the routine procedures used in our laboratories (Cortés-Delgado et al., 2016). Microbiological analyses for monitoring the populations of yeasts, acetic acid bacteria and lactic acid bacteria were carried out according to de Castro et al. (2007).

2.4. Analysis of volatile compounds

The volatile compounds were extracted using the same procedure as reported by Cortés-Delgado et al. (2016), except that the extraction temperature and extraction time were set at 40 ºC and 30 min, respectively. Olives (approximately 150 g) were pitted and then homogenized in a blender at room temperature (22-24 ºC). An aliquot (2.5 g) of homogenized pulp was placed in a 15 mL glass vial, and 7.5 mL of 30% (w/v) NaCl were added. The vial was closed and placed in a water bath adjusted to 40 ºC for 15 min in order to reach equilibrium. The extraction was performed by exposing a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) StableFlex fiber (1 cm, 50/30 µm; Supelco, Bellefonte, PA) in the headspace of the sample at 40 ºC for 30 min. The samples were gently vortexed during equilibration and extraction at 600 rpm

using a magnetic stirrer. After extraction, the fiber was removed from the vial and immediately inserted into the injection port of the GC for desorption at 250 ºC for 15 min. All analyses of the volatile compounds were made in triplicate. GC-MS analyses were performed following the procedure reported by Cortés-Delgado et al. (2016). The GC peak area of each compound was obtained from the ion extraction chromatogram (IEC) by selecting target ions for each one. These ions corresponded to base ions (m/z 100% intensity), molecular ions (M+) or another characteristic ion for each molecule. Hence, some peaks that could be co-eluted in scan mode can be integrated with a value of resolution greater than 1. Compound identification was based on mass spectra matching with the standard NIST 08 MS library and on the comparison of retention indices (RI) sourced from the NIST Standard Reference Database and from authentic reference standards when available.

2.5. Statistical analyses

All the data were compiled and calculated using a combination of Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA) and XLSTAT v. 2016 (Addinsoft, Paris, France). To compare the effect of each processing step and storage on the individual volatiles (peak areas) the paired Student´s *t*-test was used. Significant differences were determined at the $p < 0.05$ level.

3. Results and discussion

The identified headspace volatile compounds and their average peak areas after each processing step and after 8 months of storage are shown in Tables S1 and S2 for Manzanilla and Hojiblanca cultivars, respectively. A total of 62 and 76 volatile compounds were identified in Manzanilla and Hojiblanca olives, respectively. The volatile components included alcohols, carbonyl compounds, esters, heterocyclic compounds, hydrocarbons, phenols, terpenes, organosulfur compounds, acids, and ethers. Both in Manzanilla and Hojiblanca, the total peak area of identified volatile compounds showed significant changes during processing and storage (Figure 1). The changes in chemical classes of the identified volatiles, except acids, are shown in Figure 2. Some differences in the volatile composition and content between the two cultivars were noticed considering qualitative and quantitative results, as mentioned below.

3.1. Volatile compounds in fresh fruit

The chemical composition of fresh olive headspace from both cultivars was predominated by alcohols and carbonyl compounds (Figure 2). Regarding individual volatile compounds, 21 compounds were identified in the Manzanilla cultivar, whereas 28 volatiles were identified in Hojiblanca. The major volatiles were: (*Z*)-3-hexen-1-ol (17.8% in Manzanilla, 33% in Hojiblanca); (*E*)-2-hexenal (16%, 16%), 3-hexenal (sum of two isomers, 16.1%, 12.9%), 1-penten-3-one (9.8%, 8.9%), and 1-penten-3-ol (14%, 6.4%) (Tables 1 and 2). All of these compounds along with other minor C6 and C5 compounds (such as hexanal, 1-hexanol, and 1-pentanol) are known to be formed by endogenous olive enzymes through the lipoxygenase (LOX) pathway from linoleic and

linolenic acids (Ridolfi, Terenziani, Patumi, & Fontanazza, 2002). These enzymes may be activated during sample preparation for volatile analysis as a result of the operations of pitting and homogenization.

Since the degree of ripening, harvesting period, geographical origin, and extraction conditions (including the temperature of homogenization and the extraction temperature) were similar for the two olive cultivars in the present study, the different outcomes can be attributed solely to their varietal background, which results in different volatile compositions. Thus, the alcohols 1-pentanol, (*E*)-2-penten-1-ol, (*E*)-3-hexen-1-ol, and (*E*)-2-hexen-1-ol, all of them derived by LOX action as well as benzyl alcohol, (*E*,*E*)-2,4-heptadienal, pseudocumene, and (*Z*)-3-hexenyl methyl ether were found in Hojiblanca only. In fact, the total peak area from the C5 and C6 compounds derived by LOX action (data not shown) was higher in Hojiblanca compared to Manzanilla. This would indicate that Hojiblanca showed a higher enzymatic activity, as there appears to be a direct relationship between the quantity of C5 and C6 volatile compounds (aldehydes, alcohols, and ketones) and LOX activity in the olives (Ridolfi et al., 2002). In addition, the presence of (*E*,*E*)-2,4-heptadienal could be due to autoxidation reactions which did not occur in Manzanilla, presumably due to its higher content of natural antioxidants (e.g. phenolic compounds) in comparison with Hojiblanca (Ramírez, Medina, García, Brenes, & Romero, 2017). The presence of (*Z*)-3-hexenyl methyl ether (around 1%) in Hojiblanca is also worth noting. To the best of our knowledge, the occurrence of ethers in olives has not been reported in the literature to date. Further research is needed to know whether this ether could be a potential marker candidate to differentiate the Hojiblanca cultivar from other olive cultivars.

3.2. Volatile compounds in olives after the preservation step

After preservation in acid solution for 3 months, an increase in total peak area from headspace volatile compounds (without considering acetic acid) was found for both cultivars compared to fresh fruit (Figure 1), with esters and alcohols comprising the most abundant chemical classes (Figure 2). A total of 29 and 34 volatiles were identified after preservation in Manzanilla and Hojiblanca, respectively, with ethyl acetate (28%, 29%), ethanol (6.8%, 6.9%), and methyl acetate (4.3%, 3.9%) being the dominant volatiles (Tables 1 and 2). These compounds appeared during the preservation period, presumably as a result of microbial action. Yeasts and acetic acid bacteria were the only microorganisms detected in the liquid medium after preservation. This was observed in the two cultivars and the microbial counts were quite similar in both cases (Table S3). Lactic acid bacteria were not detected in any case. Consequently, ethanol was likely produced by yeast fermentation exclusively. Acetic acid bacteria are known for their ability to partially oxidize ethanol (to produce acetic acid) and a variety of sugars (Mamlouk & Gullo, 2013). Acetate esters could be synthesized by an alcohol-acyl-transferase which catalyzes the esterification of volatile alcohols with acetyl-CoA molecules (derived from acetic acid and coenzyme A) to produce volatile esters and free CoA-SH (Sabatini & Marsilio, 2008).

In addition to the above-mentioned compounds, other volatiles were formed during preservation, although in lesser amounts. Among them, octane, 1-octanol, heptanal, octanal and nonanal are worth mentioning, as they can be considered indicators of lipid

oxidation and appeared as a result of the preservation step both in Manzanilla and Hojiblanca (Tables S1 and S2). The unsaturated aldehydes (*E*)-2-octenal and (*E*)-2-decenal, other typical oxidation volatiles, were formed in Manzanilla olives but were not detected in Hojiblanca. This finding appeared to indicate a higher susceptibility to lipid oxidation in the case of the Manzanilla cultivar during the preservation step in acid solution, which is in agreement with our previous results in case of black ripe olive processing (López-López, Rodríguez-Gómez, Cortés-Delgado, Montano, & Garrido-Fernández, 2009) or Spanish-style green table olive processing (Cortés-Delgado et al., 2016; Sánchez et al., 2018). This explanation is in contrast with that mentioned for raw olives in the previous section. During the preservation step, the content of natural antioxidants such as polyphenols in olive pulp gradually decreases (Ramírez, Medina, García, Brenes, & Romero, 2017). As a consequence, the protective effect of polyphenols against lipid oxidation reactions could be less effective compared to raw olives. Octane, 1-octanol and nonanal could be formed by the breakdown of oleato 9/10-OOH, heptanal and (*E*)-2-octenal by the decomposition of linoleato 11-OOH, octanal from oleate 11-OOH, and (*E*)-2-decenal from oleato 9-OOH (Frankel, 1983). Phenylethyl alcohol, phenylacetaldehyde, benzaldehyde, and (*Z*)-3-hexenyl acetate increased significantly as a result of the preservation stage in both cultivars (TablesS1 and S2). Phenylethyl alcohol and phenylacetaldehyde could be formed by yeasts from L-phenylalanine *via* the Ehrlich pathway (Eshkol et al., 2009). In this metabolic pathway, L-phenylalanine is transaminated to phenylpyruvate by a transaminase, decarboxylated to phenylacetaldehyde by a decarboxylase, and subsequently reduced to phenylethyl alcohol by a dehydrogenase. Benzaldehyde could be produced by the

chemical oxidation of the microbially produced phenylpyruvate, although this reaction appears to be enhanced at alkaline pH in the presence of oxygen (Nierop Groot & de Bont, 1998). Since the pH of olives during the preservation step was about 4 (Table S3), it is more likely that benzaldehyde could be enzymatically produced by a peroxidase. Tzika, Sotiroudis, Papadimitriou, & Xenakis (2009) extracted and characterized a peroxidase from olives (cv. Koroneiki) and found that its pH optimum values ranged between 4.0 and 6.0. (*Z*)-3-hexenyl acetate could be synthesized by esterification of (*Z*)-3-hexen-1-ol with acetyl-CoA, which is supported by a significant decrease in the peak area of (*Z*)-3-hexen-1-ol (Tables S1 and S2).

On the other hand, most alcohols and carbonyl compounds formed by the LOX pathway in fresh olives were undetected or their concentrations decreased considerably after the preservation step in both cultivars (Tables S1 and S2), which could be attributed to the degradation or inactivation of endogenous enzymes (e.g. acyl hydrolase, lipoxygenase, and hydroperoxide lyase) due to the acidic pH of olive pulp after preservation (pH ≈ 4, Table S3). Ridolfi et al. (2002) found almost no activity of olive lipoxygenases at pH 4.

3.3. Volatile compounds in olives after the darkening step

As a result of the darkening step (which included lye treatment + air oxidation, neutralization, and color fixation) there was a considerable decrease in the total peak area of headspace volatile compounds (Figure 1). Regardless of the cultivar, hydrocarbons and carbonyl compounds were the dominant chemical families in the profile of olives after this step (Figure 2). The number of volatiles identified after

darkening was 29 and 34 for Manzanilla and Hojiblanca cultivars, respectively, with octane (56.0%, 15.4%), 3-methylbutanal (10.1%, 12%), and 2-methylbutanal (10%, 7.6%) comprising the most prominent compounds in both cultivars (Tables 1 and 2). Toluene, *p*-xylene, (*Z*)-3-hexenyl methyl ether, and (*Z*)-3-hexenyl acetate were also relatively abundant (> 5%) in Hojiblanca olives.

Both in Manzanilla and Hojiblanca olives, only a few volatiles appeared (2-methylbutanal, 3-methylbutanal, 3-ethylpyridine, 3-ethyl-4-methylpyridine) or increased significantly (octane, benzaldehyde) as a result of darkening step (Tables S1 and S2). The darkening process in black ripe olives has been mainly related to chemical oxidation browning reactions, involving the oxidation of natural *o*-diphenols in olives to *o*-quinones, followed by the transformation of *o*-quinones into different dark compounds (Marsilio, Campestre, & Lanza, 2001). Secondary reactions of the *o*-quinones can include the Strecker degradation of amino acids at room temperature (Rizzi, 2006). Thus, the formation of the Strecker aldehydes 2-methylbutanal and 3-methylbutanal (derived from the amino acids isoleucine and leucine, respectively) is an indication that such reactions induced by polyphenol oxidation occur during the darkening step of black ripe olives. The presence of the above-mentioned alkylpyridines is surprising, as pyridines are normally found in foods which have undergone thermal treatments. We confirmed this result by analyzing samples from two other elaborations of black ripe olives carried out in our laboratories (data not shown). Pyridines in foods are formed by the condensation reaction of aldehydes, ketones, α,β-unsaturated carbonyl compounds, or various derivatives of such compounds with ammonia or amino acids (Suyama & Adachi, 1980).

As mentioned above, octane is a secondary oxidation product from the oxidation of oleic acid. However, the formation of or significant increases in other typical oxidation products such as 1-octanol, octanal, nonanal, (*E*)-2-octenal, and (*E*)-2-decenal were not found. This is explained by the fact that their formation might be counteracted by their degradation during lye treatment or due to the effect of Fe^{2+} (used to fix the black color) which is oxidized to Fe^{3+} while reducing the primary oxidation products. In fact, a marked reduction in K_{270} (parameter related to the formation of trienes and other secondary oxidation products) of ripe olive fat during the darkening step has been previously reported (López-López et al., 2009). Benzaldehyde could be formed from benzyl alcohol by oxidation, which is supported by a significant decrease in the peak area of benzyl alcohol in both cultivars (Tables S1 and S2). Phenylaceldehyde is another precursor that is able to form benzaldehyde by oxidation (Chu & Yaylayan, 2008), and this is also supported by a significant decrease in (Manzanilla) or elimination of (Hojiblanca) the corresponding peak area. However, no evidence of further benzaldehyde oxidation to form benzoic acid was found, as this acid was not detected after darkening.

Regarding the compounds that were totally or partially eliminated during the darkening step, it is worth mentioning that acetic acid, isopentanol, and the esters methyl acetate and ethyl acetate disappeared from both cultivars; whereas (*Z*)-3-hexenyl acetate, hexanal, 1-hexanol, (*Z*)-3-hexen-1-ol, benzyl alcohol, and phenylethyl alcohol decreased significantly (fold change 1.3-7.1, Tables S1 and S2). Other compounds which were totally or partially lost were ethanol, phenylacetaldehyde, methyl salicylate, and dimethyl sulfide. Among the different reaction mechanisms that explain the loss of

volatiles during the darkening step we can mention neutralization (e.g. acetic acid due to lye treatment), hydrolysis (e.g. acetate esters due to lye treatment), and oxidation by air (e.g. ethanol to yield acetaldehyde, dimethyl sulfide to form dimethyl sulfoxide, which can be further oxidized to dimethyl sulfone, and benzyl alcohol to form benzaldehyde, as mentioned above). However, losses by other mechanisms (evaporation, leaching into the surrounding liquid) cannot be ruled out.

3.4. Volatile compounds in olives after the packing step

The packing step (which included the addition of fresh cover brine and a sterilization treatment) caused a significant increase in the total peak area of headspace volatile compounds compared to data before packing (Figure 1). In both cultivars, there were significant increases in most chemical classes including carbonyl compounds, esters, heterocyclic compounds, and organosulfur compounds, whereas only hydrocarbons decreased; and carbonyl compounds comprised the predominant chemical class after packing (Figure 2). Regarding individual compounds, 47 and 55 volatiles were identified after the packing of Manzanilla and Hojiblanca olives, respectively, with benzaldehyde (16%, 36%), dimethyl sulfide (16%, 11%), and ethyl acetate (10%, 12%) being the most abundant compounds (Tables 1 and 2). The aldehydes 2-methylbutanal and 3-methylbutanal were also relatively abundant in both cultivars (3.8-8.2%).
Most of the volatiles identified after packing corresponded to compounds that were formed or increased significantly in their concentrations, mostly due to the heat treatment of sterilization. With regards to the volatiles formed (> 40% of the volatiles

identified) both in Hojiblanca and Manzanilla olives (Tables S1 and S2), we can highlight: 2-methylpropanal, 2-butenal, pentanal, 2-ethyl-2-butenal, (E,E)-2,4-heptadienal, 4-ethylbenzaldehyde, (E,E)-2,4-decadienal, (E)-cinnamaldehyde, methyl acetate, ethyl acetate, methyl nicotinate, methyl 2-formylbenzoate, furfural, 3-vinylpyridine, vanillin, 6-methyl-5-hepten-2-one, and dimethyl sulfoxide. Among these compounds, the highest production corresponded to ethyl acetate (10%, 12%) followed by 3-vinylpyridine (2.6%, 4.2%) (Tables 1 and 2). The formation of aldehydes such as 2-butenal, pentanal, (E,E)-2,4-heptadienal, and (E,E)-2,4-decadienal would be indicative of oxidation reactions from linoleic and linolenic acids (Frankel, 1983). Other aldehydes formed as secondary oxidation products of fatty acids were 2-heptenal, (E)-2-octenal, and (E)-2-decenal, which were formed in Hojiblanca or increased significantly in Manzanilla (Tables S1 and S2). It is noteworthy that 2-vinyl-2-butenal was formed in a relatively high amount in Manzanilla (8.8%), but this aldehyde was not detected in Hojiblanca olives.

A higher number of pyridines were detected in Hojiblanca ripe olives compared to Manzanilla ripe olives. Thus, pyridine and 3-methylpyridine were formed in Hojiblanca, but not in Manzanilla. As mentioned above, pyridines could be formed by the condensation reaction of aldehydes, ketones, α,β-unsaturated carbonyl compounds, or various derivatives of such compounds with ammonia or amino acids (Suyama & Adachi, 1980). These heterocyclic compounds, specifically, 3-ethylpyridine, 3-vinylpyridine and 3-ethyl-4-methylpyridine have been previously identified in commercial black ripe olives (Sansone-Land et al., 2014).

Furfural could be formed as an intermediate product of the Maillard reaction when pentoses are involved (Martins, Jongen, & van Boekel, 2001). Furfuryl alcohol was found in Hojiblanca olives, but was not detected in Manzanilla. This furfural derivative might be formed during storage (it must be taken into account that sampling after packing was performed after 15 days of storage) from the reduction of furfural in the presence of Fe^{2+}, as supported by the changes found in furfural and furfuryl alcohol at longer storage, as mentioned later.

Vanillin could be derived from the thermal and oxidative breakdown of ferulic acid (Fiddler, Parker, Wasserman, & Doerr, 1967). 6-Methyl-5-hepten-2-one is regarded as a marker compound for the degradation of carotenoids (Cremer & Eichner, 2000). Dimethyl sulfoxide could be formed through the oxidation of dimethyl sulfide, as mentioned above.

Apart from the volatiles formed, more than 25% of the identified volatiles corresponded to compounds which significantly increased their concentration as a result of packing (Tables S1 and S2). Thus, in both cultivars, there was a significant increase in the peak areas of the following volatiles: 2-methylbutanal, 3-methylbutanal, benzaldehyde, hexanal, octanal, 3-ethylpyridine, 3-ethyl-4-methylpyridine, and phenol. The increase in the Strecker aldehydes 2-methylbutanal and 3-methylbutanal is another indication that the Maillard reaction occurred during the sterilization treatment (Cremer & Eichner, 2000); whereas the increase in hexanal and octanal would be related to the oxidation of linoleic and oleic acids, respectively. The noticeable increases in 3-ethylpyridine (with fold changes of 2.6 and 5.9 for Manzanilla and Hojiblanca, respectively) and 3-ethyl-4-methylpyridine (fold changes of 3.8 and 10.8), along with the formation of other

pyridines as mentioned above, confirm that high temperatures play an important role in their formation.

Among the above-mentioned volatiles, benzaldehyde merits a more detailed discussion. It is striking that the amount of benzaldehyde increased greatly with packing (fold changes of 25.7 and 79.1 for Manzanilla and Hojiblanca, respectively). Benzaldehyde was previously found in "Campo Real" unfermented table olives, a typical and specialist product from Comunidad de Madrid (Spain), when olives were subjected to a sterilization treatment (Navarro et al., 2004). Benzaldehyde could arise through the degradation of phenylpyruvate, which may be generated chemically from phenylalanine. The enzymatic generation of phenylpyruvate suggested in the preservation step (section 3.2) would not be possible in the packing step due to the sterilization treatment. It has been demonstrated that in the presence of appropriate carbonyl compounds (e.g. 4,5-epoxy-2-decenal, which has been identified as a secondary product of thermally oxidized trilinolein; Frankel, 1983), phenylalanine can undergo transamination and generate phenylpyruvic acid, which is easily oxidized to produce benzaldehyde and phenylacetaldehyde (Zamora, Navarro, Gallardo, & Hidalgo, 2006). In turn, phenylacetaldehyde could be oxidized to produce benzaldehyde according to a free radical initiated oxidative mechanism, as proposed by Chu and Yaylayan (2008).

Interestingly, benzoic acid was detected in Hojiblanca olives only. Benzoic acid could be formed from benzaldehyde oxidation. Apparently, the rate of oxidation of benzaldehyde to benzoic acid was higher in Hojiblanca (which contained 35%

benzaldehyde) than in Manzanilla (16% benzaldehyde), indicating first order dependence with substrate.

Another volatile that was formed (Hojiblanca) or increased significantly (Manzanilla) with packing, presumably due to the sterilization treatment, was dimethyl sulfide. This compound may be produced from the thermal degradation of *S*-methylmethionine (Cerny, 2015). However, references reporting the presence of this non-proteinogenic amino acid in olives have not been found.

3.5. Volatile compounds in olives after storage

The storage of black ripe olives for 8 months did not significantly change (Manzanilla) or slightly increased (Hojiblanca) the total peak area of headspace volatile compounds (Figure 1). Carbonyl compounds, which did not significantly change during storage, remained as the predominant chemical class after 8 months' storage of both cultivars (Figure 2). Regarding individual compounds, the qualitative composition of volatiles hardly varied during storage. As found after packing, the most abundant volatiles in both cultivars after storage were benzaldehyde, ethyl acetate, dimethyl sulfide, 2-methylbutanal and 3-methylbutanal (Tables 1 and 2). All of these compounds remained unchanged during the storage of Manzanilla olives, except ethyl acetate which increased significantly, whereas all of them increased in Hojiblanca, except benzaldehyde which remained unchanged (Tables S1 and S2). In fact, some 49% of the volatiles identified in Manzanilla black ripe olives remained stable during storage, whereas 21% remained unchanged in Hojiblanca. Among the minor volatiles, it is worth mentioning the

decrease in furfural, presumably due to its reduction to furfuryl alcohol, as supported by the corresponding increase in this alcohol found in Hojiblanca olives (Table S2). In addition, other aldehydes such as octanal, 2-heptenal, (E)-2-octenal, and (E)-2-decenal, which were originated mostly as a result of the sterilization treatment, were significantly decreased with the storage of both cultivars. It could be due to the reductive effect of Fe^{2+} (from the ferrous gluconate added), as mentioned above. It is also worth noting that acetic acid, which was not detected after packing, was found after 8 months' storage of Manzanilla olives but not Hojiblanca. This might be attributed to the acidification of olive pulp during storage, as indicated by the increasing values of titratable acidity and concomitant decreases in pH (Table S4), presumably due to pectin degradation. It is known that non-enzymatic β-elimination and demethoxylation reactions which occur during the storage of low-acid fruits and vegetables produce a decrease in pH (García-García, Sánchez-Gómez, & Garrido-Fernández, 2014). As a result, the pH reached 4.9 (a value close to the pK_a of acetic acid) in Manzanilla olives, which increased the concentration of the undissociated form (i.e., the volatile form) of acetic acid, but the pH remained above 6 in Hojiblanca.

4. Conclusions

The present study determined the impact of each step of black ripe olive processing on the volatile composition of two Spanish olive cultivars (Manzanilla and Hojiblanca). The volatiles were analyzed by means of headspace gas chromatography (HS-GC) and solid-phase microextraction (SPME) followed by gas chromatography-mass

spectrometry (GC-MS). Among the volatile compounds identified after the preservation step, around 60% corresponded to compounds formed during this step, mostly through enzymatic and lipid oxidation reactions; whereas the remaining compounds corresponded to olive-derived compounds (i.e. initially detected in the fresh olives). Ethyl acetate, methyl acetate, and ethanol were the dominant volatiles after the preservation step (without taking into account the acetic acid added to the preservation solution). The darkening step had a noticeable impact on the headspace volatile profile of olives, with 55% (Manzanilla) or 65% (Hojiblanca) of the volatiles being totally or partially eliminated as a result of this step. 2-Methylbutanal, 3-methylbutanal, 3-ethylpyridine, and 3-ethyl-4-methylpyridine were formed in both cultivars, and octane and benzaldehyde increased, during darkening. Around 70% of the identified constituents in the final products (i.e., packed and sterilized olives from Manzanilla or Hojiblanca cultivars) corresponded to compounds that were formed or increased significantly, likely due to different chemical reactions occurring during the sterilization treatment at 121 ºC (e.g. the Maillard reaction, oxidative degradation reactions of fatty acids and amino acids, condensation reactions of carbonyl compounds with ammonia or amino acids, and thermal degradation of other components of olives such as carotenoids and phenolic acids). Among these compounds both in Manzanilla and Hojiblanca ripe olives, the dominant headspace volatile compounds were benzaldehyde, dimethyl sulfide, and ethyl acetate. However, differences in the volatile composition and content between the two final products were found. The storage step for 8 months at room temperature had little effect on the volatile profile of black ripe olives in general,

although the Manzanilla cultivar showed better behavior with regards to the stability of individual volatiles.

Acknowledgements

This work was supported by the Ministry of Economy and Competitiveness from the Spanish government through Project AGL2014-54048-R, partially financed by the European Regional Development Fund (ERDF).

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Figure captions

Fig.1. Total peak area of headspace volatile compounds during processing and storage of black ripe olives from Manzanilla and Hojiblanca cultivars. Error bars indicate 95% confidence intervals. Peak area of acetic acid after preservation step was not considered.

Fig. 2. Changes in chemical classes of the headspace volatile compounds during processing and storage of black ripe olives from Manzanilla and Hojiblanca cultivars. Error bars indicate 95% confidence intervals. For clarity, acids were not included.

Supplementary material

Table S1: Volatile compounds identified and changes in their peak areas during processing and storage of black ripe olives (Manzanilla cultivar)

Table S2: Volatile compounds identified and changes in their peak areas during processing and storage of black ripe olives (Hojiblanca cultivar)

Table S3: Physico-chemical and microbiological characteristics of the preservation liquid of olives (Manzanilla and Hojiblanca cultivars) after 3 months of preservation

Table S4: Changes in physico-chemical characteristics of packing brines from Manzanilla and Hojiblanca ripe olives due to storage time

Table 1. Relative contents of volatile compounds during processing and storage of black ripe olives (Manzanilla cultivar)

	Content (% of total area of identified compounds)				
Compound	Fresh fruit[a]	After preservation[a]	After darkening[a]	After packing[b]	After storage[b]
Acetic acid	n.d.	58 ± 3	n.d.	n.d.	0.9 ± 0.2
Ethanol	n.d.	6.8 ± 0.1	3.2 ± 0.2	1.0 ± 0.1	0.74 ± 0.06
1-Penten-3-ol	14 ± 1	n.d.	n.d.	n.d.	n.d.
Isopentanol	n.d.	0.14 ± 0.01	n.d.	n.d.	n.d.
(*Z*)-2-Penten-1-ol	4.9 ± 0.4	n.d.	n.d.	n.d.	n.d.
1-Hexanol	0.88 ± 0.03	0.098 ± 0.006	0.55 ± 0.07	0.15 ± 0.02	0.13 ± 0.01
(*Z*)-3-Hexen-1-ol	17.8 ± 0.2	0.19 ± 0.02	0.74 ± 0.07	0.18 ± 0.02	0.15 ± 0.02
1-Heptanol	n.d.	n.d.	n.d.	0.07 ± 0.02	0.08 ± 0.03
1-Octanol	n.d.	0.020 ± 0.003	0.28 ± 0.05	0.12 ± 0.02	0.10 ± 0.01
Benzyl alcohol	n.d.	0.021±0.003	0.17 ± 0.02	0.051±0.008	0.060±0.006
Phenylethyl alcohol	0.36 ± 0.09	0.13 ± 0.01	0.38 ± 0.08	0.13 ± 0.04	0.15 ± 0.01
2-Methylpropanal	n.d.	n.d.	n.d.	0.8 ± 0.1	0.9 ± 0.1
2-Methylbutanal	n.d.	n.d.	10 ± 1	5.4 ± 0.4	6.2 ± 0.5
3-Methylbutanal	n.d.	n.d.	10.1 ± 0.7	8.2 ± 0.5	9.5 ±0.6
Pentanal	n.d.	n.d.	n.d.	1.0 ± 0.1	1.2 ± 0.2
3-Pentanone	2.5 ± 0.6	n.d.	n.d.	n.d.	n.d.
1-Penten-3-one	9.8 ± 0.5	n.d.	n.d.	n.d.	n.d.

2-Butenal	n.d.	n.d.	n.d.	3.2 ± 0.8	1.1 ± 0.2
Hexanal	0.43 ± 0.04	0.04 ± 0.02	0.22 ± 0.03	0.14 ± 0.03	0.17 ± 0.02
(*E*)-2-Pentenal	2.8 ± 0.3	n.d.	n.d.	n.d.	n.d.
3-Hexenal	16.1 ± 0.8	n.d.	n.d.	n.d.	n.d.
2-Ethyl-2-butenal	n.d.	n.d.	n.d.	0.70 ± 0.05	0.72 ± 0.09
Heptanal	n.d.	0.06 ± 0.01	0.25 ± 0.02	0.14 ± 0.03	0.14 ± 0.02
(*E*)-2-Hexenal	16 ± 1	n.d.	n.d.	n.d.	n.d.
2-Vinyl-2-butenal	n.d.	n.d.	n.d.	8.8 ± 0.9	1.05 ± 0.08
Octanal	n.d.	0.05 ± 0.03	0.5 ± 0.1	0.36 ± 0.01	0.21 ± 0.05
2-Heptenal	n.d.	n.d.	0.82 ± 0.08	0.9 ± 0.1	0.43 ± 0.06
Nonanal	n.d.	0.04 ± 0.02	0.4 ± 0.1	0.17 ± 0.03	0.15 ± 0.03
(*E*,*E*)-2,4-Hexadienal	4.1 ± 0.6	n.d.	n.d.	n.d.	n.d.
(*E*)-2-Octenal	n.d.	0.04 ± 0.01	0.32 ± 0.03	0.18 ± 0.02	0.11 ± 0.02
(*E*,*E*)-2,4-Heptadienal	n.d.	n.d.	n.d.	0.28 ± 0.03	0.11 ± 0.02
Benzaldehyde	0.25 ± 0.09	0.07 ± 0.01	1.8 ± 0.2	16 ± 1	16 ± 2
Phenylacetaldehyde	2.1 ± 0.4	0.35 ± 0.04	1.9 ± 0.2	3.0 ± 0.5	1.1 ± 0.4
(*E*)-2-Decenal	n.d.	0.19 ± 0.02	2.0 ± 0.2	0.90 ± 0.09	0.6 ± 0.1
4-Ethylbenzaldehyde	n.d.	n.d.	n.d.	0.07 ± 0.01	0.06 ± 0.01
2-Cyclohexene-1,4-dione	0.7 ± 0.1	n.d.	n.d.	n.d.	n.d.
(*E*,*E*)-2,4-Decadienal	n.d.	n.d.	n.d.	0.13 ± 0.02	0.11 ± 0.02
(*E*)-Cinnamaldehyde	n.d.	n.d.	n.d.	0.06 ± 0.01	0.06 ± 0.02
2-Phenyl-2-butenal	n.d.	n.d.	n.d.	n.d.	0.06 ± 0.01
Methyl acetate	n.d.	4.3 ± 0.4	n.d.	0.9 ± 0.2	1.5 ± 0.1
Ethyl acetate	n.d.	28 ± 3	n.d.	10 ± 2	16 ± 1

Methyl butanoate	0.5 ± 0.2	n.d.	n.d.	n.d.	n.d.
Hexyl acetate	n.d.	0.024 ± 0.001	0.39 ± 0.05	n.d.	n.d.
(*Z*)-3-Hexenyl acetate	1.97 ± 0.09	0.45 ± 0.02	3.2 ± 0.4	0.8 ± 0.1	0.53 ± 0.05
Methyl nicotinate	n.d.	n.d.	n.d.	0.27 ± 0.04	0.24 ± 0.04
Methyl salicylate	0.20 ± 0.06	0.018 ± 0.003	n.d.	n.d.	n.d.
Methyl 2-formylbenzoate	n.d.	n.d.	n.d.	1.12 ± 0.09	0.8 ± 0.2
3-Ethylpyridine	n.d.	n.d.	1.4 ± 0.2	1.3 ± 0.2	0.58 ± 0.09
Furfural	n.d.	n.d.	n.d.	1.5 ± 0.2	1.0 ± 0.1
3-Vinylpyridine	n.d.	n.d.	n.d.	2.6 ± 0.3	1.6 ± 0.2
3-Ethyl-4-methylpyridine	n.d.	n.d.	0.41 ± 0.07	0.55 ± 0.07	0.28 ± 0.06
Octane	n.d.	0.58 ± 0.05	56.0 ± 0.3	10 ± 2	17 ± 4
Toluene	n.d.	0.05 ± 0.01	1.7 ± 0.2	0.4 ± 0.1	0.5 ± 0.1
p-Xylene	n.d.	0.03 ± 0.01	0.8 ± 0.1	0.20 ± 0.04	0.25 ± 0.05
o-Xylene	n.d.	0.016 ± 0.003	0.49 ± 0.07	0.14 ± 0.04	0.12 ± 0.02
Phenol	1.6 ± 0.2	0.048 ± 0.008	0.40 ± 0.03	0.17 ± 0.03	0.18 ± 0.02
Vanillin	n.d.	n.d.	n.d.	0.10 ± 0.02	0.33 ± 0.07
6-Methyl-5-hepten-2-one	n.d.	n.d.	n.d.	0.33 ± 0.02	0.19 ± 0.05
Copaene	0.50 ± 0.06	0.043 ± 0.002	0.7 ± 0.1	0.24 ± 0.02	0.26 ± 0.05
β-Damascenone	n.d.	0.012 ± 0.002	0.19 ± 0.04	0.06 ± 0.01	0.057 ± 0.007
Dimethyl sulfide	3.7 ± 0.3	0.65 ± 0.05	0.73 ± 0.09	16 ± 1	16 ± 3
Dimethyl sulfoxide	n.d.	n.d.	n.d.	0.45 ± 0.06	0.6 ± 0.3

[a] Values are means ± standard deviation (n=3).
[b] Values are means ± standard deviation (n=9; three bottles analyzed in triplicate).
n.d. = not detected

Table 2. Relative contents of volatile compounds during processing and storage of black ripe olives (Hojiblanca cultivar)

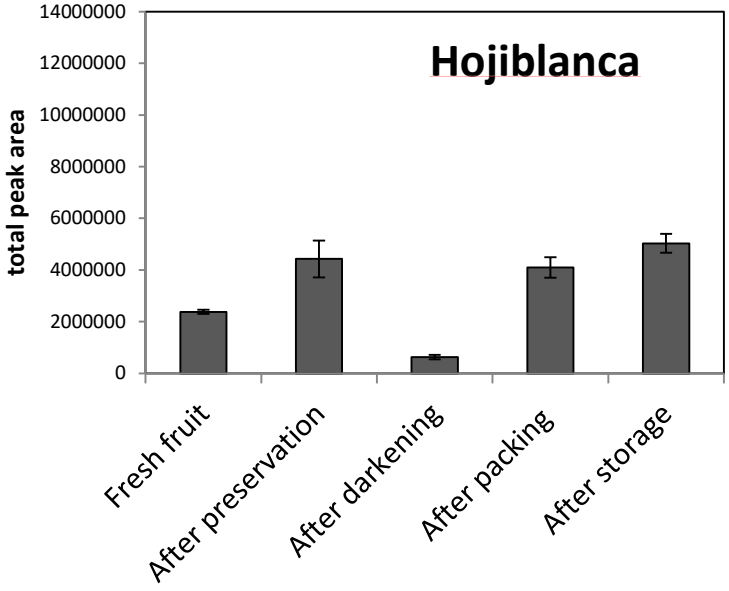
	Content (% of total area of identified compounds)				
	Fresh fruit[a]	After preservation[a]	After darkening[a]	After packing[b]	After storage[b]
Acetic acid	n.d.	54 ± 5	n.d.	n.d.	n.d.
Benzoic acid	n.d.	n.d.	n.d.	0.7 ± 0.2	0.28 ± 0.05
Ethanol	n.d.	6.9 ± 0.7	n.d.	1.7 ± 0.4	1.6 ± 0.1
1-Penten-3-ol	6.4 ± 0.1	n.d.	n.d.	n.d.	n.d.
Isopentanol	n.d.	0.15 ± 0.04	n.d.	n.d.	0.11 ± 0.02
1-Pentanol	0.14 ± 0.02	n.d.	0.31 ± 0.07	0.08 ± 0.01	0.12 ± 0.01
(*E*)-2-Penten-1-ol	0.04 ± 0.01	n.d.	n.d.	n.d.	n.d.
(*Z*)-2-Penten-1-ol	2.24 ± 0.08	n.d.	n.d.	n.d.	n.d.
1-Hexanol	2.72 ± 0.02	0.16 ± 0.01	0.84 ± 0.04	0.10 ± 0.01	0.11 ± 0.01
(*E*)-3-Hexen-1-ol	0.17 ± 0.02	n.d.	n.d.	n.d.	n.d.
(*Z*)-3-Hexen-1-ol	33 ± 1	0.23 ± 0.03	1.35 ± 0.04	0.16 ± 0.01	0.11 ± 0.01
(*E*)-2-Hexen-1-ol	0.14 ± 0.02	n.d.	n.d.	n.d.	n.d.
1-Heptanol	n.d.	n.d.	n.d.	n.d.	0.05 ± 0.01
1-Octanol	n.d.	0.02 ± 0.00	0.33 ± 0.04	0.09 ± 0.01	0.06 ± 0.01
Benzyl alcohol	0.14 ± 0.02	0.05 ± 0.00	0.49 ± 0.01	0.12 ± 0.01	0.11 ± 0.01
Phenylethyl alcohol	0.32 ± 0.02	0.17 ± 0.01	0.8 ± 0.1	0.17 ± 0.03	0.17 ± 0.02
2-Methylpropanal	n.d.	n.d.	n.d.	0.6 ± 0.2	0.90 ± 0.04
2-Methylbutanal	n.d.	n.d.	7.6 ± 0.9	3.8 ± 0.4	5.7 ± 0.4
3-Methylbutanal	n.d.	n.d.	12 ± 2	6.9 ± 0.8	7.9 ± 0.9

Pentanal	n.d.	n.d.	n.d.	1.3 ± 0.2	1.3 ± 0.3
3-Pentanone	1.50 ± 0.08	n.d.	n.d.	n.d.	n.d.
1-Penten-3-one	8.9 ± 0.3	n.d.	n.d.	n.d.	n.d.
2-Butenal	n.d.	n.d.	n.d.	0.93 ± 0.06	1.0 ± 0.2
Hexanal	2.1 ± 0.2	0.13 ± 0.04	0.29 ± 0.01	0.10 ± 0.04	0.08 ± 0.01
(*E*)-2-Pentenal	3.6 ± 0.2	n.d.	n.d.	n.d.	n.d.
2-Ethyl-2-butenal	n.d.	n.d.	n.d.	0.85 ± 0.08	0.92 ± 0.08
3-Hexenal	12.9 ± 0.9	n.d.	n.d.	n.d.	n.d.
Heptanal	n.d.	0.08 ± 0.02	0.50 ± 0.00	0.09 ± 0.03	0.08 ± 0.01
(*E*)-2-Hexenal	16 ± 1	n.d.	n.d.	n.d.	n.d.
Octanal	n.d.	0.07 ± 0.00	0.45 ± 0.08	0.26 ± 0.03	0.13 ± 0.04
2-Heptenal	n.d.	n.d.	n.d.	0.6 ± 0.1	0.3 ± 0.2
Nonanal	n.d.	0.07 ± 0.02	1.6 ± 0.4	0.17 ± 0.04	0.11 ± 0.01
(*E,E*)-2,4-Hexadienal	3.1 ± 0.3	n.d.	n.d.	n.d.	n.d.
(*E*)-2-Octenal	n.d.	n.d.	n.d.	0.10 ± 0.01	0.03 ± 0.01
(*E,E*)-2,4-Heptadienal	0.29 ± 0.04	n.d.	n.d.	0.34 ± 0.05	n.d.
Benzaldehyde	0.23 ± 0.02	0.12 ± 0.02	3.0 ± 0.4	36 ± 2	30 ± 2
Phenylacetaldehyde	0.9 ± 0.1	0.79 ± 0.03	n.d.	n.d.	n.d.
(*E*)-2-Decenal	n.d.	n.d.	n.d.	0.36 ± 0.06	0.12 ± 0.04
4-Ethylbenzaldehyde	n.d.	n.d.	n.d.	0.32 ± 0.04	0.21 ± 0.02
2-Cyclohexene-1,4-dione	0.50 ± 0.03	n.d.	n.d.	n.d.	n.d.
(*E,E)*-2,4-Decadienal	n.d.	n.d.	n.d.	0.27 ± 0.05	0.12 ± 0.02
(*E*)-Cinnamaldehyde	n.d.	n.d.	n.d.	0.24 ± 0.07	0.12±0.01
2-Phenyl-2-butenal	n.d.	n.d.	n.d.	n.d.	0.15 ± 0.02

Methyl acetate	n.d.	3.9 ± 0.7	n.d.	1.2 ± 0.3	1.7 ± 0.1
Ethyl acetate	n.d.	29 ± 4	n.d.	12 ± 2	18.0 ± 0.7
Methyl butanoate	0.16 ± 0.01	n.d.	n.d.	n.d.	n.d.
Hexyl acetate	n.d.	0.07 ± 0.01	0.85 ± 0.09	0.08 ± 0.02	0.06 ± 0.01
(*Z*)-3-Hexenyl acetate	1.0 ± 0.1	1.26 ± 0.08	5.6 ± 0.1	0.57 ± 0.09	0.48 ± 0.04
Methyl nicotinate	n.d.	n.d.	n.d.	0.11 ± 0.02	0.08 ± 0.02
Methyl salicylate	0.33 ± 0.02	0.06 ± 0.01	0.19 ± 0.04	n.d.	n.d.
Ethyl salicylate	n.d.	0.08 ± 0.01	0.50 ± 0.03	n.d.	n.d.
Methyl 2-formylbenzoate	n.d.	n.d.	n.d.	0.54 ± 0.04	0.16 ± 0.03
Pyridine	n.d.	n.d.	n.d.	0.57 ± 0.07	0.54 ± 0.07
3-Methylpyridine	n.d.	n.d.	n.d.	1.5 ± 0.2	2.1 ± 0.3
3-Ethylpyridine	n.d.	n.d.	2.85 ± 0.08	2.6 ± 0.3	1.6 ± 0.2
Furfural	n.d.	n.d.	n.d.	1.6 ± 0.2	0.62 ± 0.05
3-Vinylpyridine	n.d.	n.d.	n.d.	4.2 ± 0.6	2.9 ± 0.4
3-Ethyl-4-methylpyridine	n.d.	n.d.	1.1 ± 0.1	1.8 ± 0.2	1.3 ± 0.2
Furfuryl alcohol	n.d.	n.d.	n.d.	0.09 ± 0.02	0.12 ± 0.01
Octane	n.d.	0.15 ± 0.01	15.4 ± 0.3	0.80 ± 0.09	2.0 ± 0.6
Toluene	n.d.	1.66 ± 0.09	12.0 ± 0.9	0.6 ± 0.1	0.27 ± 0.07
p-Xylene	n.d.	0.97 ± 0.02	8.3 ± 0.4	0.5 ± 0.1	0.6 ± 0.2
o-Xylene	n.d.	0.54 ± 0.04	4.5 ± 0.3	0.32 ± 0.08	0.37 ± 0.04
Styrene	n.d.	0.13 ± 0.02	2.5 ± 0.2	0.19 ± 0.05	0.3 ± 0.1
Pseudocumene	0.18 ± 0.01	0.27 ± 0.01	2.9 ± 0.1	0.25 ± 0.06	0.24 ± 0.04
p-Creosol	n.d.	0.03 ± 0.01	n.d.	n.d.	n.d.
Phenol	0.82 ± 0.06	0.09 ± 0.02	1.2 ± 0.2	0.38 ± 0.05	0.16 ± 0.01

Vanillin	n.d.	n.d.	n.d.	0.12 ± 0.01	0.18 ± 0.03
6-Methyl-5-hepten-2-one	n.d.	n.d.	n.d.	0.27 ± 0.04	0.15 ± 0.07
Copaene	0.28 ± 0.05	0.24 ± 0.04	3.1 ± 0.9	0.9 ± 0.2	0.57 ± 0.06
α-Muurolene	n.d.	0.01 ± 0.00	0.24 ± 0.08	0.08 ± 0.02	0.04 ± 0.01
β-Damascenone	n.d.	0.07 ± 0.02	1.2 ± 0.1	0.17 ± 0.04	0.13 ± 0.02
Dimethyl sulfide	1.1 ± 0.1	0.08 ± 0.02	n.d.	11 ± 2	13 ± 1
Dimethyl sulfoxide	n.d.	n.d.	n.d.	0.6 ± 0.2	0.38 ± 0.05
(*Z*)-3-Hexenyl methyl ether	0.91 ± 0.08	0.47 ± 0.03	7.5 ± 0.1	0.36 ± 0.06	0.47 ± 0.08
Benzyl methyl ether	n.d.	0.17 ± 0.01	1.5 ± 0.1	0.14 ± 0.02	0.11 ± 0.01

[a] Values are means ± standard deviation (n=3).
[b] Values are means ± standard deviation (n=9; three bottles analyzed in triplicate).
n.d. = not detected


Hojiblanca
total peak area
14000000
12000000
10000000
8000000
6000000
4000000
2000000
0
Fresh fruit
After preservation
After darkening
After packing
After storage

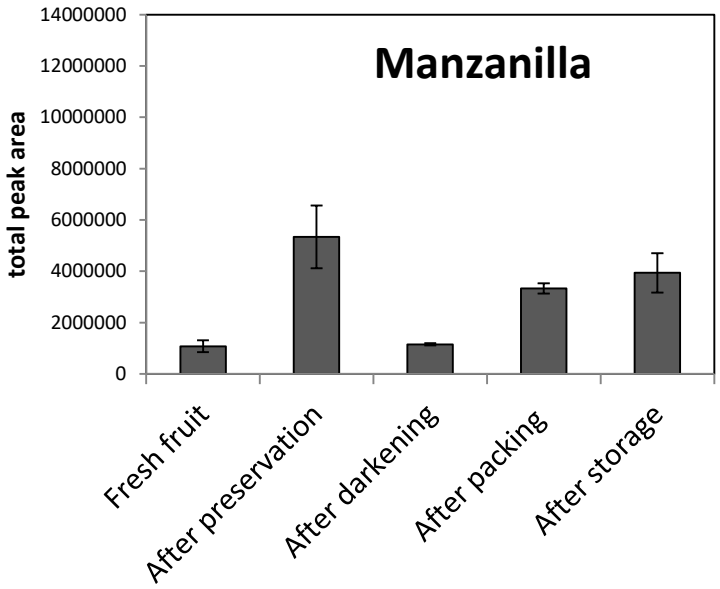

Manzanilla
total peak area
14000000
12000000
10000000
8000000
6000000
4000000
2000000
0
Fresh fruit
After preservation
After darkening
After packing
After storage

Figure 1

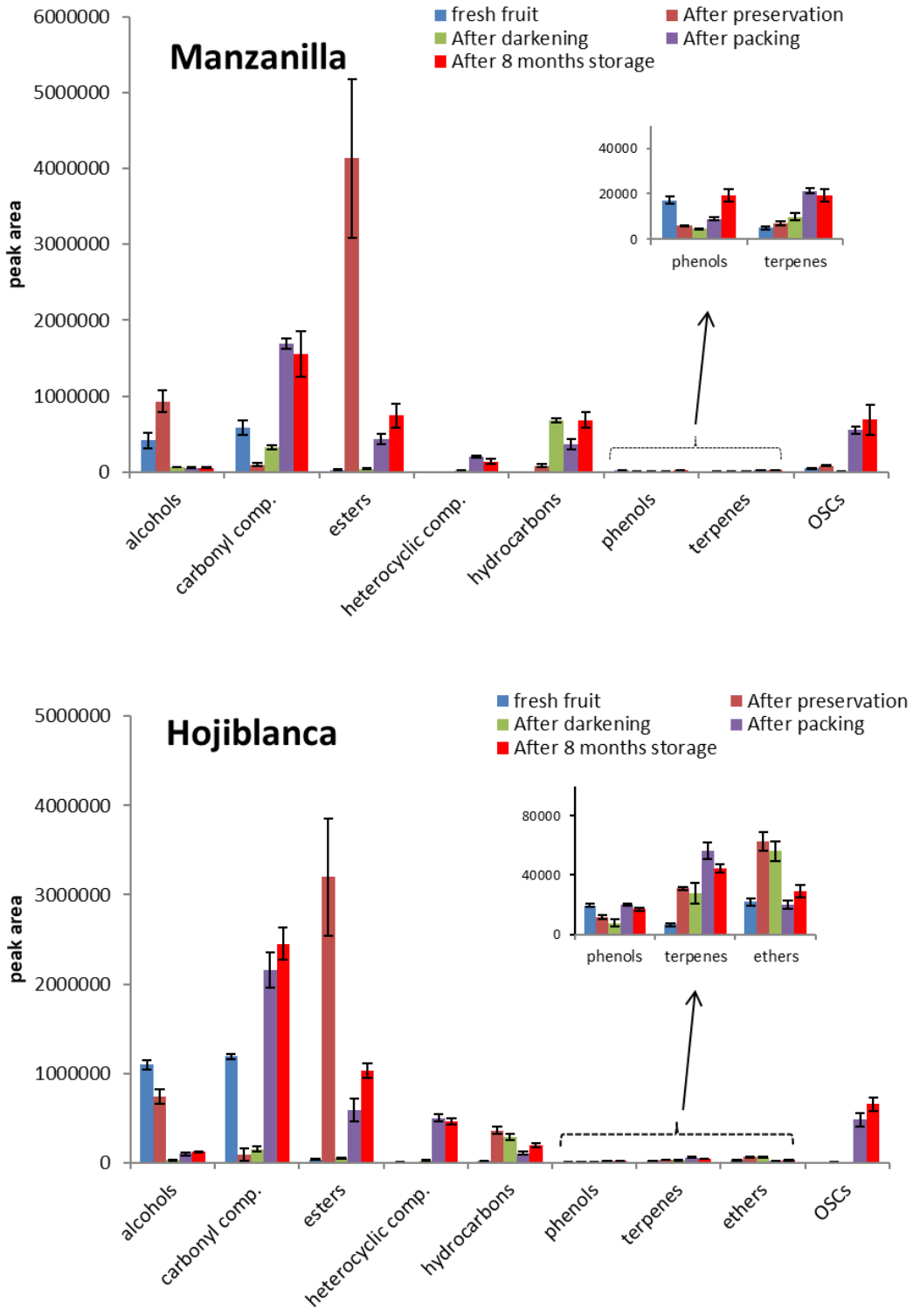

Manzanilla
fresh fruit
After preservation
After darkening
After packing
After 8 months storage
peak area
6000000
5000000
4000000
3000000
2000000
1000000
0
40000
20000
0
phenols
terpenes
alcohols
carbonyl comp.
esters
heterocyclic comp.
hydrocarbons
phenols
terpenes
OSCs
Hojiblanca
fresh fruit
After preservation
After darkening
After packing
After 8 months storage
peak area
5000000
4000000
3000000
2000000
1000000
0
80000
40000
0
phenols
terpenes
ethers
alcohols
carbonyl comp.
esters
heterocyclic comp.
hydrocarbons
phenols
terpenes
ethers
OSCs

Figure 2

Table S1. Volatile compounds identified and changes in their peak areas during processing and storage of black ripe olives (Manzanilla cultivar)

			Fresh fruit		After preservation		After darkening		After packing		After storage	
Compound	IEC (*m/z*) [a]	ID[b]	Average area	SD[c]	Average area	SD[c]	Average area	SD[c]	Average area	SD[d]	Average area	SD[d]
acetic acid	60	1	n.d.		7272375[e] (**F**)	783368	n.d. (**E**)		n.d.		42537 (**F**)	19819
ethanol	45	1	n.d.		851478 (**F**)	115348	36333 (↓**23.4**)	3374	33681 (**n.s.**)	2824	29523 (**n.s.**)	9570
1-penten-3-ol*	57	1	153537	39812	n.d. (**E**)		n.d.		n.d.		n.d.	
isopentanol	55	1	n.d.		17491 (**F**)	4017	n.d. (**E**)		n.d.		n.d.	
(Z)-2-penten-1-ol*	68	2	53461	13305	n.d. (**E**)		n.d.		n.d.		n.d.	
1-hexanol**	56	1	9618	1920	12358 (**n.s.**)	2154	6310 (↓**2.0**)	670	4946 (↓**1.3**)	500	5193 (**n.s.**)	1830
(Z)-3-hexen-1-ol*	67	1	193558	35757	23908 (↓**8.1**)	4451	8554 (↓**2.8**)	615	5960 (↓**1.4**)	648	6171 (**n.s.**)	2469
1-heptanol	70	1	n.d.		n.d.		n.d.		2298 (**F**)	475	3177 (↑**1.4**)	796
1-octanol	84	1	n.d.		2588 (**F**)	656	3257 (**n.s.**)	474	3976 (**n.s.**)	652	3720 (**n.s.**)	937
benzyl alcohol	108	1	n.d.		2616 (**F**)	166	1951 (↓**1.3**)	260	1690 (**n.s.**)	275	2405 (**n.s.**)	893
phenylethyl alcohol	91	1	3832	512	16120 (↑**4.2**)	835	4370 (↓**3.7**)	834	4397 (**n.s.**)	1170	5805 (**n.s.**)	2047
2-methylpropanal	72	2	n.d.		n.d.		n.d.		26994 (**F**)	4937	35104 (**n.s.**)	15310
2-methylbutanal	57	1	n.d.		n.d.		114628 (**F**)	19674	180914 (↑**1.6**)	25412	248270 (**n.s.**)	92275
3-methylbutanal	44	2	n.d.		n.d.		117381	12217	273577	25946	378148	126450

							(**F**)		(↑**2.3**)		(**n.s.**)	
Pentanal**	44	1	n.d.		n.d.		n.d.		33215 (**F**)	5779	43826 (↑**1.3**)	9274
3-pentanone**	86	2	26592	1995	n.d. (**E**)		n.d.		n.d.		n.d.	
1-penten-3-one*	84	1	106239	19243	n.d. (**E**)		n.d.		n.d.		n.d.	
2-butenal	70	1	n.d.		n.d.		n.d.		106049 (**F**)	23205	40958 (↓**2.6**)	5853
hexanal**	82	1	4665	1215	5575 (**n.s.**)	1431	2542 (↓**2.2**)	284	4582 (↑**1.8**)	895	6446 (↑**1.4**)	1646
(E)-2-pentenal*	55	1	30985	6716	n.d. (**E**)		n.d.		n.d.		n.d.	
3-hexenal*	69	2	165403	7967	n.d. (**E**)		n.d.		n.d.		n.d.	
2-ethyl-2-butenal	98	2	n.d.		n.d.		n.d.		23257 (**F**)	2151	29206 (**n.s.**)	10897
heptanal	70	1	n.d.		7927 (**F**)	3648	2841 (↓**2.8**)	329	4606 (↑**1.6**)	736	5373 (**n.s.**)	1447
(E)-2-hexenal*	83	1	171772	39327	n.d. (**E**)		n.d.		n.d.		n.d.	
2-vinyl-2-butenal	67	2	n.d.		n.d.		n.d.		291124 (**F**)	23095	40830 (↓**7.1**)	9883
octanal	84	1	n.d.		9228 (**F**)	3425	5601 (**n.s.**)	1121	11991 (↑**2.1**)	1260	8138 (↓**1.5**)	2019
2-heptenal	83	1	n.d.		n.d.		9464 (**F**)	1291	29948 (↑**3.2**)	3080	16296 (↓**1.8**)	3451
nonanal	98	1	n.d.		3075 (**F**)	846	4214 (**n.s.**)	1321	5643 (**n.s.**)	771	5607 (**n.s.**)	1296
(E,E)-2,4-hexadienal	81	1	43743	8801	n.d. (**E**)		n.d.		n.d.		n.d.	
(E)-2-octenal	55	1	n.d.		4552	721	3635	247	6025	613	4160	691

					(**F**)		(**n.s.**)		(↑**1.7**)		(↓**1.4**)	
(E,E)-2,4-heptadienal	81	1	n.d.		n.d.		n.d.		9262 (**F**)	801	3961 (↓**2.3**)	532
benzaldehyde	106	1	3505	756	8035 (↑**2.3**)	815	21239 (↑**2.6**)	1886	546418 (↑**25.7**)	27990	610795 (**n.s.**)	174837
phenylacetaldehyde	91	1	23877	8636	44496 (↑**1.9**)	6490	22061 (↓**2.0**)	2142	99334 (↑**4.5**)	18130	43158 (↓**2.3**)	15636
(E)-2-decenal	70	1	n.d.		23718 (**F**)	4911	22729 (**n.s.**)	2573	29967 (↑**1.3**)	2375	24005 (↓**1.2**)	4852
4-ethylbenzaldehyde	134	3	n.d.		n.d.		n.d.		2426 (**F**)	292	2580 (**n.s.**)	866
2-cyclohexene-1,4-dione	110	3	7741	89	n.d. (**E**)		n.d.		n.d.		n.d.	
(E,E)-2,4-decadienal	81	1	n.d.		n.d.		n.d.		4327 (**F**)	481	4140 (**n.s.**)	502
(E)-cinnamaldehyde	131	3	n.d.		n.d.		n.d.		2003 (**F**)	283	2672 (**n.s.**)	1419
2-phenyl-2-butenal	146	2	n.d		n.d		n.d		n.d		2308 (**F**)	676
methyl acetate	74	2	n.d.		541100 (**F**)	96956	n.d. (**E**)		30871 (**F**)	8131	58412 (↑**1.9**)	21114
ethyl acetate	43	1	n.d.		3530450 (**F**)	826437	n.d. (**E**)		331153 (**F**)	84785	624449 (↑**1.9**)	206344
methyl butanoate	87	1	5588	1477	n.d. (**E**)		n.d.		n.d.		n.d.	
hexyl acetate*	56	1	n.d.		3020 (**F**)	494	4442 (↑**1.5**)	442	n.d. (**E**)		n.d.	
(Z)-3-hexenyl acetate*	67	1	21461	4489	56569 (↑**2.6**)	7423	36393 (↓**1.6**)	3261	26391 (↓**1.4**)	3941	21035 (**n.s.**)	6222
methyl nicotinate	106	2	n.d.		n.d.		n.d.		8980 (**F**)	1532	9370 (**n.s.**)	3542
methyl salicylate	120	1	2044	350	2298	120	n.d.		n.d.		n.d.	

					(n.s.)		**(E)**					
methyl 2-formylbenzoate	133	3	n.d.		n.d.		n.d.		37264 **(F)**	2705	28267 **(↓1.3)**	4390
3-ethylpyridine	107	2	n.d.		n.d.		16593 **(F)**	1377	42661 **(↑2.6)**	3920	23452 **(↓1.8)**	10007
furfural	96	1	n.d.		n.d.		n.d.		49785 **(F)**	4774	37801 **(↓1.3)**	8755
3-vinylpyridine	105	3	n.d.		n.d.		n.d.		87706 **(F)**	6776	65059 **(↓1.3)**	23327
3-ethyl-4-methylpyridine	106	3	n.d.		n.d.		4724 **(F)**	689	18296 **(↑3.8)**	2147	11427 **(↓1.6)**	4796
octane	85	1	n.d.		73069 **(F)**	16320	645676 **(↑8.8)**	21989	340366 **(↓1.9)**	88368	648760 **(↑1.9)**	156230
toluene	91	1	n.d.		6245 **(F)**	1687	19157 **(↑3.1)**	1673	14404 **(↓1.3)**	2961	20549 **(↑1.4)**	4865
p-xylene	91	1	n.d.		2769 **(F)**	494	8904 **(↑3.2)**	1503	6770 **(↓1.3)**	1347	9405 **(↑1.4)**	1705
o-xylene	91	1	n.d.		1983 **(F)**	73	5716 **(↑2.9)**	710	4738 **(↓1.2)**	1259	4662 **(n.s.)**	1162
phenol	94	1	17206	1396	5974 **(↓2.9)**	150	4587 **(↓1.3)**	244	5624 **(↑1.2)**	679	6991 **(n.s.)**	2047
vanillin	151	1	n.d.		n.d.		n.d.		3350 **(F)**	944	12341 **(↑3.7)**	2226
6-methyl-5-hepten-2-one	108	1	n.d.		n.d.		n.d.		10962 **(F)**	977	7212 **(↓1.5)**	801
copaene	119	2	5021	488	5440 **(n.s.)**	729	7632 **(↑1.4)**	1221	8121 **(n.s.)**	732	9990 **(n.s.)**	3101
β-damascenone	121	1	n.d.		1537 **(F)**	241	2205 **(↑1.4)**	350	2087 **(n.s.)**	291	2223 **(n.s.)**	649
dimethyl sulfide	62	1	44637	5434	81732 **(↑1.3)**	8972	8810 **(↓9.3)**	1354	536737 **(↑60.9)**	53537	674150 **(n.s.)**	300373
dimethyl sulfoxide	63	1	n.d.		n.d.		n.d.		14936	1674	16791	11353

									(F)		**(n.s.)**	
∑ E					10		5		1		0	
∑ F					18		5		19		2	
∑ ↑					5		8		14		9	
∑ ↓					2		11		7		14	
∑ n.s.					4		5		7		24	
Number of identified volatiles (∑ F + ∑ ↑ + ∑ ↓ + ∑ n.s.)					29		29		47		49	

[a] Ion extraction chromatogram, *m/z* used to obtain the GC peak area of each compound.
[b] Identification: 1, identified, mass spectrum and RI were in accordance with standards; 2, tentatively identified, mass spectrum matched in the standard NIST 2008 library and RI matched with literature; 3, tentatively identified, mass spectrum agreed with the standard NIST 2008.
[c] Standard deviation (n=3).
[d] Standard deviation (n=9, 3 bottles analyzed in triplicate).
n.d. = not detected.
[e] Most acetic acid was from the preservation liquid used. Possible acetic acid formed by acetic acid bacteria was unknown.
In parenthesis, change of peak area of a given compound as a result of the processing step: E = elimination; F = formation; ↑ = significant increase ($p<0.05$); ↓ = significant decrease ($p<0.05$); n.s. = not significant change ($p > 0.05$).
*Compound produced in fresh olives by LOX action from linolenic acid.**Compound produced in fresh olives by LOX action from linoleic acid.

Table S2. Volatile compounds identified and changes in their peak areas during processing and storage of black ripe olives (Hojiblanca cultivar)

			Fresh fruit		After preservation		After darkening		After packing		After storage	
Compound	IEC (*m/z*) [a]	ID[b]	Average area	SD[c]	Average area	SD[c]	Average area	SD[c]	Average area	SD[d]	Average area	SD[d]
acetic acid	60	1	n.d.		5311790[e] (**F**)	1112149	n.d. (**E**)		n.d.		n.d.	
benzoic acid	105	1	n.d.		n.d.		n.d.		26563 (**F**)	6672	13888 (↓**1.9**)	1658
ethanol	45	1	n.d.		667130 (**F**)	59400	n.d. (**E**)		71001 (**F**)	19414	79511 (**n.s.**)	4310
1-penten-3-ol*	57	1	153220	5454	n.d. (**E**)		n.d.		n.d.		n.d.	
isopentanol	55	1	n.d.		15006 (**F**)	2576	n.d. (**E**)		n.d.		5680	1004
1-pentanol**	55	1	3304	572	n.d. (**E**)		1978 (**F**)	659	3356 (↑**1.7**)	533	5844 (↑**1.7**)	998
(E)-2-penten-1-ol*	86	2	892	144	n.d. (**E**)		n.d.		n.d.		n.d.	
(Z)-2-penten-1-ol*	68	2	53754	1115	n.d. (**E**)		n.d.		n.d.		n.d.	
1-hexanol**	56	1	65176	1530	15636 (↓**4.2**)	2136	5245 (↓**3.0**)	906	4195 (**n.s.**)	504	5441 (↑**1.3**)	666
(E)-3-hexen-1-ol*	67	2	4124	312	n.d. (**E**)		n.d.		n.d.		n.d.	
(Z)-3-hexen-1-ol*	67	1	801570	45006	22431 (↓**35.7**)	3669	8428 (↓**2.7**)	1143	6516 (↓**1.3**)	684	5582 (↓**1.2**)	784
(E)-2-hexen-1-ol*	82	2	3437	339	n.d. (**E**)		n.d.		n.d.		n.d.	
1-heptanol	70	1	n.d.		n.d.		n.d.		n.d.		2728 (**F**)	575
1-octanol	84	1	n.d.		2154	4	2067	506	3553	260	2762	394

					(**F**)		(**n.s.**)		(↑**1.7**)		(↓**1.3**)	
benzyl alcohol	108	1	3275	520	4842 (↑**1.5**)	350	3061 (↓**1.6**)	418	4690 (↑**1.5**)	496	5507 (↑**1.2**)	632
phenylethyl alcohol	91	1	7652	682	16912 (↑**2.2**)	2135	5133 (↓**3.3**)	677	6956 (↑**1.4**)	1196	8290 (↑**1.2**)	1102
2-methylpropanal	72	2	n.d.		n.d.		n.d.		23350 (**F**)	8055	45209 (↑**1.9**)	4747
2-methylbutanal	57	1	n.d.		n.d.		46823 (**F**)	5005	156245 (↑**3.3**)	26759	287997 (↑**1.8**)	48071
3-methylbutanal	44	2	n.d.		n.d.		73057 (**F**)	19173	281857 (↑**3.9**)	57034	398226 (↑**1.4**)	79697
pentanal**	44	1	n.d.		n.d.		n.d.		54722 (**F**)	12084	64359 (**n.s.**)	13688
3-pentanone**	86	2	35997	2797	n.d. (**E**)		n.d.		n.d.		n.d.	
1-penten-3-one*	82	2	212264	8094	n.d. (**E**)		n.d.		n.d.		n.d.	
2-butenal	70	1	n.d.		n.d.		n.d.		37795 (**F**)	4915	49385 (↑**1.3**)	10648
hexanal**	82	1	49405	5115	12756 (↓**3.9**)	6121	1793 (↓**7.1**)	371	3820 (↑**2.1**)	1101	3879 (**n.s.**)	839
(E)-2-pentenal*	55	1	85913	4668	n.d. (**E**)		n.d.		n.d.		n.d.	
2-ethyl-2-butenal	98	2	n.d.		n.d.		n.d.		34243 (**F**)	4325	46277 (↑**1.4**)	8213
3-hexenal*	69	2	307210	28151	n.d. (**E**)		n.d.		n.d.		n.d.	
heptanal	70	1	n.d.		8385 (**F**)	3128	3065 (**n.s.**)	532	3362 (**n.s.**)	1006	4069 (**n.s.**)	788
(E)-2-hexenal*	83	1	377158	16098	n.d. (**E**)		n.d.		n.d.		n.d.	
octanal	84	1	n.d.		6803	1243	2777	948	10590	1797	6318	1627

					(**F**)		(**n.s.**)		(↑**3.8**)		(↓**1.7**)	
2-heptenal	83	1	n.d.		n.d.		n.d.		26063 (**F**)	6018	16318 (↓**1.6**)	8365
nonanal	98	1	n.d.		7000 (**F**)	1718	9876 (**n.s.**)	3135	6804 (**n.s.**)	1055	5717 (↓**1.2**)	680
(E,E)-2,4-hexadienal	81	1	74437	4565	n.d. (**E**)		n.d.		n.d.		n.d.	
(E)-2-octenal	55	1	n.d.		n.d.		n.d.		3984 (**F**)	494	1472 (↓**2.7**)	603
(E,E)-2,4-heptadienal	81	1	6794	1163	n.d. (**E**)		n.d.		13915 (**F**)	2140	n.d. (**E**)	
benzaldehyde	106	1	5441	719	11712 (↑**2.2**)	1912	18383 (↑**1.6**)	2472	1453748 (↑**79.1**)	162202	1491048 (**n.s.**)	147487
phenylacetaldehyde	91	1	22655	3232	77383 (↑**3.4**)	12668	n.d. (**E**)		n.d.		n.d.	
(E)-2-decenal	70	1	n.d.		n.d.		n.d.		14521 (**F**)	3291	5715 (↓**2.5**)	1769
4-ethylbenzaldehyde	134	3	n.d.		n.d.		n.d.		12717 (**F**)	1431	10539 (↓**1.2**)	1359
2-cyclohexene-1,4-dione	110	3	11933	422	n.d. (**E**)		n.d.		n.d.		n.d.	
(E,E)-2,4-decadienal	81	1	n.d.		n.d.		n.d.		11063 (**F**)	2097	5980 (↓**1.9**)	943
(E)-cinnamaldehyde	131	3	n.d.		n.d.		n.d.		9468 (**F**)	2318	5820 (↓**1.6**)	515
2-phenyl-2-butenal	146	2	n.d.		n.d.		n.d.		n.d.		7360 (**F**)	1347
methyl acetate	74	2	n.d.		352778 (**F**)	101979	n.d. (**E**)		49675 (**F**)	15633	82915 (↑**1.7**)	7297
ethyl acetate	43	1	n.d.		2821549 (**F**)	561253	n.d. (**E**)		490998 (**F**)	140914	908550 (↑**1.9**)	111322
methyl butanoate	87	1	3740	216	n.d.		n.d.		n.d.		n.d.	

					(**E**)							
hexyl acetate*	56	1	n.d.		6673 (**F**)	578	5267 (↓**1.3**)	248	3371 (↓**1.6**)	776	3245 (**n.s.**)	518
(Z)-3-hexenyl acetate*	67	1	23274	3636	122227 (↑**5.3**)	9822	34783 (↓**3.5**)	5036	23235 (↓**1.5**)	4724	24189 (**n.s.**)	2201
methyl nicotinate	106	2	n.d.		n.d.		n.d.		4484 (**F**)	527	3850 (**n.s.**)	903
methyl salicylate	120	1	7918	740	5625 (↓**1.4**)	334	1178 (↓**4.8**)	188	n.d. (**E**)		n.d.	
ethyl salicylate	120	1	n.d.		7383 (**F**)	384	3098 (↓**2.4**)	238	n.d. (**E**)		n.d.	
methyl 2-formylbenzoate	133	3	n.d.		n.d.		n.d.		22118 (**F**)	3320	8066 (↓**2.7**)	1264
pyridine	79	2	n.d.		n.d.		n.d.		22855 (**F**)	1690	26968 (↑**1.2**)	3970
3-methylpyridine	93	2	n.d.		n.d.		n.d.		60797 (**F**)	11723	103532 (↑**1.7**)	13556
3-ethylpyridine	107	2	n.d.		n.d.		17769 (**F**)	2560	105062 (↑**5.9**)	14171	79899 (↓**1.3**)	12367
furfural	96	1	n.d.		n.d.		n.d.		64137 (**F**)	4607	31405 (↓**2.0**)	4291
3-vinylpyridine	105	3	n.d.		n.d.		n.d.		170329 (**F**)	21245	148106 (**n.s.**)	23146
3-ethyl-4-methylpyridine	106	3	n.d.		n.d.		6616 (**F**)	1321	71767 (↑**10.8**)	8252	66204 (**n.s.**)	9643
furfuryl alcohol	98	2	n.d.		n.d.		n.d.		3427 (**F**)	728	6159 (↑**1.8**)	549
octane	85	1	n.d.		14875 (**F**)	1526	95766 (↑**6.4**)	13503	32307 (↓**3.0**)	4173	104071 (↑**3.2**)	34155
toluene	91	1	n.d.		160832 (**F**)	17403	74530 (↓**2.2**)	8037	24565 (↓**3.0**)	6178	13208 (↓**1.9**)	2864
p-xylene	91	1	n.d.		94636	12022	51572	6193	18623	5561	30961	9245

					(**F**)		(↓**1.8**)		(↓**2.8**)		(↑**1.7**)	
o-xylene	91	1	n.d.		52072 (**F**)	4698	27890 (↓**1.9**)	1997	12910 (↓**2.2**)	3048	18590 (↑**1.4**)	1713
styrene	104	1	n.d.		12794 (**F**)	2322	15534 (**n.s.**)	2023	7408 (↓**2.1**)	1591	15904 (↑**2.1**)	5654
pseudocumene	105	1	4206	118	26327 (↑**6.3**)	2742	18126 (↓**1.5**)	1759	10003 (↓**1.8**)	2523	11829 (**n.s.**)	1537
p-creosol	138	1	n.d.		2908 (**F**)	483	n.d. (**E**)		n.d.		n.d.	
phenol	94	1	19659	799	8956 (↓**2.2**)	896	7745 (**n.s.**)	2355	15324 (↑**2.0**)	907	7802 (↓**2.0**)	646
vanillin	151	1	n.d.		n.d.		n.d.		4859 (**F**)	698	9140 (↑**1.9**)	1509
6-methyl-5-hepten-2-one	108	1	n.d.		n.d.		n.d.		11214 (**F**)	2606	7218 (↓**1.6**)	3199
copaene	119	2	6594	1105	22746 (↑**3.4**)	246	18911 (**n.s.**)	5866	35255 (↑**1.9**)	5876	28708 (↓**1.2**)	2498
α-muurolene	161	1	n.d.		1258 (**F**)	83	1490 (**n.s.**)	572	3174 (↑**2.1**)	524	2160 (↓**1.5**)	339
β-damascenone	121	1	n.d.		6905 (**F**)	850	7552 (**n.s.**)	459	6931 (**n.s.**)	1232	6450 (**n.s.**)	855
dimethyl sulfide	62	1	26333	3698	7905 (↓**3.3**)	1585	n.d. (**E**)		453865 (**F**)	91650	636912 (↑**1.4**)	114145
dimethyl sulfoxide	63	1	n.d.		n.d.		n.d.		25079 (**F**)	4990	19296 (↓**1.3**)	3888
(Z)-3-hexenyl methyl ether	82	2	21959	2248	46315 (↑**2.1**)	5019	46638 (**n.s.**)	5383	14556 (↓**3.2**)	2802	23712 (↑**1.6**)	6194
benzyl methyl ether	121	2	n.d.		16289 (**F**)	1015	9558 (↓**1.7**)	513	5620 (↓**1.7**)	654	5582 (**n.s.**)	784
∑ E					15		8		2		1	
∑ F					20		8		26		3	

∑ I					8		2		14		21	
∑ D					6		14		11		20	
∑n.s.					0		10		4		13	
Number of identified volatiles (∑ F + ∑ I + ∑ D + ∑n.s.)					34		34		55		57	

[a] Ion extraction chromatogram, *m/z* used to obtain the GC peak area of each compound.
[b] Identification: 1, identified, mass spectrum and RI were in accordance with standards; 2, tentatively identified, mass spectrum matched in the standard NIST 2008 library and RI matched with literature; 3, tentatively identified, mass spectrum agreed with the standard NIST 2008.
[c] Standard deviation (n=3).
[d] Standard deviation (n=9, 3 bottles analyzed in triplicate).
n.d. = not detected.
[e] Most acetic acid was from the preservation liquid used. Possible acetic acid formed by acetic acid bacteria was unknown.
In parenthesis, change of peak area of a given compound as a result of the processing step: E = elimination; F = formation; ↑ = significant increase ($p<0.05$); ↓ = significant decrease ($p<0.05$); n.s. = not significant change ($p > 0.05$).
*Compound produced in fresh olives by LOX action from linolenic acid.**Compound produced in fresh olives by LOX action from linoleic acid.

Table S3. Physico-chemical and microbiological characteristics of the preservation liquid of olives (Manzanilla and Hojiblanca cultivars) after 3 months of preservation

	Manzanilla	Hojiblanca
Physico-chemical parameter		
pH	4.00	3.85
Titratable acidity (% acetic acid)	1.49	1.40
Combined acidity (N)	0.074	0.066
Microbial population (log cfu/mL)		
Lactic acid bacteria	n.d.	n.d.
Yeasts	4.93	4.64
Acetic acid bacteria	4.85	4.74

n.d.= not detected (log cfu/mL < 1.3)

Table S4. Changes in physico-chemical characteristics of packing brines from Manzanilla and Hojiblanca ripe olives due to storage time[a]

Physico-chemical parameter	Manzanilla ripe olives		Hojiblanca ripe olives	
	Initial[b]	8 months storage	Initial[b]	8 months storage
pH	6.12 ± 0.05	4.92 ± 0.27	6.71 ± 0.02	6.13 ± 0.19
Titratable acidity (g L^{-1} lactic acid)	0.50 ± 0.00	2.80 ± 0.26	0.33 ± 0.06	0.77 ± 0.23
Combined acidity (eq L^{-1})	0.037 ± 0.002	0.037 ± 0.001	0.054 ± 0.002	0.061 ± 0.007
Salt (g L^{-1} NaCl)	19.9 ± 0.7	17.5 ± 0.8	18.8 ± 0.6	16.3 ± 0.3

[a] Values are means ± SD of three replicate bottles

[b] After 15 days of storage