Effect of post-fermentation and packing stages on the volatile composition of Spanish-style green table olives

Antonio Higinio Sánchez, Antonio López-López, Amparo Cortés-Delgado, Víctor Manuel Beato, Eduardo Medina, Antonio de Castro, Alfredo Montaña*

Food Biotechnology Department, Instituto de la Grasa-CSIC, Pablo Olavide University
Campus, building 46, Utrera road, km 1, 41013 Seville, Spain

*Tel.: +34 954611550, fax: +34 954616790, e-mail corresponding author (A. Montaña):
amontano@ig.csic.es

E-mail addresses for co-authors:

Antonio Higinio Sánchez: ahiginio@ig.csic.es

Antonio de Castro: amillan@cica.es

Antonio López-López: all@cica.es

Amparo Cortés-Delgado: acortes@cica.es

Víctor Manuel Beato: vmbeagal@ig.csic.es

Eduardo Medina: emedina@ig.csic.es

Running title: Volatile composition of Spanish-style green table olives

Abstract

The volatile profile of Spanish-style green table olives after fermentation and the changes in volatile compounds that occurred as a result of the post-fermentation and subsequent packing stage were explored by solid phase micro-extraction (SPME) and gas chromatography coupled to mass spectrometry (GC-MS). Three olive cultivars (Manzanilla, Gordal, and Hojiblanca) were processed and olive samples were taken at three different times throughout the elaboration: after fermentation, after post-fermentation, and after packing. A total of 132 volatile compounds were identified, including 10 phenols, 25 alcohols, 11 acids, 39 esters, 8 hydrocarbons, 14 carbonyl compounds, 17 terpenes, and 6 other compounds. A varying number of compounds from each chemical family underwent significant changes because of the post-fermentation and packing stages. Among them, some typical reaction products of lipid oxidation (e.g. (E)-2-decenal and (E,E)-2,4-decadienal) increased with the post-fermentation in Manzanilla cultivar, and also as a result of packing in all three cultivars.

Keywords: table olives; fermentation; post-fermentation; packing; olive cultivar; volatile compounds; SPME; GC-MS

41 **Highlights**

- 42 ● More than 130 compounds were identified in Spanish-style green table olives.
- 43 ● The contents of volatiles were affected by post-fermentation and packing stages.
- 44 ● Significant differences in volatile compounds between olive cultivars were found.
- 45 ● **Lipid oxidation likely occurred as a result of packing in the cultivars studied.**

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1. Introduction

Among the different types of table olives commercialized worldwide, the Spanish-style green table olive is especially valued for its unique and pleasant flavor. This vegetable product is considered the main fermented vegetable product in western countries. Its processing consists of a treatment with alkaline lye (1.8-2.5%, w/v NaOH) to hydrolyze the bitter glucoside oleuropein, followed by a washing step to remove the excess alkali. A solution of NaCl (10-13%, w/v) is added, and a lactic acid fermentation takes place (Rejano, Montaña, Casado, Sánchez, & de Castro, 2010). Although research about processing and the nutritional composition of the final product has been intense in the past 20 years, surprisingly there are relatively few published studies about olive flavor, the most critical attribute of food quality. The flavor of table olives is closely related to both the qualitative and quantitative composition of volatile **and non-volatile** compounds, and can be influenced by a number of factors, including olive cultivar, fruit ripeness stage, and processing method (Sabatini and Marsilio, 2008).

A few studies have been published on volatile composition of Spanish-style green olives, all of them being carried out after several months of brining once olives were totally fermented (Montaña, Sánchez, & Rejano, 1990; Iraqi, Vermeulen, Benzekri, Bouseta, & Collin, 2005; Sabatini & Marsilio, 2008; Cano-Lamadrid et al., 2015; Cortés-Delgado, Sánchez, de Castro, López-López, Beato, & Montaña, 2016). At the industry, after fermentation, fruits are usually kept in the fermenter until they are marketed either in bulk with their own fermenting brine or packed in small containers. Post-fermentation time in the fermenter can last several months, including summer, a time which is frequently referred to as the “curing” stage (Fernández-Díez et al., 1985). It is known that the right combination of brine concentration, which must be increased

to more than 8% before summer time, and pH (values below 4.2) helps to guarantee the correct preservation during this stage (Rejano et al., 2010), but the influence of post-fermentation on the volatile profile of olives has not yet been studied. Usually, during this stage, the brine in the fermenter is periodically topped up with fresh brine to offset any losses through evaporation or spillage (operation referred to as “requerido” in the jargon of the Spanish olive industry). This operation **could dilute the fermenting brine and produce losses in** the acidity and flavor of fruits, which would affect the olive quality (Fernández-Díez et al., 1985). In addition, at packing time, the fruits are usually washed with water and then packed in small containers using fresh acidified brine as cover liquor. Also, as in the case of packing in glass bottles, the cover liquor is usually added hot ($> 60\text{ }^{\circ}\text{C}$) in order to achieve and maintain a vacuum inside the bottles. As a result of these operations, the volatile profiles of packed olives could significantly differ from olives prepared in bulk.

The objective of this study was to investigate the changes in the volatile compounds of Spanish-style green table olives as a result of the post-fermentation stage and the subsequent packing stage. The study was carried out using the most popular olive cultivars (Manzanilla, Hojiblanca, and Gordal) dedicated in Spain to this type of table olives.

2. Materials and Methods

2.1. Materials and chemicals

Olives of the three cultivars were harvested in the Province of Seville (Spain) at their mature-green stage and transported to our laboratories to be processed. Processing was

carried out in cylindrical fermenters made of polyethylene (5.2 kg fruits plus 3.4 L liquid capacity). Packing was carried out in cylindrical glass bottles (type “B250”, 125 g fruits plus 120 mL brine capacity), which were supplied by Juvasa Co. (Dos Hermanas, Seville, Spain).

All volatile compounds used as reference standards were purchased from Sigma-Aldrich (St Louis, MO). Deionized water was obtained from a Milli-Q system (Millipore, Bedford, MA). All other chemicals and solvents were of analytical or chromatographic grade from various suppliers (Panreac, Barcelona, Spain; VWR, Barcelona, Spain; Merck, Darmstadt, Germany).

2.1. Olive processing

After washing with tap water to eliminate plant materials and superficial contaminants, the olives from each variety were placed in two replicate fermenters and subjected to the typical treatments of Spanish-style green olive preparation (alkaline treatment, water washing, and brining). The alkaline treatment was performed using a lye solution of 1.8-1.9% w/v NaOH. The olives remained in this solution until the lye had penetrated two-thirds of the way through the flesh (around 5 h duration for Manzanilla and 8 h duration for Gordal and Hojiblanca). After that, a long-period water washing (15-17 h duration) was applied to eliminate the NaOH residues. Then, the olives were covered with brine (12.2-12.8% NaCl) and allowed to ferment at ambient temperature. Once the olives were totally fermented (after 7 months of brining), the salt level and pH in the brine were adjusted to approximately 9% and 3.9, respectively, by adding solid NaCl and lactic acid solution (90% w/v) to guarantee good preservation during the post-fermentation stage. After 11 months of brining, the olives from the two replicate

fermenters were pooled and conditioned in tap water for 4 days before packing. This conditioning step was performed in order to reach the low pH values (< 3.5) necessary to guarantee proper long-term preservation of the packed product. Finally, the olives were packed using acidified brine as cover liquor. Ten replicate bottles for each olive variety were prepared. The acidified brine consisted of lactic acid and NaCl to give equilibrium values of 0.5 g/100 mL titratable acidity and 5.0 g/100 mL NaCl. This cover brine was added hot (≈ 70 °C), as usually done at the industry.

2.2. Sampling

Olive samples from each fermenter were taken for analysis of volatile compounds and major fermentation end-products after 7 and 11 months of brining. Values reported in tables and figures are means of two replicate fermenters, each analyzed in duplicate. Sampling of packed olives was performed after 2 months of storage at room temperature. Three replicate bottles of each sample were drained and the pooled olives and brine were taken for analysis, which was carried out in triplicate. Apart from that, brine sampling was carried out during the fermentation and post-fermentation stages to control the main physicochemical and microbiological characteristics.

2.3. Physico-chemical and microbiological analyses

The pH and titratable acidity of brines were measured using a Metrohm 670 Titro processor (Herisau, Switzerland). Titratable acidity was determined by titrating to pH 8.3 with 0.2 mol L⁻¹ NaOH and expressed as lactic acid.

The culturable populations of the principal groups of micro-organisms were determined by plating the brines, and their decimal dilutions (in 0.9 % NaCl), on the appropriate solid media by using a Spiral Plater (Don Whitley Sci. Ltd., Shipley, England). De Man, Rogosa, Sharpe (Biokar diagnostics, Beauvais, France) MRS agar with and without 0.02% sodium azide (Sigma-Aldrich) was used for the lactic acid bacteria (LAB) determination, VRBG agar (Biokar) for *Enterobacteriaceae*, and oxytetracycline-glucose-yeast extract (Oxoid Ltd., Basingstoke, England) agar was used for yeasts. MRS plates were incubated under anaerobic conditions (AnaeroGen, Oxoid). All plates were incubated at 32 °C for up to 5 days, and the numbers of colony forming units were computed by using a Scan 500 (Interscience, St Nom la Bretèche, France) colony counter.

In addition to the above, microbial genomic DNA was extracted from the brines after the post-fermentation stage as described by Medina et al. (2016) and sent to the Sequencing and Bioinformatic Service of FISABIO (Valencia, Spain) for massive sequencing of 16S rDNA and ITS amplicons using a MySeq Illumina platform. A Bioinformatic analysis was also carried out at FISABIO. Taxonomic affiliations of 16S rDNA and ITS datasets were assigned using the RDP_classifier from the Ribosomal Database Project (Wang, Garrity, Tiedje, & Cole, 2007; Cole et al., 2009). For bacterial assignation, the proper training set was used, whereas for ITS sequences, the UNITE fungal classification database (<https://unite.ut.ee/>) was used, which is also included in the RDP classifier.

2.4. HPLC analyses

The major fermentation end-products in olive juice were analyzed by HPLC. The olives were pitted and blended with a homogenizer (Braun, Kronberg, Germany) without added liquid. The homogenate was squeezed through cheesecloth and then centrifuged for 30 min at 12000 x g using a Sorvall RC-5 super-speed centrifuge (Du Pont Instruments, Newton, CT). One portion of the aqueous supernatant was frozen until analysis by HPLC. Organic acids (lactic, acetic and succinic acids) and ethanol were analyzed using a C18 column and deionized water (pH adjusted to 2.2 using concentrated H₃PO₄) as the mobile phase and a refractive index detector (Sánchez, de Castro, Rejano, & Montaña, 2000).

2.5. Analysis of volatile compounds

Volatile compounds were analyzed by solid phase micro-extraction (SPME) and gas chromatography coupled to mass spectrometry (GC-MS) following the procedure **previously reported by Cortés-Delgado et al. (2016). Briefly, the olives (approximately 200 g) were pitted and homogenized, and 2.5 g of homogenized pulp were placed in a** 15 mL glass vial. After the addition of a 7.5 mL of NaCl solution (300 g L⁻¹), 100 µL of 3-octanol (2 mg L⁻¹) as internal standard, and a stirring bar (for stirring at 600 rpm), the vial was closed and placed in a water bath adjusted to 60 °C. Headspace volatile compounds were extracted for 60 min on a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (1cm, 50/30 µm; Supelco, Bellefonte, PA). Volatile compounds adsorbed on the SPME fiber were desorbed at 265 °C for 15 min in the injector port of a GC interfaced with a mass detector (internal ionization source: 70 eV) with a scan range from m/z 30 to 400 (GC model 7890A and mass detector model 5975C, Agilent Technologies, Santa Clara, CA).

Separation was achieved on a VF-WAX MS capillary column (30 m x 0.25 mm x 0.25 μm film thickness) from Agilent. The initial oven temperature was 40 °C (5 min), then 40-195 °C at 3 °C min^{-1} , and then 195-240 °C at 10 °C min^{-1} and held there for 15 min. The carrier gas was helium at a constant flow of 1 mL min^{-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 NIST Standard Reference Database and from authentic reference standards when available. For the determination of the RI, a C7-C30 *n*-alkanes series was used, and the values were compared, when available, with values reported in the literature for similar chromatographic columns. The volatile compounds were quantified by comparison of peak areas in the ion extraction chromatogram (IEC), which was obtained by selecting target ions for each compound to that of internal standard (3-octanol) (semi-quantitative determination). These ions corresponded to base ion (m/z 100% intensity), molecular ion (M^+) or another characteristic ion for each molecule. All analyses of the volatile compounds were made in triplicate using different vials. The method repeatability computed as relative standard deviations (RSD) were in the range 5-48%, with an average value of 18.1%. This value is within the expected precision for analyte concentrations between 10 and 100 $\mu\text{g kg}^{-1}$ and is lower than the expected precision (30%) for a concentration of 1 $\mu\text{g kg}^{-1}$, according to the AOAC criteria (AOAC, 2016). Odor thresholds (OTs) in water for volatile compounds were taken from references in van Gemert (2003). Odor descriptors for volatile compounds were taken from Flavornet (www.flavornet.org) and Pherobase (www.pherobase.com) online databases.

2.6. Statistical data analysis

The means of the total contents of the different chemical classes and their 95% confidence intervals were calculated using a Microsoft Excel spreadsheet (Microsoft). One-way analysis of variance (ANOVA) was applied to test the effect of sampling time on the individual compounds for each olive cultivar. Prior to ANOVA, the homogeneity of variances was checked with the Levene test and normality of the variables was checked by the Shapiro-Wilk test, both at a significance level of 5%. When these two conditions were fulfilled, the classic ANOVA (F-test) was applied and the Duncan test was used for mean comparisons. In case of heterogeneity of variances, assuming that normality was fulfilled, the Welch test was used to determine whether there was a significant difference between the means and the Games-Howell post hoc test was used for mean comparisons. Significant differences were determined at the $p < 0.05$ level. These statistical analyses were performed using SPSS software v. 23.0 (IBM Corp., Armonk, NY, USA).

3. Results and Discussion

3.1. Chemical and microbiological characteristics of samples

Titrateable acidity progressively increased during fermentation, with a concomitant decrease in pH, until pH values of 4.1-4.3 were reached after 7 months of brining (Figure 1). At this point, in order to prevent microbial spoilage during the post-fermentation stage, lactic acid and NaCl were added to each fermenter, as mentioned in the Materials and Methods section. This explains the additional increases in acidity (and concomitant decreases in pH) between 200 and 250 days as shown in Figure 1. During the post-fermentation stage until 11 months of brining there was a decrease in titrateable

acidity with a concomitant increase in pH in all cases, with this change being particularly intense in Gordal olives. In fact, final pH reached 4.6 in this cultivar. As discussed later, there are diverse microbial causes for this acidity decrease.

The evolution of LAB and yeasts is displayed in Figure 2. LAB were present from the start of the fermentation in all cultivars, and their populations were higher than the number of yeasts, which is a sign that fermentation proceeds adequately. Only the last analysis showed an important LAB decrease in one of the Manzanilla fermenters. *Enterobacteriaceae* were present in all cultivars, but their growth was short and did not reach high numbers (data not shown).

The concentrations of major end-products (lactic acid, acetic acid, succinic acid, and ethanol) after the stages of fermentation, post-fermentation, and packing are shown in Table 1. The concentrations of these compounds after fermentation were within the normal ranges for the industrially fermented green olives of Manzanilla, Gordal and Hojiblanca cultivars reported by Montaña, Sánchez, López-López, de Castro, & Rejano (2010). However, a higher concentration of lactic acid would be expected after the post-fermentation time for all cultivars because of the quantities of lactic acid added after fermentation. The differences between the expected and the actual lactic acid concentrations after post-fermentation were lesser for Manzanilla and Hojiblanca olives than for Gordal olives, which registered the most important decrease. Apart from lactic acid, the concentration of acetic acid also diminished significantly through the post-fermentation period, albeit the drop was similar for the three cultivars. The decrease of lactic acid concentration in fermented vegetables may have different causes, all of them provoked by microbial metabolism unless intentional dilution has been carried out. Medina et al. (2016) compiled information related to the *Lactobacillus buchneri* capacity of metabolize lactic acid increasing the initial acidic pH and facilitating the

growth of other microorganisms (*Propionibacterium*, *Pectinatus*, and others), in relation to fermented cucumber spoilage. *Propionibacterium* has also been related to the lactic acid consumption in Spanish-style green olives (Montaño, Sánchez, Casado, de Castro, & Rejano, 2003). However, an increase of acetic acid concentration would be expected, which was not found (Table 1). Apart from these bacteria, yeasts have also the capability of depleting not only lactic acid but also other organic acids, in this case using oxidative pathways when they grow on the brine surface in contact with air (Ruiz Cruz, & González Cancho, 1969; Medina et al., 2016). Taking into account the diverse potential origins, it was considered relevant to carry out a genomic analysis in order to know the relative DNA abundance of the different microorganisms that were present at the post-fermentation stage. The results are presented in Table S1. When comparing the microbial DNA relative abundance with the variation of lactic acid concentration through the post-fermentation period, the most striking difference between Gordal and Manzanilla/Hojiblanca cultivars was the high presence of *Cardiobacteriaceae* DNA in Gordal brines (44.54 %) opposite to the other cultivars (<1 %). *Suttonella*, a genus within *Cardiobacteriaceae*, has been found in some instances of cucumber (Breidt et al., 2013) and olive (Golomb et al., 2013) spoilages. Unfortunately, no information on lactic acid metabolism by this bacterial family has been found in the literature. A low percentage (0.05) of *Propionibacteriaceae* DNA was found in Gordal brines, whereas it was not found in the other cultivars. Regarding yeasts, *Pichia membranifaciens*, a typical surface film-forming organism, was the most abundant species in the three cultivars. On the whole, it is reasonable to assume the combined effect that the different microorganisms may have had. Film-forming yeast, particularly *Pichia membranifaciens*, could consume part of lactic and acetic acids in all fermenters,

whereas bacteria, presumably from the *Cardiobacteriaceae* family, would have increased the consumption of lactic acid only in the case of Gordal olives.

The concentrations of acetic acid, succinic acid, and ethanol significantly decreased in all cases (Table 1) during the post-fermentation stage. As mentioned above, acetic acid can be metabolized by yeasts growing on the brine surfaces. Their respiratory pathways in this case do not give rise to compounds dissolved in the brines, but CO₂ and water. Similarly, decreases in succinic acid and ethanol could be related to yeast activity (Wickerham & Burton, 1948). However, it cannot be ruled out that esterification reactions could also have occurred (Sabatini & Marsilio, 2008). Also, it must be taken into account that, in our case, the post-fermentation period occurred between early May and early September, with ambient temperatures above 30 °C for two or more months, and closures of fermenters were not hermetic, so ethanol loss by evaporation cannot be excluded. Finally, decrease in all the compound concentrations displayed in Table 1 were obvious as result of packing operations (preconditioning in water, addition of hot cover brine), which would originate losses in hydrosoluble compounds by leaching.

3.2. Volatile compounds

Based on SPME-GC-MS, 132 volatile compounds were identified in the present study. The retention indices (RI) along with the selected ions to obtain the GC peak area, the identification criteria, and the odor thresholds for all identified compounds are shown in Table S2. With the exception of 13 compounds (6-methyl-5-hepten-2-ol, (E)-2-octen-1-ol, (Z)-3-nonen-1-ol, homovanillyl alcohol, propyl butanoate, isobornyl acetate, ethyl phenylacetate, benzyl propanoate, triacetin, methylpropenal, (E)-rose

oxide, β -citronellol, and p-vinylanisole) all the compounds were previously identified in one or various types of table olives, including Spanish-style (Montaño et al., 1990; Sabatini & Marsilio, 2008; Iraqi et al., 2005; Cano-Lamadrid et al., 2015; Cortés-Delgado et al., 2016; Sánchez, de Castro, López-López, Cortés-Delgado, Beato, & Montaño, 2017), California-style (Sansone-Land et al., 2014), Greek-style (Sabatini, Mucciarella, & Marsilio, 2008; Collin, Nizet, Muls, Iraqi, & Bouseta, 2008; Randazzo et al., 2014; Bleve et al., 2014, 2015; De Angelis et al., 2015; Martorana et al., 2015), Tunisian-style (Dabbou et al., 2012), “Campo Real” unfermented olives from the Comunidad de Madrid (Spain) (Navarro, de Lorenzo, & Pérez, 2004), and “alcaparras” stoned olives from the Trás-os-Montes region (Portugal) (Malheiro, de Pinho, Casal, Bento, & Pereira, 2011).

3.2.1. Changes in the total contents of different chemical families

The identified compounds were grouped into different chemical classes :acids, alcohols, aldehydes/ketones, esters, hydrocarbons, phenols, terpenes, and other compounds. In agreement with our previous work (Cortés-Delgado et al., 2016), phenols, alcohols, acids, and esters were the predominant classes in the headspace **profile of Spanish-style green table olives. On average, Gordal and Hojiblanca olives had higher contents of phenols, acids, esters, and terpenes than Manzanilla olives (Table 2). The content of alcohols was higher in Hojiblanca olives (mean value 1295 $\mu\text{g kg}^{-1}$) compared to Manzanilla (621 $\mu\text{g kg}^{-1}$) and Gordal (639 $\mu\text{g kg}^{-1}$), which agrees with the higher content of ethanol in Hojiblanca measured by HPLC (Table 1). Samples of Gordal olives had the highest content of esters (848 $\mu\text{g kg}^{-1}$) and acids (750 $\mu\text{g kg}^{-1}$). Mean contents of alcohols, acids, and esters significantly decreased, but those of**

hydrocarbons increased, with sampling time (Table 2). This factor did not have a significant influence on the mean contents of the remaining chemical classes. The interaction between sampling time and olive cultivar is depicted in Figure 3. It can be seen that in general the volatile profile for each olive cultivar was not significantly affected by the post-fermentation stage compared to contents after fermentation. The only exceptions were the content of carbonyl compounds, which increased in Manzanilla olives, and the content of volatile acids which decreased in Hojiblanca. As commented below, the increase of carbonyl compounds with the post-fermentation stage in Manzanilla olives could be indicative of lipid oxidation. The packing stage had significant effects on various chemical classes compared to contents after the post-fermentation stage. Thus, as result of packing, Manzanilla olives decreased in acids, alcohols, and esters, but increased in hydrocarbons and terpenes; Gordal olives decreased in esters, but increased in aldehydes/ketones and hydrocarbons; and Hojiblanca olives decreased in acids, alcohols, and esters, but increased in hydrocarbons.

3.2.2. Changes in individual volatile compounds

Table 3 summarizes the data of the volatile compounds encountered in the studied Spanish-style green olives, before and after the post-fermentation and packing stages.

Ten volatile phenols were identified, of which p-cresol was the most abundant in all three cultivars at any stage along their elaboration process. The contents of 4-ethyl phenol and o-guaiacol were relatively important in Gordal and Hojiblanca olives, respectively. As result of the post-fermentation stage, p-cresol increased in Manzanilla

olives compared to its content after fermentation, but the increase was not significant in Gordal or Hojiblanca olives. The content of 4-ethyl phenol remained unchanged with the post-fermentation in all three cultivars. Ortho-guaiacol increased in Manzanilla and Gordal olives, but the concentration change was not significant in Hojiblanca olives. Among the minor phenols, p-propyl guaiacol and vanillin showed a significant increase with the post-fermentation stage in all three cultivars. The increase in volatile phenols can be attributed to microbial activity during the post-fermentation stage. Thus, vanillin could have been formed by microbial transformation of ferulic acid (Rao & Ravishankar, 2000), which may be formed during fermentation stage (Benincasa, Muccilli, Amenta, Perri, & Romeo, 2015). In general, the packing stage did not have a significant effect on individual phenols compared to the concentrations obtained after post-fermentation (Table 3). In all samples, p-creosol (smoky, sweet, and spicy odor) presented concentrations one or two orders of magnitude higher than its odor threshold ($10 \mu\text{g kg}^{-1}$ in water, Table S2). Therefore, this phenol could be a probable contributor to the aroma of Spanish-style green olives. Ortho-guaiacol (smoke, sweet, and medicine; threshold $2.5 \mu\text{g kg}^{-1}$ in water) could be an odor active compound in Gordal and Hojiblanca olives. In addition, 4-ethyl phenol (must odor, threshold $42 \mu\text{g kg}^{-1}$ in water) could contribute to the aroma of Gordal olives. However, it must be stressed that the procedure used for quantification in the present study was semi-quantitative and is not able to provide accurate data on actual concentration of volatiles in the olive matrix.

Within the family of alcohols, 25 compounds were identified. Phenylethyl alcohol was the dominant alcohol in all samples, with concentrations ranging between 275 and $456 \mu\text{g kg}^{-1}$ after fermentation (Table 3). As a result of the post-fermentation stage, there was a significant increase in phenylethyl alcohol, although this increase was not significant in Gordal olives. This alcohol could be formed by yeasts from L-

phenylalanine (Eshkol et al., 2009). The remaining alcohols, in general, remained unchanged or decreased in concentration with post-fermentation. Among the alcohols that significantly decreased their concentration, we can highlight ethanol and 1-propanol (in all the three cultivars), 2-butanol and (Z)-3-hexen-1-ol (Manzanilla, Hojiblanca), and benzyl alcohol (Gordal, Hojiblanca). As discussed in section 3.1, decrease in ethanol could be attributed to a combination of mechanisms (yeast metabolism, esterification reactions, evaporation). Most individual alcohols decreased or remained unchanged as a result of packing. Only 1-heptanol and 1-octanol increased during this stage in all three cultivars. These two alcohols could be formed as a result of lipid (oleic acid) oxidation (Schaich, 2013). In general, the odor thresholds in water for alcohols (Table S2) were high. Therefore, their contribution to the olive aroma would likely be minor.

Eleven volatile acids were identified in the present study. Acetic and propanoic acids were the dominant ones after fermentation, the latter acid being particularly abundant in Gordal olives (Table 3). The formation of acetic acid in olives occurs during the alkaline treatment and during the fermentation stage (Sánchez et al., 2000). Propanoic acid is frequently formed after a prolonged time in brine as result of the growth of *Propionibacterium* species, which is characterized by the formation of propanoic and acetic acids at the expense of lactic acid (Montaño et al., 2003). As a result of the post-fermentation stage, acetic acid significantly decreased (as discussed in section 3.1), but propanoic acid did not significantly change in any of the three cultivars. On the other hand, isobutanoic and 2-methylbutanoic acids appeared to increase in all three cultivars. Butanoic acid increased with post-fermentation in Manzanilla and Gordal olives, but apparently this did not occur in Hojiblanca. It can be noted that the concentration of this acid presented a high variability between the duplicate fermenters, particularly in Hojiblanca (Table 3). The formation of these short

fatty acids is likely due to microbial activity during the post-fermentation stage (Medina et al., 2016). In general, the short-chain fatty acids (C2-C5) were partially lost as a result of the packing process. However, medium-chain fatty acids (C6-C10) were hardly affected by packing. This could be related to the lower solubility in water of medium-chain fatty acids compared to short-chain fatty acids. Since odor thresholds of volatile acids (Table S2) were much higher than their concentrations, the odor contributions of these compounds could be low or negligible.

Thirty-nine esters were identified in the present study, of which ethyl and methyl esters were the most numerous. After fermentation, ethyl acetate and triacetin were the dominant esters in Manzanilla olives; propanoate esters (mainly, propyl propanoate, ethyl propanoate and methyl propanoate) along with propyl acetate and ethyl acetate were the dominant esters in Gordal olives; and ethyl acetate, ethyl propanoate, and ethyl butanoate were the most abundant in Hojiblanca olives (Table 3). The considerably higher concentration of propyl propanoate in Gordal compared to Manzanilla or Hojiblanca is noteworthy. This fact is consistent with the relatively high contents of 1-propanol and propanoic acid in Gordal olives, as mentioned above. Acetate esters and propanoate esters could be synthesized by an alcohol acyltransferase (AAT), which catalyzes the esterification of volatile alcohols with acetyl CoA and propionyl CoA, respectively, to produce volatile esters and free CoA-SH (Sabatini & Marsilio, 2008). In Manzanilla olives, concentrations of individual esters increased (17 esters) or remained unchanged (16 esters) as a result of the post-fermentation stage, while only ethyl acetate decreased. In Gordal and Hojiblanca olives, there were almost equal number of increases and decreases in individual esters, but most of them (21 and 27 esters, respectively) remained unchanged. Ethyl acetate decreased with the post-fermentation stage in all three cultivars. Ester losses can be attributed to hydrolytic reactions. In

wines, decreases in ethyl acetate and other esters have been attributed to oxidation reactions during storage, these reactions being due to dissolved oxygen or to oxidative species present in the wine (Patrianakou and Roussis, 2013). However, these oxidation reactions are less probable in case of fermented olives as microorganisms would consume oxygen (both dissolved oxygen and oxygen entering into the fermenter through closure), thereby preventing oxidation reactions (Sánchez, López-López, Beato, de Castro, & Montaña, 2017). In general, individual esters decreased or remained unchanged as a result of packing. Only ethyl lactate increased as a result of packing in all cases. This ester could be formed from lactic acid and ethanol when the hot cover brine was added to olives at packing. Various esters could contribute to the aroma of Spanish-style green table olives due to their low odor thresholds (Table S2). For instance, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, and ethyl 3-cyclohexenecarboxylate present odor thresholds in water of 0.09, 0.01, and 0.003 $\mu\text{g kg}^{-1}$ respectively. These esters could contribute to the fruity notes of samples.

Eight hydrocarbons were identified in the present study. Octane, decane, and 2-bornene were the predominant hydrocarbons after fermentation in all cases, although styrene was also relatively abundant in Gordal and Hojiblanca olives (Table 3). In all three cultivars, individual hydrocarbons increased or remained unchanged as a result of the post-fermentation stage, except for decane, which significantly decreased in Gordal and Hojiblanca olives. The packing stage appeared to increase the concentration of several hydrocarbons in Manzanilla and Gordal olives, but it had little influence in Hojiblanca olives. Octane increased with packing in all three cultivars. This can be attributed to lipid oxidation, as octane is one of the secondary oxidation products of oleic acid (Schaich, 2013). In general, the odor thresholds of hydrocarbons (Table S2)

were quite higher than their contents in all samples. Therefore, these compounds would not likely contribute to the aroma of Spanish-style green olives.

Within the chemical class of aldehydes/ketones, 14 compounds were identified. After fermentation, benzaldehyde and (E)-2-decenal were the most abundant in Manzanilla olives, but (E)-2-heptenal and 1-octen-3-one were also abundant in Gordal and Hojiblanca olives (Table 3). As result of post-fermentation, in Manzanilla olives benzaldehyde remained unchanged, while there was formation of 4 aldehydes, namely, pentanal, nonanal, (E,E)-2,4-heptadienal, and phenylacetaldehyde, and increases in other carbonyl compounds such as octanal, 1-octen-3-one, (E)-2-octenal, (E)-2-decenal, and (E,E)-2,4-decadienal. Most of the latter compounds have been used as indicators of lipid oxidation in other foods (Cleary & McFeeters, 2006; Jansen, 2015). Therefore, the increases in the concentration of these aldehydes in Manzanilla olives can be considered to be an indicator of lipid oxidation during the post-fermentation stage. In Gordal and Hojiblanca olives, benzaldehyde content significantly decreased, but other carbonyl compounds remained unchanged. As a result of packing, in Manzanilla olives again there were increases in (E)-2-decenal and (E,E)-2,4-decadienal, but octanal and 1-octen-3-one decreased. In Gordal olives and Hojiblanca olives, the changes in individual carbonyl compounds included increases in various aldehydes ((E)-2-decenal and (E,E)-2,4-decadienal, among others). Therefore, it is likely that lipid oxidation occurred during packing operations and/or during storage time in all three cultivars. Regarding the probable contributors to the aroma of Spanish-style green olives, various carbonyl compounds can be highlighted due to their low odor thresholds (Table S2). Among them, we can mention: octanal (fat, soap, lemon, and green notes; threshold $0.9 \mu\text{g kg}^{-1}$), 1-octen-3-one (mushroom odor, threshold $0.05 \mu\text{g kg}^{-1}$), (E)-2-octenal (green, nut,

fat; threshold 0.2 $\mu\text{g kg}^{-1}$), and (E,E)-2,4-decadienal (fried note, lemon, citrus; 0.1 $\mu\text{g kg}^{-1}$).

Seventeen terpenes/terpenoids were identified, with dihydroedulan and linalool being the predominant terpenes in Manzanilla olives. Gordal olives were characterized by a relatively high content of α -terpineol, whereas copaene was the most abundant in Hojiblanca (Table 3). The above mentioned terpenes remained unchanged as a result of the post-fermentation stage in all three cultivars. In general, the packing stage did not have a significant effect on individual terpenes. β -Damascenone which has an apple, rose and honey odor showed concentrations that were two orders of magnitude higher than its odor threshold (0.0013 $\mu\text{g kg}^{-1}$ in water; Table S2). Therefore, β -damascenone could be a probable contributor to the overall aroma of Spanish-style green table olives at any stage of their elaboration process. Interestingly, this carotenoid-derived compound has been reported to have the highest odor activity value among the volatiles detected in California-style black ripe olives (Sansone-Land et al., 2014), which have sensory characteristics quite different from those of Spanish-style green table olives.

Finally, 2 sulfur compounds (dimethyl sulfide and dimethyl sulfoxide), 1 lactone (butyrolactone), and 3 other compounds (theaspirane, p-vinyanisole, and 1,4-dimethoxybenzene) were identified. After fermentation, dimethyl sulfide and the two isomers of theaspirane (A and B) were the predominant compounds, which did not show significant changes as a result of the post-fermentation stage in all three cultivars (Table 3). The packing stage did not have a significant effect on the above-mentioned compounds, with the exception of butyrolactone, which disappeared with the packing stage and 1,4-dimethoxybenzene, which appeared to increase in all three cultivars. The fact that dimethyl sulfide was not affected by post-fermentation or packing stage in any case suggests that anaerobic conditions were maintained inside the fermenters during

post-fermentation period as well as inside the bottles during storage. Dissolved oxygen in olive brine both before and after packing stage could be consumed by microorganisms, thereby preventing oxidation reactions (Sánchez, López-López, Beato, de Castro, & Montaña, 2017). However, in the absence of microbial activity (e.g. in case of pasteurized packed olives), the presence of oxygen can originate different oxidation reactions (e.g. oxidation of dimethyl sulfide to form dimethyl sulfoxide, oxidation of phenolic compounds leading to brown pigments, etc.) (Sánchez et al., 2017). Dimethyl sulfide (cabbage and sulfur notes; threshold $0.3 \mu\text{g kg}^{-1}$) could contribute to the aroma of olives in all cases.

4. Conclusions

In the present study, more than 130 volatile compounds from different chemical classes were identified in the pulp of Spanish-style green table olives. The post-fermentation stage had little or non-significant effects on the total contents of the different chemical classes in samples obtained from Manzanilla, Gordal and Hojiblanca cultivars. However, a number of compounds of each chemical family suffered significant changes compared to their concentrations after fermentation, the changes being especially numerous in esters and alcohols. Significant increases in several carbonyl compounds (octanal, 1-octen-3-one, (E)-2-octenal, (E)-2-decenal, and (E,E)-2,4-decadienal) were found in Manzanilla olives as a consequence of post-fermentation, which could be indicative of lipid oxidation. However, these compounds remained stable in Gordal and Hojiblanca olives.

Regarding the packing stage, it had a significant influence on the total contents of various chemical families compared to the contents after the post-fermentation stage.

Alcohols, acids, esters, hydrocarbons, and carbonyl compounds were the most affected by packing. Lipid oxidation appeared to occur as a result of packing stage, as significant increases in some typical reaction products of fatty acids oxidation ((E)-2-decenal, (E,E)-2,4-decadienal, 1-heptanol, 1-octanol, octane) were found in all three cultivars.

Acknowledgements

This work was supported in part 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

Figure 1.Changes in pH and titratable acidity during the fermentation and post-fermentation stages of Spanish-style green olives of the Manzanilla, Gordal, and Hojiblanca cultivars. Points are the means of duplicate fermentations. Where error bars (which denote the range of data) are not visible, determinations were within the range of the symbols on the graph.

Figure 2.Changes in microbial populations (lactic acid bacteria and yeasts) during the fermentation and post-fermentation stages of Spanish-style green olives of the Manzanilla, Gordal, and Hojiblanca cultivars. Points are the means of duplicate fermentations. Where error bars (which denote the range of data) are not visible, determinations were within the range of the symbols on the graph.

Figure 3.Chemical classes of the volatile compounds in Spanish-style green olives from Manzanilla, Gordal, and Hojiblanca cultivars at three different times of elaboration process: after fermentation (7 months of brining), after post-fermentation (11 months of brining), and after packing (2 months of storage). Before packing, the values are means **of two replicate fermenters, each analyzed in triplicate**. In packed olives, the content of **three bottles was pooled and olives analyzed in triplicate**. Error bars indicate 95% confidence intervals.

Supplementary material

Table S1: Relative abundance (%) of the preponderant bacterial genera or families, and yeast genera, obtained by pyrosequencing analysis after 11 months of brining

Table S2: Volatile compounds in the headspace of Spanish-style green table olives identified in the present study

Table 1. Major end-products in juice of Spanish-style green table olives of Manzanilla, Gordal, and Hojiblanca cultivars at three different times along the elaboration process

Olive cultivar	Sampling time	Concentration (g L ⁻¹) ^a			
		Lactic acid	Acetic acid	Succinic acid	Ethanol
Manzanilla	After fermentation ^b	9.54 (0.01)b	1.80 (0.04)c	0.41 (0.001)c	0.25 (0.02)b
	After post-fermentation ^c	11.7 (0.9)c	0.8 (0.1)b	0.21 (0.05)b	0.07 (0.02)a
	After packing ^d	6.30 (0.01)a	0.10 (0.001)a	0.03 (0.001)a	ND
Gordal	After fermentation	7.9 (0.2)b	2.7 (0.4)c	0.4 (0.4) c	0.99 (0.06)c
	After post-fermentation	5.7 (1.5)a	1.8 (0.3)b	0.20 (0.01)b	0.16 (0.03)b
	After packing	5.4 (0.01)a	0.3 (0.001)a	0.04 (0.001)a	0.02 (0.001)a
Hojiblanca	After fermentation	12.4 (0.8)b	1.9 (0.2)c	0.41 (0.04)c	1.1 (0.2)c
	After post-fermentation	12.9 (0.3)b	1.1 (0.1)b	0.20 (0.03)b	0.4 (0.1)b
	After packing	6.6 (0.01)a	0.1 (0.001)a	0.07 (0.002)a	0.04 (0.002)a

^a Values are means with standard deviations in parentheses. Data in the same column with different letters for each olive cultivar are significantly different ($p < 0.05$). ND = not detected. ^b Analysis performed after 7 months of brining. Values are means of two replicate fermenters, each analyzed in duplicate. ^c Analysis performed after 11 months of brining. Values are means of two replicate fermenters, each analyzed in duplicate. ^d Analysis performed after 2 months of packing. Values are means of triplicate analyses from a composite sample, which was made up of three bottles.

Table 2

Table 2. Main effects of olive cultivar and sampling time on the different chemical classes of volatile compounds in Spanish-style green table olives^a

Chemical class	Olive cultivar			P ^b	Sampling time			P
	Manzanilla	Gordal	Hojiblanca		After fermentation	After post-fermentation	After packing	
Phenols	525 (150)a	1334 (399)b	1268 (243)b	***	935 (416)	1175 (515)	1029 (413)	NS
Alcohols	621 (164)a	639 (156)a	1295 (410)b	***	899 (412)b	954 (471)b	583 (119)a	**
Acids	256 (103)a	750 (162)c	581 (279)b	***	651 (265)c	523 (254)b	276 (211)a	***
Esters	96 (40)a	848 (348)c	490 (199)b	***	578 (394)b	529 (380)b	148 (92)a	***
Hydrocarbons	114 (54)b	57 (17)a	119 (39)b	- ^c	88 (38)a	78 (30)a	159 (51)b	***
Aldehydes/ketones	59 (25)a	71 (28)a	100 (33)b	**	70 (41)	75 (22)	97 (29)	NS
Terpenes	48 (13)a	100 (24)b	99 (18)b	***	84 (32)	80 (34)	87 (20)	-
Others	66 (18)b	31 (10)a	81 (22)c	-	55 (27)	60 (31)	70 (18)	-

^a Values are means (μg kg⁻¹) with standard deviations in parentheses. Means with different letters within a row for each effect are significantly different (P < 0.05).

^b Probability that there is a difference between means: NS, no significant difference between means (P > 0.05); * significant at the 5% level; ** significant at the 1% level; *** significant at the 0.1% level.

^c -, ANOVA test was not performed because normality conditions were violated in at least one group.

Table 3

Table 2. Volatile compounds in the headspace of Spanish-style green table olives of Manzanilla, Gordal and Hojiblanca cultivars at three different times along the elaboration process: 7 months of brining (A), 11 months of brining (B), and 2 months of packing (C)

Compound	Concentration of identified compounds ^a											
	Manzanilla cv.				Gordal cv.				Hojiblanca cv.			
	A	B	C	P ^b	A	B	C	P	A	B	C	P
Volatile phenols												
o-Guaiacol	0.7 (0.2)a	1.8 (0.7)b	1.9 (0.4)b	**	2.6 (0.4)a	5 (2)b	6 (1)b	**	100 (106)	66 (69)	49 (9)	NS
p-Creosol	361 (103)a	580 (173)b	587 (62)b	*	952 (134)	1291 (464)	1375 (178)	NS	1075 (121)	1214 (259)	921 (115)	NS
Phenol	1.8 (0.5)a	4 (2)b	2.7 0.8)ab	*	5 (1)	6 (2)	5.8 (0.6)	NS	15 (14)	11 (9)	8.1 (0.9)	- ^c
p-Ethyl guaiacol	1.9 (0.5)a	2.9 (0.9)ab	3.2 (0.3)b	*	7 (1)a	10 (3)b	11 (2)b	*	3.5 (0.3)	5 (1)	3.6 (0.2)	NS
p-Cresol	10 (3)	14 (7)	12 (1)	NS	14 (3)	19 (7)	19 (3)	NS	19 (5)	20 (6)	14 (1)	NS
p-Propyl guaicol	1.2 (0.3)a	2.0 (0.6)b	2.3 (0.1)b	**	2.1 (0.5)a	6 (2)b	6 (1)b	**	2.6 (0.4)a	3.7 (0.8)b	3.1(0.1)ab	*
Eugenol	1.0 (0.3)	1.4 (0.6)	1.7 (0.2)	NS	1.9 (0.4)	2.5 (0.8)	3.1 (0.5)	NS	1.5 (0.2)	1.8 (0.5)	1.5 (0.1)	NS
4-Ethyl phenol	12 (3)	18 (7)	20 (2)	NS	110 (15)	123 (40)	157 (25)	NS	33 (4)	31 (6)	26 (2)	NS
Vanillin	0.7 (0.3)a	3 (2)b	4.6 (0.3)b	**	2.3 (0.3)a	5 (1)b	4.8 (0.5)b	**	2.6 (0.4)a	4 (1)b	5.4 (0.5)c	***
Tyrosol	24 (11)	19 (12)	14 (3)	-	11 (3)	9 (15)	3 (1)	-	32 (18)b	14 (12)ab	5 (3)a	*
Alcohols												
Ethanol	30 (11)b	13 (8)a	3.3 (0.5)a	**	94 (12)b	23 (9)a	8.0 (0.5)a	***	216 (33)c	71 (25)b	13 (2)a	***
2-Butanol	43 (12)b	18 (9)a	6 (1)a	***	12 (1)b	9 (4)ab	5.0 (0.9)a	*	63 (12)c	30 (15)b	7 (1)a	***
1-Propanol	6 (1)c	3 (1)b	1.1 (0.5)a	***	157 (30)c	77 (27)b	25.2 (0.4)a	***	43 (5)c	17 (6)b	3.8 (0.4)a	***
Isobutanol	0.6 (0.2)	0.4 (0.5)	ND	NS	0.5 (0.1)b	0.2 (0.2)a	ND	**	2.5 (0.5)b	1.9 (0.8)b	0.6 (0.1)a	**
1-Butanol	0.5 (0.2)	ND	ND		1.2 (0.1)	2 (1)	1.5 (0.2)	NS	5 (4)	1.2 (0.9)	ND	NS
Isopentanol	8 (4)a	23 (12)b	11 (2)ab	*	9 (1)	8 (4)	7.0 (0.4)	NS	34 (6)	67 (26)	33 (8)	NS
3-Methyl-3-buten-1-ol	0.2 (0.4)	ND	ND		0.9 (0.2)b	0.4 (0.2)a	ND	**	1.4 (0.3)b	0.8 (0.3)a	ND	**
1-Pentanol	0.8 (0.5)	0.7 (0.2)	ND	NS	1.0 (0.2)b	0.6 (0.3)a	1.0 (0.2)b	*	1.4 (0.4)b	0.7 (0.2)a	0.7 (0.2)a	**
2-Heptanol	ND	1.2 (0.6)	1.6 (0.2)	NS	ND	1 (1)	2.0 (0.3)	NS	ND	ND	1.8 (0.4)	

3-Methyl-2-buten-1-ol	3 (1)b	0.2 (0.3)a	ND	**	1.4 (0.2)	ND	ND		4.3 (0.9)	ND	ND	
3-Methyl-1-pentanol	ND	0.5 (0.6)	0.7 (0.2)	NS	0.6 (0.2)a	0.7 (0.6)ab	0.92 (0.01)b	*	0.3 (0.1)	ND	0.3 (0.1)	NS
1-Hexanol	9 (5)	5 (2)	4.9 (0.7)	-	10 (1)b	4 (2)a	7.0 (0.8)ab	**	23 (6)	10 (7)	8 (2)	-
(Z)-3-Hexen-1-ol	69 (27)b	22 (14)a	14 (2)a	**	28 (4)b	17 (9)ab	13.0 (0.7)a	***	79 (19)b	29 (12)a	16 (4)a	***
1-Octen-3-ol	6 (2)	7 (1)	7 (1)	NS	10 (5)	10 (5)	19 (2)	NS	10 (3)	8 (4)	13 (4)	NS
1-Heptanol	5 (2)a	7 (3)a	12 (1)b	**	4.8 (0.7)a	7 (5)a	14 (1)b	**	7 (1)a	7 (5)a	14 (3)b	*
6-Methyl-5-hepten-2-ol	3 (1)	2 (1)	3.4 (0.7)	-	1.9 (0.3)	2 (1)	3.5 (0.1)	-	2.3 (0.4)	3 (1)	2.7 (0.3)	NS
2-Ethyl-1-hexanol	3 (1)a	3 (1)a	6.2 (0.7)b	***	19 (5)	15 (6)	27 (3)	NS	4.0 (0.7)a	4 (2)a	6.3 (0.9)b	*
1-Octanol	8 (2)a	11 (3)a	24 (3)b	***	7 (1)a	8 (3)a	20 (1)b	***	9 (1)a	8 (3)a	17 (3)b	**
(E)-2-Octen-1-ol	2.6 (0.9)b	1.5 (0.3)ab	1.0 (0.2)a	*	2 (1)	2.1 (0.8)	3.8 (0.3)	NS	2.1 (0.6)	1.6 (0.6)	1.6 (0.7)	NS
1-Nonanol	7 (2)a	10 (3)b	14 (1)b	**	4.9 (0.8)a	7 (2)a	10 (1)b	*	5.5 (0.8)	7 (2)	7.3 (0.6)	NS
(Z)-3-Nonen-1-ol	0.7 (0.2)	1.0 (0.3)	ND	NS	ND	ND	ND		0.5 (0.1)	0.7 (0.3)	ND	NS
2-Decen-1-ol	8 (2)a	13 (3)b	16 (1)b	**	2.5 (0.6)a	6 (3)ab	9 (1)b	**	6 (1)	5 (1)	5.6 (0.1)	NS
Benzyl alcohol	62 (18)	73 (35)	35 (4)	-	75 (10)b	50 (17)a	35 (4)a	**	434 (71)c	263 (89)b	120 (6)a	***
Phenylethyl alcohol	290 (79)a	699 (310)b	368 (46)a	*	275 (34)	361 (117)	267 (30)	NS	456 (87)a	952 (277)b	438 (26)a	**
Homovanillyl alcohol	1.7 (0.7)	2 (1)	1.1 (0.1)	NS	1.8 (0.5)	2 (2)	0.41 (0.09)	NS	6 (4)b	3 (2)ab	0.8 (0.4)a	*
Acids												
Acetic acid	213 (82)c	134 (96)b	50 (7)a	*	325 (78)b	89 (27)a	85 (10)a	***	444 (48)c	133 (58)b	41 (5)a	***
Propanoic acid	69 (21)b	79 (32)b	25 (4)a	*	459 (61)	580 (145)	449 (59)	NS	230 (23)b	216 (85)b	57 (5)a	***
Isobutanoic acid	ND	8 (4)b	3.1 (0.7)a	*	0.5 (0.5)a	4 (1)b	3.01 (0.05)b	***	2 (1)a	17 (5)c	4.1 (0.2)b	***
Butanoic acid	0.6 (0.7)a	4 (2)b	2.4 (0.4)b	**	5 (2)a	13 (3)b	8 (1)a	**	95 (100)	46 (50)	14 (2)	NS
2-Methylbutanoic acid	22 (6)	53 (26)	26 (5)	-	3.4 (0.7)a	29 (10)b	28 (3)b	***	23 (5)	78 (22)	37 (7)	-
Hexanoic acid	7 (2)	12 (6)	8 (1)	-	4.3 (0.8)a	9 (4)ab	10 (1)b	**	14 (3)	16 (5)	11 (1)	-
Heptanoic acid	2.6 (0.8)	4 (2)	4.6 (0.9)	NS	1.0 (0.4)a	3 (2)b	4.2 (0.7)b	**	1.4 (0.3)a	2.4 (0.5)b	2.3 (0.3)b	**
(E)-3-Hexenoic acid	0.4 (0.3)	1.8 (0.9)	0.9 (0.1)	-	0.2 (0.1)	0.3 (0.1)	0.40 (0.09)	NS	0.6 (0.2)	1.2 (0.6)	0.5 (0.1)	NS
Octanoic acid	4 (1)a	7 (3)b	10.3 (0.6)c	**	5 (2)a	10 (3)b	16 (3)c	**	9 (4)	13 (6)	13 (0.8)	NS
Nonanoic acid	1.3 (0.4)a	2.2 (0.8)b	3.4 (0.3)c	**	1.1 (0.5)	1.3 (0.5)	2.2 (0.3)	NS	0.6 (0.1)	1.3 (0.6)	1.2 (0.4)	-
Decanoic acid	1.6 (0.4)a	2.3 (0.9)a	4.2 (0.3)b	***	0.5 (0.2)a	0.7 (0.1)a	1.5 (0.3)b	***	1 (1)	1.2 (0.7)	1.4 (0.5)	-

Benzoic acid	1.3 (0.5)a	5 (2)b	3.9 (0.9)b	*	1.6 (0.4)a	2.2 (0.8)ab	3.1 (0.6)b	*	4.8 (0.9)	8 (4)	5 (1)	NS
Esters												
Ethyl acetate	34 (23)b	5 (4)a	1.8 (0.4)a	*	64 (14)c	4 (2)b	1.20 (0.08)a	***	163 (33)b	38 (24)a	9 (2)a	***
Methyl propanoate	5 (2)b	8 (3)b	1.1 (0.3)a	**	53 (5)b	47 (24)b	8.5 (0.2)a	*	20 (4)b	32 (15)b	1.8 (0.4)a	**
Ethyl propanoate	ND	4 (1)	ND		71 (22)b	39 (18)a	ND	*	124 (26)	89 (68)	ND	NS
Propyl acetate	6 (9)	1.5 (0.6)	1 (1)	-	123 (28)c	30 (13)b	5.0 (0.4)a	***	11 (4)	12 (9)	5 (2)	-
Methyl butanoate	1.6 (0.6)	1.4 (0.3)	1.3 (0.1)	NS	1.7 (0.3)	2 (0.9)	1.5 (0.3)	-	9 (8)ab	4 (2)b	1.6 (0.3)a	*
Methyl 2-methylbutanoate	2.3 (0.6)a	5 (2)b	1.3 (0.1)a	**	1.0 (0.1)	2.0 (0.9)	1.3 (0.1)	NS	3.0 (0.7)	11 (8)	1.6 (0.1)	-
Methyl 3-methylbutanoate	ND	5 (2)b	0.5 (0.1)a	**	1.0 (0.4)	2 (1)	0.40 (0.07)	NS	2.0 (0.4)	14 (11)	0.8 (0.1)	-
Ethyl butanoate	ND	ND	ND		0.1 (0.1)a	0.9 (0.5)b	ND	**	59 (62)	14 (10)	ND	NS
Propyl propanoate	ND	3 (1)	3.2 (0.5)	NS	559 (119)b	604 (214)b	175 (29)a	*	13 (7)	54 (52)	43 (11)	NS
Ethyl 2-methylbutanoate	1.7 (0.7)	1.6 (0.9)	0.5 (0.5)	-	0.2 (0.2)a	0.9 (0.4)b	ND	**	8 (2)	19 (19)	6 (1)	NS
Ethyl 3-methylbutanoate	0.6 (0.3)a	1.5 (0.7)b	0.3 (0.1)a	**	0.4 (0.6)	1.0 (0.5)	ND	NS	4.8 (0.9)	21 (22)	9 (2)	NS
Isoamyl acetate	3 (1)	3 (1)	4.7 (0.7)	NS	7 (7)	9 (8)	ND	NS	23 (9)	34 (25)	ND	NS
Propyl butanoate	ND	0.2 (0.3)	ND		10 (2)b	15 (10)b	4.1 (0.5)a	**	15 (15)	19 (14)	24 (5)	NS
Methyl hexanoate	2 (1)	3 (2)	0.4 (0.1)	-	11 (2)b	5 (3)a	ND	**	10 (2)	8 (7)	0.6 (0.1)	NS
Ethyl hexanoate	1.1 (0.3)	0.8 (0.4)	ND	NS	0.4 (0.3)ab	1.0 (0.5)b	0.21 (0.00)a	*	9 (2)c	5 (2)b	0.53 (0.05)a	***
Methyl (E)-3-hexenoate	0.2 (0.2)	0.4 (0.3)	ND	NS	0.9 (0.2)b	0.4 (0.4)a	0.06 (0.01)a	**	0.8 (0.2)	0.6 (0.4)	ND	NS
Hexyl acetate	0.4 (0.2)	0.4 (0.5)	ND	NS	1.3 (0.2)b	0.9 (0.5)ab	0.30 (0.06)a	***	3.7 (0.7)	4 (3)	5 (1)	-
Ethyl (E)-3-hexenoate	ND	ND	ND		ND	ND	ND		0.5 (0.2)	0.7 (0.6)	ND	NS
(Z)-3-Hexenyl acetate	ND	7 (2)	ND		7 (2)b	4 (2)a	3.5 (0.4)a	**	13 (2)	15 (10)	18 (5)	-
Methyl lactate	5 (2)	6 (4)	3.3 (0.6)	-	2.6 (0.6)	0.4 (0.4)	2.5 (0.4)	-	8 (2)b	6 (2)b	2.9 (0.2)a	*
Ethyl lactate	0.6 (0.2)a	0.4 (0.4)a	1.1 (0.3)b	*	1.0 (0.2)a	1 (1)a	2.9 (0.3)b	**	6 (1)b	3 (1)a	6 (1)b	***
Methyl octanoate	ND	6 (2)b	1.5 (0.1)a	*	6 (3)b	7 (3)b	1.8 (0.2)a	**	8 (4)	15 (12)	2.5 (0.3)	NS
Ethyl octanoate	1.0 (0.3)	1.2 (0.5)	0.46 (0.02)	-	2.8 (0.8)b	2.4 (0.7)b	0.44 (0.06)a	**	15 (8)	13 (10)	5.5 (0.5)	NS
Isoamyl lactate	ND	0.8 (0.4)	1.0 (0.2)	NS	ND	ND	0.5 (0.1)		ND	5 (1)	3.7 (0.8)	NS
Isobornyl acetate	2 (2)	3 (3)	4.0 (0.3)	NS	1.5 (0.6)a	4 (2)ab	6 (2)b	**	0.8 (0.5)a	4 (1)c	1.9 (0.1)b	**

Methyl decanoate	1.1 (0.3)a	3 (1)b	1.4 (0.2)a	**	0.6 (0.1)b	1.1 (0.2)c	0.26 (0.01)a	**	1.3 (0.9)	2 (1)	1.04 (0.07)	NS
Methyl benzoate	1.1 (0.4)a	4 (1)b	ND	**	1.4 (0.1)	2.6 (1)	ND	NS	4.0 (0.8)a	15 (5)b	ND	**
Ethyl decanoate	0.7 (0.2)	1.1 (0.4)	0.7 (0.3)	NS	1.3 (0.7)	0.8 (0.3)	0.52 (0.09)	NS	2 (1)ab	4.1 (0.8)b	1.8 (0.2)a	*
Ethyl benzoate	0.8 (0.9)ab	1.3 (0.5)b	0.52 (0.03)a	*	2.6 (0.6)	1.7 (0.9)	ND	NS	9 (1)a	18 (8)b	6.7 (0.9)a	*
Benzyl acetate	3 (1)a	6 (2)b	5.9 (0.7)b	*	6 (1)c	4 (2)b	0.7 (0.1)a	**	14 (3)	22 (10)	20 (3)	NS
Propyl benzoate	ND	ND	ND		1.7 (0.4)a	5 (2)b	5.0 (0.5)b	***	ND	1.1 (0.5)	1.1 (0.1)	NS
Methyl salicylate	2.7 (0.8)a	5 (1)b	4.7 (0.4)b	**	1.6 (0.4)b	1.1 (0.3)ab	0.7 (0.2)a	*	10 (1)	11 (4)	9 (1)	-
Ethyl phenylacetate	ND	ND	ND		0.5 (0.2)	ND	ND		1.6 (0.3)	ND	1.9 (0.1)	NS
Benzyl propanoate	ND	ND	ND		4 (1)a	13 (4)b	5 (0.7)a	**	ND	3.0 (0.9)	2.8 (0.2)	NS
Ethyl salicylate	0.8 (0.2)a	1.5 (0.5)b	1.8 (0.2)b	**	1.8 (0.3)b	1.8 (0.7)ab	1.07 (0.08)a	**	3.5 (0.4)a	6 (2)b	5.5 (0.4)b	*
Methyl hydrocinnamate	3.5 (0.9)b	8 (2)c	0.5 (0.2)a	***	35 (7)c	13 (6)b	1.1 (0.3)a	***	12 (2)	12 (3)	0.60 (0.07)	-
Ethyl hydrocinnamate	ND	1.6 (0.3)	ND		20 (4)a	73 (18)b	ND	**	18 (2)b	12 (4)a	ND	**
Triacetin	27 (18)	41 (24)	ND	NS	ND	3 (3)	ND		22 (6)	25 (17)	ND	NS
Ethyl 3-cyclohexenecarboxylate	ND	0.6 (0.3)	0.8 (0.3)	NS	0.3 (0.4)	0.2 (0.1)	ND	NS	0.9 (0.2)b	0.8 (0.3)b	0.4 (0.1)a	*
Hydrocarbons												
Octane	39 (12)a	36 (23)a	88 (14)b	**	3.4 (0.9)a	4 (3)a	8 (1)b	*	11 (3)a	9 (6)a	32 (5)b	***
Decane	10 (5)	9 (3)	11 (1)	NS	23 (8)b	11 (5)a	24 (3)b	*	63 (7)b	22 (9)a	14 (2)a	***
Toluene	2 (1)	9 (2)	13 (1)	-	1.7 (0.5)	7 (1)	11.7 (0.3)	-	1.6 (0.2)	11 (10)	10.4 (0.6)	-
p-Xylene	2.0 (0.8)	8 (4)	13 (1)	-	1.7 (0.2)	6 (2)	10.2 (0.6)	-	3.2 (0.6)	10 (10)	14 (3)	-
o-Xylene	1.2 (0.3)	3 (1)	4.4 (0.5)	-	0.8 (0.1)	2.2 (0.5)	3.5 (0.1)	-	1.3 (0.1)	3 (3)	4.1 (0.5)	-
Styrene	0.4 (0.3)a	1.2 (0.6)b	2.1 (0.3)c	***	12 (3)	13 (5)	15 (2)	NS	22 (5)	16 (14)	20 (3)	-
Pseudocumene	ND	2.7 (0.7)a	3.7 (0.4)b	*	1.3 (0.2)a	3.0 (0.4)b	3.90 (0.08)c	***	ND	3 (1)	3.6 (0.3)	-
2-Bornene	27 (8)a	47 (16)b	72 (7)c	***	6.5 (0.9)a	9 (3)ab	14 (1)b	**	33 (5)	50 (23)	61 (5)	-
Aldehydes/Ketones												
Methylpropenal	1.1 (0.5)a	1.8 (0.4)b	ND	*	0.8 (0.4)a	1.0 (0.5)a	5.1 (0.8)b	***	2 (1)	2 (1)	3 (2)	-
Pentanal	ND	1.5 (0.4)	1.3 (0.3)	NS	3 (2)	1.4 (0.6)	5.2 (0.6)	-	5 (1)	3 (2)	6 (1)	-
Hexanal	0.2 (0.1)	0.30 (0.04)	ND	NS	0.1 (0.1)	0.2 (0.2)	0.74 (0.05)	-	0.3 (0.1)ab	0.1 (0.2)a	0.42 (0.01)b	*

Heptanal	0.6 (0.5)	0.5 (0.2)	0.5 (0.2)	-	1 (1)	0.3 (0.1)	ND	NS	1 (1)	0.3 (0.2)	0.6 (0.2)	-
Octanal	1.7 (0.5)a	3.1 (0.8)b	1.7 (0.5)a	**	3 (1)	3 (1)	3.4 (0.4)	NS	4 (1)	3 (1)	2.1 (0.9)	NS
1-Octen-3-one	4 (1)a	7 (2)b	2.2 (0.6)a	**	9 (6)	11 (4)	13 (2)	NS	9 (3)	7 (4)	7 (3)	NS
(E)-2-Heptenal	3 (3)	15 (5)	10 (3)	-	15 (8)a	21 (8)a	35 (6)b	*	17 (6)	17 (10)	28 (14)	NS
Nonanal	ND	1.8 (0.2)	2.1 (0.4)	NS	1.1 (0.4)	1.5 (0.5)	2.0 (0.1)	NS	1.3 (0.3)	1.6 (0.4)	1.8 (0.4)	NS
(E)-2-Octenal	0.9 (0.3)a	2.6 (0.5)b	2.8 (0.5)b	***	1.5 (0.8)a	3 (1)a	4.2 (0.4)b	*	1.8 (0.6)a	1.7 (0.9)a	5 (1)b	**
(E,E)-2,4-Heptadienal	ND	6 (1)b	2.0 (0.6)a	**	4 (2)a	6 (2)ab	9 (1)b	*	4 (3)	6 (4)	6 (4)	NS
Benzaldehyde	9 (5)	9 (4)	5 (1)	NS	16 (3)b	8 (4)a	5.12 (0.03)a	**	46 (6)b	15 (3)a	10 (1)a	***
Phenylacetaldehyde	ND	8 (4)	13 (2)	NS	ND	2 (1)a	3.1 (0.3)b	*	15 (3)	12 (3)	10 (1)	NS
(E)-2-Decenal	12 (3)a	23 (3)b	34 (4)c	***	7 (5)a	10 (4)a	21 (2)b	**	12 (4)a	10 (7)a	27 (7)b	**
(E,E)-2,4-Decadienal	0.5 (0.1)a	2.0 (0.6)b	4.2 (0.4)c	***	0.5 (0.2)a	0.3 (0.4)a	2.8 (0.5)b	***	0.6 (0.2)a	0.8 (0.4)a	4.1 (0.4)b	***
Terpenes												
Limonene	2 (2)a	3 (3)ab	6.5 (0.8)b	**	0.5 (0.5)a	3 (2)b	ND	*	1.0 (0.3)	1.4 (0.5)	1.4 (0.1)	NS
(E)- β -Ocimene	ND	1.0 (0.2)a	2.5 (0.3)b	**	0.7 (0.1)	1.2 (0.8)	1.0 (0.2)	-	1.4 (0.3)	1.0 (0.7)	1.6 (0.5)	NS
6-Methyl-5-hepten-2-one	4 (1)	3.3 (0.5)	2.5 (0.4)	NS	3 (1)	2.6 (0.9)	3.5 (0.2)	-	7 (1)	4 (2)	5 (2)	-
(E)-Rose oxide	ND	0.6 (0.2)	0.9 (0.1)	NS	0.9 (0.3)a	ND	2.7 (0.1)b	***	0.9 (0.2)a	2 (1)ab	2.3 (0.2)b	*
Linalool oxide	1.3 (0.4)	3 (1)	1.6 (0.2)	-	0.7 (0.2)	2 (1)	1.3 (0.1)	NS	3.8 (0.8)a	5.9 (0.7)b	4 (1)a	**
(+)-Cycloisosativene	ND	ND	ND		1.0 (0.2)b	0.7 (0.2)a	1.6 (0.2)c	***	1.0 (0.2)	1.2 (0.4)	1.2 (0.1)	-
Copaene	1.6 (0.6)	2.2 (0.8)	2.6 (0.1)	-	8 (1)a	11 (4)ab	13 (2)b	*	20 (4)	25 (7)	26 (1)	NS
Dihydroedulan	10 (3)a	12 (4)ab	16 (2)b	*	4.1 (0.6)a	5 (2)ab	5.5 (0.4)b	*	7 (2)	7 (3)	6.7 (0.4)	NS
Linalool	8 (3)	9 (4)	13 (1)	NS	2.7 (0.4)	3 (2)	4.2 (0.6)	-	6.4 (0.8)	7 (3)	7.8 (0.8)	NS
Caryophyllene	ND	ND	ND		0.3 (0.1)	0.2 (0.2)	ND	NS	0.30 (0.05)	ND	ND	
α -Terpineol	5 (2)	6 (2)	7.7 (0.7)	NS	57 (8)	53 (19)	61 (8)	NS	14 (2)	12 (3)	11 (1)	NS
α -Muurolene	ND	0.2 (0.1)	0.21 (0.03)	NS	1.3 (0.2)	1.6 (0.4)	1.9 (0.3)	NS	3.1 (0.4)	3 (1)	3.0 (0.3)	NS
β -Citronellol	0.4 (0.3)b	0.1 (0.1)a	0.2 (0.1)ab	*	1.2 (0.3)	1.4 (0.4)	1.6 (0.3)	NS	1.7 (0.7)	1.7 (0.6)	1.5 (0.1)	-
β -Damascenone	3.0 (0.8)a	4 (1)a	6.8 (0.5)b	**	2.6 (0.4)	3 (1)	3.5 (0.6)	NS	6.6 (0.8)	6 (3)	8 (1)	NS
Isogeraniol	1.6 (0.5)	1.5 (0.6)	1.7 (0.1)	NS	2.7 (0.4)	1.8 (0.7)	1.9 (0.3)	NS	3.4 (0.3)b	2.0 (0.6)a	1.8 (0.1)a	***
Geraniol	5 (1)	4 (2)	3.4 (0.2)	NS	11 (2)b	4 (2)a	3.5 (0.6)a	***	21 (2)b	8 (3)a	5.3 (0.3)a	***

Iridomyrmecin	1.7 (0.5)	2.1 (0.9)	2.0 (0.3)	NS	6.4 (0.9)b	4 (1)a	4.3 (0.6)a	*	9 (5)	8 (2)	5.5 (0.6)	-
Other compounds												
Dimethyl sulfide	27 (12)	31 (26)	28 (2)	NS	10 (1)	7 (4)	9 (2)	NS	25 (2)b	21 (11)ab	12.4 (0.1)a	***
Theaspirane A	13 (4)a	17 (6)ab	23 (2)b	*	5.7 (0.9)a	7 (2)ab	8.2 (0.7)b	**	21 (3)	23 (9)	24 (2)	NS
Theaspirane B	14 (3)a	18 (6)ab	24 (3)b	*	6.0 (0.9)a	7 (2)ab	9.0 (0.9)b	*	24 (4)	25 (10)	25 (1)	NS
Dimethyl sulfoxide	5 (2)	4 (3)	1.6 (0.5)	-	1.3 (0.5)	0.8 (0.3)	1.0 (0.2)	NS	3.9 (0.8)c	1.7 (0.7)b	0.8 (0.1)a	***
Butyrolactone	ND	1.5 (0.9)	ND		0.7 (0.1)	0.9 (0.3)	ND	NS	1.7 (0.2)	1.4 (0.3)	ND	NS
p-Vinylanisole	ND	ND	ND		0.4 (0.1)a	0.9 (0.3)b	1.0 (0.1)b	**	1.7 (0.2)a	4 (1)b	3.7 (0.6)b	**
1,4-Dimethoxybenzene	ND	3.7 (0.7)a	8.3 (0.8)b	***	ND	4 (2)a	8.5 (0.1)b	**	0.4 (0.5)	4 (1)	5.8 (0.4)	-

^a Concentrations are expressed as $\mu\text{g kg}^{-1}$ of 3-octanol (standard deviations in parentheses). For each cultivar, means in the same row labelled with different letters are significantly different ($P < 0.05$). Means in columns A and B are from two replicate fermenters, each analyzed intriplicate; in column C, means are from triplicate analyses from a composite sample, which was made up of three bottles; ND, not detected (limit of detection 0.05).

^b Probability that there is a difference between means; NS, no significant difference between means ($P > 0.05$); * significant at the 5% level; ** significant at the 1% level; *** significant at the 0.1% level.

^c -, ANOVA test was not performed because normality conditions were violated in at least one group.

Figure 1
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Highlights

- More than 130 compounds were identified in Spanish-style green table olives.
- The contents of volatiles were affected by post-fermentation and packing stages.
- Significant differences in volatile compounds between olive cultivars were found.
- **Lipid oxidation likely occurred as a result of packing in the cultivars studied.**