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

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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.

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 (1.82.5%, *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, Montaño, 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 qualiy. 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 & 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 (Cano-Lamadrid et al., 2015; Cortés-Delgado et al., 2016; Iraqi, Vermeulen, Benzekri, Bouseta, & Collin, 2005; Montaño, Sánchez, & Rejano, 1990; Sabatini & Marsilio, 2008). 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

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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 °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, Billerica, MA). All other chemicals and solvents were of analytical or chromatographic grade from various suppliers (Panreac, Barcelona, Spain; VWR, Barcelona, Spain; Merck, Darmstadt, Germany).

2.2. 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 is usually done in the industry.

2.3. 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.

2.4. Physicochemical 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 microorganisms 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, UK) 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 (Cole et al., 2009; Wang, Garrity, Tiedje, & Cole, 2007). 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.

2.5. 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*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 a refractive index detector with deionized water (pH adjusted to 2.2 using concentrated H_3PO_4) as the mobile phase (Sánchez, de Castro, Rejano, & Montaño, 2000).

2.6. Analysis of volatile compounds

Volatile compounds were analyzed by solid-phase microextraction (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})