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Combined use of nitrogen and coatings to improve the quality of mechanically harvested Manzanilla olives

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ABSTRACT

The combined effect of an edible coating and a nitrogen atmosphere on the quality of Manzanilla olives mechanically harvested and processed as Spanish-style green olives was assessed. The percentage of olives free of any brown spots ranged between 35–50%, 10–25% and 50–65% for fruit directly processed, storage under nitrogen and coated and storage under nitrogen respectively. Moreover, olives stored in the open air developed brown spots due to the oxidation of oleuropein. By contrast, the anoxic conditions prevented oleuropein from undergoing enzymatic oxidation but not from its enzymatic hydrolysis. Hence, the phenolic derivative HyEDA was formed in olives stored under nitrogen, and this substance was rapidly oxidized in the open air to give rise to brown spots although to a lesser extent in the coated fruit. Therefore, the postharvest storage of coated olives under nitrogen can be a good method to prevent bruise damage in mechanically harvested fruit.

1. Introduction

Among table olive varieties (*Olea europaea* L.), the Manzanilla is one of the most susceptible to bruising during harvesting and postharvest handling (Jiménez-Jiménez et al., 2013; Zipori, Dag, Tugendhaft, & Birger, 2014). These olives are traditionally picked by hand which is a very high cost operation and requires many workers. For this reason, mechanical harvesting is highly demanded by farmers (Ferguson, 2006; Gambella, Dimauro, & Paschino, 2013; Saracoglu, Ucer, & Ozarslan, 2011). In Spain, low prone to bruising fruit such as the Hojiblanca variety are mechanically harvested as well as most olives intended for oil extraction. However, fruit damage has limited the use of this technology for the Manzanilla variety, particularly when they must be processed as Spanish-style green olives.

Bruising damage consists of a browning of the olive area mainly affected by the fruit-fruit and fruit-branch impact during mechanical harvesting. The cellular tissue is disrupted, leading to the release of polyphenoloxidase (PPO) and its phenolics substrate, resulting in physical contact. In the presence of oxygen, oleuropein, which is the major phenolic compound in olive pulp, is oxidized, giving rise to the formation of brown polymers (García et al., 2008; Ramírez, García-García, De Castro, Romero, & Brenes, 2013; Sánchez, Romero, Ramírez, & Brenes, 2013).

Early on, Ben-Shalom, Harel, and Mayer (1978) found that dipping the injured olives in a week NaOH solution (0.2–0.4%) could prevent the formation of the brown spots, and this method has been confirmed later by other researchers (Rejano, Sánchez, & Vega, 2008; Zipori et al., 2014). Nevertheless, the alkaline solution must be refrigerated to prolong the postharvest period by at least 10–12 h. In addition, leaves and small branches must be removed at the groves before the olives are covered with the weak alkaline solution to avoid blockage into the pumps. An alternative method to preserve the olive quality of damaged fruit is based on the use of a nitrogen atmosphere (Segovia-Bravo, García-García, López-López, & Garrido-Fernández, 2012). Bruising was negligible on olives stored under these anoxic conditions for 6 h but brown spots rapidly appeared after the exposure of the fruit to open air conditions (Sánchez et al., 2013). It seemed that oxygen penetrated into the olive flesh very fast and the oxidative reactions started again once the nitrogen atmosphere was released. Upon the arrival of the olives to the factories, at least 15 min are necessary to cover them with the NaOH solution used for their processing as Spanish-style green olives, and therefore strategies for reducing browning during this period are mandatory.

The effects of edible coatings on retarding browning in harvested fruit have been extensively reported (Chauhan, Raju, Singh, & Bawa, 2011; Hernández-Muñoz, Almenar, Del Valle,

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Velez, & Gavara, 2008; Pereira, Machado, & de Costa, 2013). They provide a semi-permeable barrier to oxygen resulting in browning inhibition that has been suggested for glycerol coated olives (Sánchez et al., 2013). Hence, the aim of this work was to investigate the combined use of coatings and nitrogen atmosphere during the postharvest of the Manzanilla variety to optimize the quality of these fruits processed as Spanish-style green olives.

2. Materials and methods

2.1. Experiments simulating mechanical harvesting

2.1.1. Preliminary survey of coatings

Fruit of the Manzanilla variety (*O. europaea* L.) were hand-harvested from an olive grove located in the province of Seville (Spain) and transported to the laboratory within 1 h. They had an optimal green-yellow colour and were macroscopically free of damage and disease. Simulated bruising was carried out in a controlled manner by allowing the fruit to drop freely onto the cement floor from a height of a 2.5 m. The damaged fruit were dipped in several coating solutions for 5 s and stored under a nitrogen atmosphere for 3 h. Subsequently, the olives were exposed to the open air for 15 min and submerged in a 0.5 M NaOH solution (lye) for 7 h until the alkali penetrated two-thirds of the way to the pit. Finally, the olives were washed with tap water for 12 h and exposed to the open air for 15 min in order to visually detect brown spots on the fruit surface by table olive experts. The coatings used in this experiments were 1% glycerol, and several commercial coatings employed for citrus products such as Citrosol 686 (Carnauba + Shellac), Citrosol 680 (Carnauba + Shellac), Citrosol 652 (Polyethylene + Shellac) and Citrosol 642 (Polyethylene + Shellac) (Productos Citrosol S. A., Valencia, Spain). These coatings are marketed for maintaining the commercial quality of fruits, and particularly to control postharvest diseases.

2.1.2. Study of phenolic compounds and PPO in damaged olives

Simulated bruised olives of the Manzanilla variety were left in the open air for 24 h or alternatively under nitrogen atmosphere for 23 h plus one hour in the open air.

In another experiment, bruised olives were stored (i) under a nitrogen atmosphere for 23 h, (ii) under a nitrogen atmosphere for 23 h plus one hour in the open air, and (iii) dipped in a Citrosol 642 solution for 5 s, left under the nitrogen atmosphere for 23 h and finally in the open air for 3 h.

Phenolic compounds were analysed in the bruised and unbruised areas of the damaged olives according to the method described elsewhere (Sánchez et al., 2013). Briefly, these substances were extracted from the olive pulp with dimethyl sulfoxide (DMSO). Around 0.1 g of olive pulp from bruised and unbruised areas were put into contact with 0.5 mL DMSO, vortexed for 1 min and sonicated for 5 min. After 30 min, the mixture was centrifuged at 6000*g* for 5 min, and 0.25 mL of the supernatant were diluted with 0.5 mL DMSO and 0.25 mL of 0.2 mM syringic acid (internal standard). Finally, the mixture was filtered through 0.22 μm pore size nylon filter and 20 μL were injected into the chromatograph. All analyses were run in triplicate.

Polyphenol oxidase (PPO) activity was also determined in the bruised and unbruised areas of the damaged olives. The enzyme extraction was carried out from a protein precipitate as described elsewhere (Sciancalepore & Longone, 1984). Acetone powders were obtained from 50 g of olive pulp homogenized with 100 mL of cold acetone (−30 °C) containing 2.5 g of polyethylene glycol. The residue was re-extracted three times with 100 mL of cold acetone, obtaining a white powder that was dried overnight at room temperature to remove residual acetone. The acetone powder (0.5 g) was suspended in 20 mL of a 0.1 M phosphate buffer, containing 1 M KCl and the pH was adjusted at 6.2 units with NaOH. The suspension was stirred at 4 °C for 30 min and then centrifuged at 15,550*g* for 20 min at 4 °C. The pellet was discarded and the supernatant divided into two aliquots; one was used as the active crude enzymatic extract, and the other was boiled for 30 min to obtain the denatured enzymatic extract.

The PPO activity was determined spectrophotometrically by using a Shimadzu UV-1800 spectrophotometer as described elsewhere (Hornero-Méndez, Gallardo-Guerrero, Jarén-Galán, & Mínguez-Mosquera, 2002). All measurements of PPO activity were carried out with 4-methylcatechol as substrate by measuring the change in absorbance at 410 nm at 25 °C for 10 min at intervals of 5 s. The incubation mixture contained 0.5 mL of enzyme preparation and 2.5 mL of 0.1 M sodium citrate buffer at pH 5 containing 0.02 M of substrate. The assay mixture with the denatured enzymatic extract served as the control. One unit of enzymatic activity was defined as the amount of the enzyme giving, under the above-mentioned conditions, a change in absorbance of 0.05 unit AU/min (e.a.u.). Data were expressed as e.a.u./mL of enzymatic extract. All reactions were carried out in duplicate.

2.2. Postharvest storage and processing of mechanically harvested olives

2.2.1. Plant material

Olives of the Manzanilla variety were mechanically harvested by trunk shakers from olive groves located in the province of Seville (Spain) during the season 2012/2013. Leaves and small branches were removed at the groves and the olives were transported in less than 30 min to the factory. All olives were harvested at their optimal green-yellow surface colour.

2.2.2. Experiment A

Upon arrival at the olive factory, 4 kg of fruit were covered with a 0.5 M NaOH solution and maintained until the alkali penetrated two-thirds of the way to the pit of the olives (ca. 7 h) (Control 0 h). Another lot of damaged fruit was left in the open air for 5 h before the alkali treatment (Control 5 h). Three lots of olives were dipped in 5% glycerol, 100% Citrosol 642 and 50% Citrosol 642 for 5 s before the fruit were stored under a nitrogen atmosphere in 5 L stainless steel containers for 5 h. After the storage period in the inert atmosphere, these olives were left in the open air for 15 min and dipped in the alkali solution.

All the fruit submitted to the alkaline treatment was then washed with tap water for 12 h and subsequently covered with a 12% NaCl solution where spontaneous lactic acid fermentation occurred for months.

All experiments were run in duplicate, and the quality analyses were carried out after 8 months of fermentation.

2.2.3. Experiment B

Because of the great variability among olive batches, a new experiment was design to confirm results obtained in Experiment A but treatment with glycerol was eliminated. The damaged fruit was treated directly with the NaOH solution (Control) or stored under a nitrogen atmosphere for 5 h. In the latter case, two lots of the olives were dipped in 100% Citrosol 642 or 50% Citrosol 642 solutions for 5 s previously to the inert atmosphere storage. Olives were left in the open air for 15 min and dipped in the alkali solution. All fruits were then processed as mentioned in Experiment A.

This Experiment B was run with olives from two different groves (batches A and B) and in duplicate. The quality analyses were carried out after 8 months of fermentation.