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Study of microbiological quality of controlled atmosphere packaged 'Ambrunés' sweet cherries and subsequent shelf-life

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ABSTRACT

The objectives of this study were to evaluate the effect of different controlled atmospheres, containing 3% O_2 + 10% CO_2, 5% O_2 + 10% CO_2 and 8% O_2 + 10% CO_2, on changes in microbial population of 'Ambrunés' sweet cherries throughout storage during 30 days and subsequent shelf-life, as well as to identify the main genera of yeast, mould, lactic acid bacteria, *Staphylococcus* spp., *Pseudomonas* spp., *Enterobacteriaceae* spp., and coliforms. The results indicated that controlled atmospheres with 5% O_2 + 10% CO_2 and 8% O_2 + 10% CO_2 were highly effective to control the growth of mesophilic aerobic bacteria, psychrotrophs, *Pseudomonas* spp., yeasts, and moulds after 15 days of storage, showing, in the case of yeasts and moulds, counts that ranged between <1 and 1.75 log CFU/g. The genera of yeasts, moulds, lactic acid bacteria, *Enterobacteriaceae* spp., and coliforms identified were *Aureobasidium* spp., *Penicillium* spp., *Leuconostoc* spp., and *Rahnella* spp., respectively. In addition, the genera *Staphylococcus* spp. and *Pseudomonas* spp. were also identified. On the other hand, cherries of Stage 3 ripening presented the highest counts for all microbial groups.

1. Introduction

The commercial designation "Picota" embraces four traditional late varieties of sweet cherry from the "Valle del Jerte" (Cáceres, Spain) harvested without stems. These are: 'Ambrunés', 'Pico Limón', 'Pico Negro', and 'Pico Colorado'. These cultivars are marketed under the protected designation of origin (PDO) "Cereza del Jerte". The "Cereza del Jerte" sweet cherry is a high quality fruit of *Prunus avium* L. varieties. The most widely studied is 'Ambrunés' because it is the most important in terms of production (Bernalte et al., 1999; Serradilla et al., 2011; Tudela et al., 2004).

During postharvest life, sweet cherries are highly perishable, and this represents a problem for the packing houses. For this reason, storage at low temperature is a necessary postharvest tool to stop fruit deterioration (Alique et al., 2005; Bernalte et al., 2003; Petracek et al., 2002). However, with refrigeration it is only possible to maintain sweet cherries for 16 or 17 days with an optimal quality (Bernalte et al., 2003; Serrano et al., 2005).

Postharvest losses are mainly due to fungal spoilage due to the genera *Penicillium*, *Botrytis*, *Rhizopus*, *Mucor*, *Cladosporium*, *Alternaria*, and *Monilia* that can gravely damage sweet cherries, in particular during longer storage periods (Chand-Goyal and Spotts, 1996; Valero and Serrano, 2010). Additionally, some of these moulds can be toxigenic or pathogenic. They could produce mycotoxins while growing on fruits (Stinson et al., 1980) even during refrigeration, (Tournas and Stack, 2001) or they could cause infections or allergies. Yeasts also form part of the natural microflora of fruits (Venturini et al., 2002). It has been observed that the role of yeast proliferation in the quality degradation of strawberries during refrigerated storage had a great influence on consumer's final acceptation (Ragaert et al., 2006). Other microbial groups such as mesophilic aerobic, psychrotrophs, lactic acid bacteria (LAB), *Staphylococcus* spp., *Enterobacteriaceae* spp., and *Pseudomonas* spp. could also be involved in the spoilage of vegetables (Abadias et al., 2008; Badosa et al., 2008), although the available information is very scarce. It has been reported that different microbial groups, such as LAB, *Pseudomonas* spp., and *Enterobacteriaceae* spp. have great importance in texture breakdown because these bacteria are related to the production of pectinolytic enzymes (Fraaije et al., 1997; Juven et al., 1985; Liao et al., 1997). In addition, pathogenic bacteria, such as *Salmonella* spp., have also been detected in grapes (Badosa et al., 2008).

Currently, controlled and modified atmospheres, together with low temperatures, are used to control fungal growth (Petracek et al., 2002; Prusky et al., 1997; Remón et al., 2000; Tian et al., 2001, 2004). It has been reported that moulds and Gram-negative aerobic bacteria are highly sensitive to CO_2, while low O_2 levels inhibit growth of most aerobic microorganisms, although low O_2 appears to have limited

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fungistatic properties and is less effective than elevated CO_2 (Conte et al., 2009; Tian et al., 2001). However, there are no published data on the use of controlled atmospheres to extend the postharvest life of 'Picota' type sweet cherries. In a controlled atmosphere, it is necessary to optimise CO_2 and O_2 concentrations for each cultivar and temperature during storage for cherry preservation.

The objective of our research was to examine the microbiological changes during cold storage under different controlled atmospheres, in particular, 3% O_2 + 10% CO_2, 5% O_2 + 10% CO_2, and 8% O_2 + 10% CO_2 to develop a system for maintaining microbiological quality during cold storage and prolonging postharvest life of 'Ambrunés' sweet cherry.

2. Materials and methods

2.1. Plant material

The samples of sweet cherry (*P. avium* L.) used in this study were obtained from fourteen-year-old sweet cherry trees of the 'Ambrunés' cultivar on *P. avium* L. rootstock, from an experimental orchard at an altitude of 400 m above sea level in Cabrero (lat. 40º 06′ 40″ N, long. 5º 53′ 20″ W), in the "Valle del Jerte" (Cáceres, Spain). Fruits were harvested totally at random from multiple trees and transported to the distribution centre in less than 1 h. They were then hydrocooled at a water temperature of 1 °C in a 1000 L immersion hydrocooler equipped with a water recirculation system. Sodium hypochlorite was added to the water to achieve a chlorine concentration of 100 μL L^{-1}. Then the cherries were grouped into 3 commercial ripening stages, based on the size and colour of the fruit, by an I20 automatic colour sorter (Multiscan Technologies, Alicante, Spain), with the following colour parameter setting: Stage 1: 34–49 (I20 automatic colour sorter, Multiscan Technologies, Alicante, Spain) corresponding to mean values of $L^* = 35.68$, $a^* = 34.03$, $b^* = 14.16$, $C^* = 36.89$, and hue = 22.33 (Minolta CR-400 Chroma Meter, Osaka, Japan); Stage 2: 25.5–34 ($L^* = 31.15$, $a^* = 22.47$, $b^* = 7.79$, $C^* = 23.78$, and hue = 18.94); and Stage 3: 0–25.5 ($L^* = 28.51$, $a^* = 14.62$, $b^* = 3.28$, $C^* = 14.98$, and hue =12.67).

2.2. Storage conditions

Fruits (500 g approx.) were packaged in polypropylene punnets and wrapped with macroperforated (mp) film with five holes (ø =9 mm). Storage conditions were as follows: control (without control atmosphere) and controlled atmosphere containing 3% O_2 + 10% CO_2, 5% O_2 + 10% CO_2 and 8% O_2 + 10% CO_2. Punnets, except in the control, were placed in controlled atmosphere cabinets, (530 dm^3) mod Control-Tec Research, with an atmosphere analyzer controlled by software (Tecnidex, Valencia, Spain). These cabinets were located inside refrigerator chambers. Initial O_2 and CO_2 levels in the cabinets were established by a flow-through system, mixing N_2, CO_2 and air via pressure regulators, automatically controlled and regulated by the analyzer. A total of 156 punnets were classified into 5 different lots:

(1) Lot 1: 1 batch of 4 punnets for each ripening stage was randomly selected and analysed immediately after the hydrocooling treatment (0 days of storage).
(2) Lot 2: 3 batches of 4 punnets for each ripening stage were randomly selected and stored at 1 °C, 95% (RH) in darkness for 15, 21, and 30 days under controlled atmosphere containing 3% O_2 and 10% CO_2.
(3) Lot 3: 3 batches of 4 punnets for each ripening stage were randomly selected and stored at 1 °C, 95% (RH) in darkness for 15, 21, and 30 days under controlled atmosphere containing 5% O_2 and 10% CO_2.
(4) Lot 4: 3 batches of 4 punnets for each ripening stage were randomly selected and stored at 1 °C, 95% (RH) in darkness for 15, 21, and 30 days under controlled atmosphere containing 8% O_2 and 10% CO_2.
(5) Lot 5 (Control): 3 batches of 4 punnets for each ripening stage were randomly selected and stored at 1 °C, 95% (RH) in darkness for 15, 21, and 30 days under ordinary atmosphere.

In all analyses, 4 punnets (for each ripening stage, storage date and treatment) were sampled at random from refrigerator chambers, from which 2 were analysed immediately after cold storage and 2 after a shelf life period under an ordinary atmosphere that consisted of 2 days at 5 °C (for transport simulation) and 2 days of storage at 20 °C.

2.3. Physicochemical analysis

2.3.1. Titratable acidity and pH

Titratable acidity (TA) and pH were measured for each independent homogenate per batch, obtained from 25 fresh pitted fruits from each punnet (n = 4) and homogenised using an Omni Mixer homogeniser (Omni International, Marietta, GA, USA). TA and pH were determined in 5 g aliquots diluted to 50 mL with de-ionised water from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Analyses were conducted using a 716 DMS Titrino automatic titrator (Metrohm, Herisau, Switzerland). Samples were titrated with 0.1 mol/L NaOH up to pH 8.1. Results are expressed as g malic acid per 100 g fresh weight (FW).

2.4. Microbial counts

For the microbial counts and isolates, three independent replicates of 10 g of sweet cherry sample per batch were homogenised in 90 mL sterile 0.1% (p/v) peptone water in a Stomacher (Lab Blender, Model 4001, Seward Medical, London, UK) for 30 s. Serial 10-fold dilutions were prepared from the same solution with 0.1% (p/v) peptone water, and 0.1 mL of aliquots were inoculated onto agar plates under the following conditions. The mesophilic and psychrotrophic aerobic bacteria counts were done on Plate Count Agar (PCA, Oxoid, Unipath, Basingstoke, UK) for 48 h at 30 ± 1 °C and 7 days at 5 ± 1 °C, respectively. Total enterobacteria (Gram-negative and cytochrome oxidase-negative) were inoculated on Violet Red Bile Glucose Agar (VRBGA; Oxoid), the plates were covered with a layer of the same medium before incubation at 30 °C ± 1 °C for 24 h, and colonies that were rose-coloured and surrounded by a halo of purple precipitate were counted. Violet Red Bile Agar (VRBA) was used for coliform counts and the inoculated plates of this medium were also covered with a layer of the same medium before incubation at 37 °C for 48 h. Typical dark red colonies (>0.5 mm in diameter) surrounded by a zone of precipitated bile acids were considered as coliforms for the counts. *Staphylococcus* spp. on Baird Parker Agar Base (BP, Oxoid) was also used, supplemented with potassium tellurite and egg yolk emulsion at 37 °C for 48 h, and black colonies were counted. Lactic acid bacteria (LAB) were determined on MRS Agar (Oxoid) with pH adjusted to 5.6 with acetic acid (10%), incubating at 37 °C for 2 days under anaerobic conditions. *Pseudomonas* spp. were plated on *Pseudomonas* Agar Base (*Ps*AB, Oxoid) for 48 h at 30 ± 1 °C. Yeasts and moulds were counted on Potato Dextrose Agar (PDA, Oxoid) and incubated at 25 ± 1 °C for 4 days. For proper counting, plates with 30 to 300 colony forming units (CFUs) were considered.

2.5. Microbial identification

2.5.1. Identification of the strains by 16S rRNA sequence analysis

The different colonies isolated from the sweet cherries were identified by 16S rRNA gene sequencing analysis. Five colonies from the plates with the highest dilutions were isolated at random on 5 mL of nutrient broth to collect strains. Twenty-two isolates from MRS and thirty from *Ps*AB, BP, VRBGA, and VRBA agar respectively, were sequenced directly. The DNA was obtained and subjected to the 16S rRNA gene sequence analysis (Benito et al., 2008a,b; Ruiz-Moyano