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Instability profile of fresh packed "seasoned" Manzanilla-Aloreña table olives

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ABSTRACT

The shelf life of cracked "seasoned" Manzanilla-Aloreña table olives is short; containers may swell and the fruits become progressively brownish. Respiration of cracked fruits continued for a period longer than 48 h after picking and the carbon dioxide produced may be an initial cause of spoilage. As processing progressed, the microbial loads in the olives increased due to successive washings and, together with the microbial load from the added ingredients, led to relatively high viable counts of yeasts and LAB at packing. During shelf life, this microflora showed an increase in viable counts which could also eventually lead to swelling spoilage. This behaviour was favoured by the presence of residual fermentable substrates and a decrease in potassium sorbate over time in both brine and flesh. At the same time, colour darkened, titratable acidity increased and pH decreased. The application of two additional washings to the cracked olives before packing was favourable because fermentable material and polyphenols were removed; however, fruits with as low as possible respiration rates, stronger washing treatments, ingredients with low or no microbial viable counts and higher preservative concentrations would be necessary to achieve complete stabilization of the product.

1. Introduction

The cracked, green, "seasoned" Manzanilla-Aloreña is a table olive specialty that is gaining the favour of consumers and increasing its production, which reached 7,000,000 kg in the 2005/2006 season, due to a progressive interest in traditional and natural foodstuffs on the part of consumers. These fruits are picked in the green maturation stage, sorted and graded by size, washed, and cracked. Then, part of the crop is directly packed and the rest is stored in brine (11 g NaCl/100 mL) and packed throughout the year according to demand. The packing line consists of a washing bath, followed by filling equipment for fruits, where the olives are mixed with the homemade seasoning material (garlic, red pepper strips, fennel, and thyme), and a filling device for brine. Finally, the tops of the containers are fixed by hand and the product is commercialized in different size jars or plastic containers (0.9–2.7 kg olives) (Garrido Fernández et al., 2002). Unfortunately, the packed "seasoned" Manzanilla-Aloreña olives have a rather limited shelf life because the freshness of the product, especially its natural green colour, which is highly appreciated by the market, is rapidly lost and the production of gas leads to the swelling of containers and the clouding of brines (Garrido Fernández et al., 2002). So, complete stabilization of the final product is not achieved under the current packing conditions.

An initial origin of the gas found in freshly packed products could be the respiration process of the fruits themselves (Forcier, Raghavan, & Gariépy, 1987). García, Durán, and Garrido (1982) showed that this process may be responsible for most of the carbon dioxide (CO_2) produced during the first days of ripe olive processing. Romero, Brenes, García, and Garrido (1996) also reported CO_2 production and O_2 consumption by Hojiblanca olives stored in sterile water. The respiration process (CO_2 production and O_2 consumption) of cracked olives has not yet been studied.

Previous works have shown the presence of fermentative yeasts such as *Saccharomyces cerevisiae* and *Issatchenkia occidentalis* in the brines of cracked packed "seasoned" table olives (Arroyo-López, Durán Quintana, Ruiz Barba, Querol, & Garrido Fernández, 2006). Other microorganisms such as lactic acid bacteria (LAB) and *Enterobacteriaceae* were also present in the final packed products (Arroyo-López et al., 2005). However, a systematic study to identify the key microbiological and physicochemical factors related to the changes produced during shelf life, which, eventually, may lead to the spoilage of this specialty, is not yet available.

The addition of potassium sorbate as a preservative for green olives is well known practice (Marsilio & Chichelli, 1992; Rodríguez de la Borbolla & Fernández Díez, 1961). Turantas et al. (1999) used this preservative during the fermentation process of naturally black

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olives with a marked reduction in the yeast viable counts and a slight stimulation of the LAB viable counts. The use of this weak acid is also common in "seasoned" table olives (Garrido Fernández, Fernández Díez, & Adams, 1997). Polyphenols have a marked inhibitory effect on microorganisms related to table olives (Durán, García, Brenes, & Garrido, 1994) and their polymerization may contribute to browning (Garrido Fernández et al., 1997).

The aim of this work was to examine the factors which might be involved in the quality deterioration and eventual spoilage of fresh, packed, cracked "seasoned" Manzanilla-Aloreña olives. The study comprises (1) respiration and the colour of fresh, whole and cracked, fruits; (2) changes in the microbial viable counts in fruits during conditioning; (3) microbial viable counts present in the "seasoning" material; and (4) microbial viable counts and physicochemical changes in products subjected or not to additional washings, packed with or in the absence of potassium sorbate.

2. Material and methods

2.1. Respiratory activity of fresh fruits

The method applied was similar to that used by Romero et al. (1996) to determine the CO_2 production of Hojiblanca olives in fruits exposed to air. A respirometer model Micro-Oxymax O_2/CO_2 (Columbus Instruments, Ohio, USA) was used to determine the CO_2 produced and O_2 consumed by fresh Manzanilla-Aloreña olives when exposed to air. Five intact (fresh, whole) or cracked olives ($\approx$25 g) were cleaned and placed in sterile glass jars and incubated at 25 °C. The jars were closed and connected to the respirometer to determine the consumption of O_2 and the production of CO_2 by measuring their concentrations in the atmosphere surrounding the fruits every 4 h for 2 days. After each measurement, the air in the jars was replaced with fresh, dry air. So the respiration of the olives was tested in what can be considered an open system. Respiratory activity was expressed as millimols (mmol) CO_2 or O_2, produced and consumed (respectively), per hour and kilogram of fruits (mmol/kg*h). All experiments were carried out in duplicate. The respiratory quotient (RQ) was calculated from the equation: RQ = Rate CO_2 produced/Rate O_2 consumed.

2.2. Microbiological analysis of fruit surface and brine throughout processing

The method applied to determine the microbial load on the surface of the fruits was similar to that used by Pelagatti (1978) to determine the LAB associated to the olive surface. Fresh, hand picked Manzanilla-Aloreña olives, transported to the factory within 12 h after harvesting in 1000 kg bins (wire netting walls), were collected upon arrival at the processing plant (Aceitunas Bravo S.A., Alhaurin el Grande, Málaga, Spain). Five olives ($\approx$25 g) were placed into a 200 mL sterile glass bottle with 50 mL of sterile peptone water (0.1 g/100 mL). After 2 h of shaking, this solution was plated onto different culture media using a spiral plate maker model dwScientific (Don Whitley Scientific Limited, England). Samples of fruits were also analyzed after the different processing phases (first washing, cracking, second washing, and packing).

The microbiological analyses included yeasts, LAB, *Enterobacteriaceae*, and aerobic mesophilic. *Enterobacteriaceae* were counted on VRBD (Crystal-violet Neutral-Red bile glucose) agar (Merck, Darmstadt, Germany), LAB on MRS (de Man, Rogosa and Sharpe) agar (Oxoid LTD, Basingstoke, England) with 0.02 g/100 mL sodium azide (Sigma, St. Louis, USA), and yeasts on YM (yeast–malt–peptone–glucose) agar (Difco™, Becton and Dickinson Company, Sparks, MD, USA) supplemented with 0.005 g/100 mL gentamicin sulphate. Aerobic mesophilic microorganisms were numbered on PCA (Tryptone–glucose–yeast extract) agar (Oxoid LTD, Basingstoke, England). Those catalase-positive colonies with suspicious morphology (rough and with a diameter of 5 mm or more after 24 h of incubation) were examined under a phase contrast microscope (Olympus Optical Co., LTD Tokyo, Japan) to look for spores. Those showing spores were considered *Bacillus* spp according to Sneath (1986). All plates were incubated aerobically at 30 °C for 48 h and then counted using a CounterMat v.3.10 (IUL, Barcelona, Spain). The cell concentrations were calculated as recommended by the manufacturers of both pieces of equipment and expressed as $\log_{10}$ cfu/g.

Samples of seasoning material and filling brines were also plated onto similar culture media to determine their microbial loads. All analyses were carried out in duplicate.

2.3. Packing procedure

The study focused on the packing of fresh cracked "seasoned" Manzanilla-Aloreña olives under usual industrial conditions (Aceitunas Bravo S.A., Alhaurín el Grande, Málaga, Spain), but other alternative processes were also investigated. A scheme of the design showing the different treatments that comprised the experiment is shown in Fig. 1. Common operations to all treatments were the initial fresh olive washing with tap water, cracking and second washing with tap water. Cracking was achieved in an automatic machine consisting in two parallel iron plates, which separation was adjustable; the top plate was attached to a piston which, periodically lowed this plane to the minimum separation distance (slightly greater than the olive pit diameter) while breaking the olives without affecting the pit. Then, part of the olives were additionally washed two times for 12 h with 11 g NaCl/100 mL brine (washed fruits, W) and the rest were packed directly (unwashed fruits, U, or usual conditions). Each fruit type, added

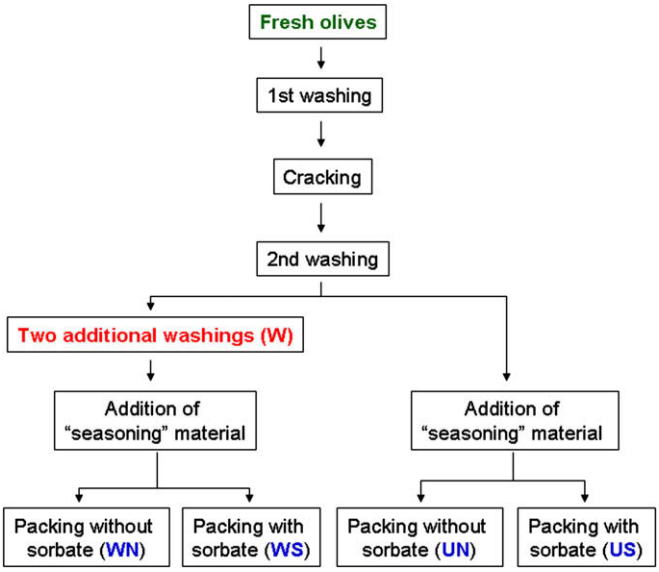

Fig. 1. Scheme of the experimental design. It includes the usual industrial practices and modified treatments. Common first and second washings were accomplished with tap water. Additional washings were carried out with brine (11 g NaCl/100 mL) for 12 h. The whole experiment was then composed of four different treatments whose factors were washing system (W, additional two washings; U, unwashed) and preservation method (S, packed with potassium sorbate; N, packed without preservatives). The "seasoning" material consisted of natural garlic, fennel, and thyme as well as preserved red pepper strips. The approximate proportion was of 200 g "seasoning"/kg of final product. Concentration of potassium sorbate in the initial packing brine was 0.02 g/100 mL. Packing containers had 0.9 kg olives and 0.7 L brine.