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Evaluation of different conditions to enhance the performances of *Lactobacillus pentosus* OM13 during industrial production of Spanish-style table olives

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ABSTRACT

The main objective was to set up a methodology to improve the high volume production of green table olives, cv. Nocellara del Belice. *Lactobacillus pentosus* OM13 was applied during three different industrial processes of table olives as follows: trial one (IOP1) was subjected to an addition of lactic acid until a brine level of pH 7.0 was reached; trial two (IOP2) subjected to same addition of lactic acid as in trial one plus nutrient adjuvant and trial three (IOP3) subjected to same addition as trial two, but with the strain *L. pentosus* OM13 acclimatized in brine for 12 h before inoculation. These trials were compared against two untreated controls (spontaneously fermented and addition of *L. pentosus* OM13 only).

Within the third day of fermentation, the pH of the brines decreased significantly, reaching pH 4.85 for trial three, pH 5.15 for trial two, and pH 5.92 for trial one. The pH of both controls decreased more slowly, and had values below pH 5.0 only after the fifteenth day of fermentation (control one) and the sixty-fifth day of fermentation (control two). Trial three reached the highest lactic acid bacteria (LAB) concentration on the third day of fermentation. After six days of fermentation, all trials showed similar values of LAB counts that were significantly higher compared to control number one. The result from genotypic identification showed that *L. pentosus* OM13 was the most frequently isolated in the inoculated trials. *Lactobacillus plantarum*, *Lactobacillus coryniformis* and *Pediococcus pentosaceous* were also detected at very low concentrations. Homoguaiacol, 2-butanol, 4-ethylphenol, phenylethyl alcohol and 4-ethylphenol were the volatile organic compounds detected at the highest levels in all experimental trials. Trial three showed a higher concentration of squalene that was not detected in other trials. The highest sensory scores of green olive aroma and overall satisfaction were found for all experimental olives, especially for those of trial one and trial two, that differed significantly from the untreated controls.

This study provides evidence that the addition of lactic acid, nutrient adjuvants and, most importantly, the acclimatization of LAB cells significantly shortens the acidification process of olive brine, and improves safety and sensory quality. Shorter acidification processes result in a more rapid transformation of table olives, with reduced commodity loss and lower costs of production compared to conventional manufacturing protocols.

1. Introduction

The use of starter cultures is widely applied in food fermentations (Corsetti et al., 2012), including table olive production (Roig and Hernández, 1991; Rodríguez-Gómez et al., 2013). Several

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studies have been performed to select the most adapted lactic acid bacteria (LAB) to be used as starters for table olive production (Servili et al., 2006; Aponte et al., 2012; Rodríguez-Gómez et al., 2013; Zago et al., 2013). The spontaneous fermentation, although still widely applied for table olive transformation, is an uncontrolled biological process.

The main purpose of using starter cultures during table olive manufacturing is to drive the fermentation and inhibit the development of spoilage microorganisms, such as pseudomonads, Enterobacteriaceae and staphylococci. In the absence of consistent levels of LAB, undesired microbial groups can rapidly increase and negatively affect the final product. Starter cultures may stabilize the manufacturing process in terms of chemico-physical, microbiological and sensory quality of table olives. The technological characteristics of starter cultures for table olive production include mainly the ability to grow at 15 °C, in presence of different concentrations of salt and phenolic compounds. Therefore, high levels of viability and efficiency of starter cultures are required to create the rapid drop of brine pH during the production of hydrogen sulphide; the β-glucosidase, lipolytic and proteolytic activities (Rodríguez-Gómez et al., 2010). Furthermore, the use of starter cultures may improve the food shelf-life by inhibition of spoilage microorganisms based on nutrient competition (Fernández-Díez et al., 1985; Garrido-Fernández et al., 1997; Durán Quintana et al., 1999; Holzapfel, 2002; Devlieghere et al., 2004; Silvestri et al., 2009), and should improve aroma and flavor of final products not only by inhibition of the spoilage microorganisms that interfere with the process of aroma generation, but also directly through their metabolism (Aponte et al., 2010). Until now, *Lactobacillus plantarum* (Lu et al., 2003) and *Lactobacillus pentosus* (Rodríguez-Gómez et al., 2013) have been the primary LAB species commonly applied to produce fermented table olives (Hurtado et al., 2012). However, some yeasts, such as *Wickerhamomyces anomalus*, *Saccharomyces cerevisiae* and *Pichia membranifaciens* have been selected as starters for this kind of production (Garrido-Fernández et al., 1997; Bautista-Gallego et al., 2011; Arroyo-López et al., 2012).

In order to enhance the activity of the starter cultures, the addition of some specific nutrient is suggested. Panagou et al. (2003) demonstrated that the death rate of Enterobacteriaceae and pseudomonads in presence of *L. pentosus* was more rapid in brines supplemented with glucose. Roig and Hernández (1991) suggested that enrichment of the brine with a nutritive supplement at the time of inoculation may increase the viability and efficiency of starter cultures, creating the rapid drop of brine pH. Within the published literature, however, little information is available on the use of supplements and adjuvants for fermenting table olives.

The main objective of this work was to set up a methodology to improve the high volume production of green table olives. To this end, the strain *L. pentosus* OM13 [selected previously (Aponte et al., 2012) and proved to be promising at pilot-plant scale (Martorana et al., 2015)], was applied during the industrial process, after an acclimatization procedure and in presence of supplements and adjuvants. The study was carried out with drupes of the cultivar Nocellara del Belice, and microbiological, chemical and sensory parameters were evaluated.

2. Materials and methods

2.1. Olive processing, experimental design and sample collection

A total of 250 tons of olives “Nocellara del Belice” were harvested at maturity. The drupes were selected, calibrated, washed by water and processed according to Sevillian technology, as follows: the drupes were supplemented with lye (2.6 °Bè) to remove the bitterness and, after 8 h, three sequential washings were performed with complete water replacement.

The bulk olives were transferred into 12.5 ton fiberglass vats [10 t of drupes and 2.5 t of brine (10% w/v NaCl)] and divided into three aliquots representing three experimental trials named IOP1, IOP2 and IOP3 (Fig. 1). Trial IOP1 was subjected to an addition of lactic acid (80% w/v, Purac, Biochem, Netherlands) until pH of the brine was 7.0. Trial IOP2 was added with lactic acid as in trial IOP1 plus 2 kg/t of the nutrient LBO2014 (Lallemand, Inc., Montreal, Canada) consisting of glucose, fructose and yeast autolysates for LAB nutritional requirements. Both trials IOP1 and IOP2 were inoculated with *L. pentosus* OM13 (150 g/t of olives) in freeze-dried form (Lallemand, Inc., Montreal, Canada) containing approximately 1.1×10^9 colony forming units CFU/g. This inoculum allowed a cell concentration of the starter strain of about 10^7 CFU/ml as confirmed by plate counts (Martorana et al., 2015). Trial IOP3 was subjected to the same additions as trial IOP2, but the starter culture *L. pentosus* OM13 was acclimatized in brine (6% w/v NaCl) for 12 h at room temperature before inoculation. Also for this trial the cell density reached in brine was approximately 10^7 CFU/ml.

Two untreated controls (C1 and C2) were also included in the experimental design. Both untreated controls did not receive any supplement. The fermentation of C1 was carried out by the indigenous drupe microorganisms. C2 was inoculated with the freeze-dried *L. pentosus* OM13 as reported above for trials IOP1 and IOP2.

The fermentation of all trials was performed at room temperature for 195 d and periodically monitored. Samples of brine (approximately 50 ml) were collected before addition of adjuvants and starter cultures, soon after their addition and after 3, 6, 9, 15, 35, 65, 143 and 195 d of fermentation. The experiment was performed in duplicate (two vats per trial). Two independent productions were performed in two consecutive weeks during October 2014 at the Geolive SAS company located in Castelvetrano (Trapani province, Siciliy, Italy) (37° 36′ 46″ N/12°50′ 52″ E).

2.2. Physico-chemical and microbiological analyses

Brine pH was measured using pH meter Russell RL060P (Thermo Fisher Scientific, Beverly, MA). The samples of brine were serially diluted in Ringer's solution (Sigma-Aldrich, Milan, Italy). Mesophilic rod LAB on de Man-Rogosa-Sharpe (MRS) agar added with cycloeximide (10 mg/ml), to avoid yeast growth. Yeasts, Enterobacteriaceae, pseudomonads, staphylococci and coagulase-positive staphylococci (CPS) were investigated as reported by Martorana et al. (2015). All analyses were performed in triplicate.

2.3. Isolation and phenotypic grouping of LAB

LAB were isolated after growth on MRS agar. At least five colonies per morphology were randomly collected from the agar plates and purified to homogeneity after several subculturing steps onto MRS agar. After microscopic inspection, three isolates (from each sample) sharing the same cellular morphology were stored at −80 °C. All isolates were subjected to Gregersen KOH method (Gregersen, 1978) and the test for the enzyme catalase (5%, w/v, H_2O_2). The isolates were grouped according to their cell morphology and disposition, growth at 15 and 45 °C and metabolism type (Martorana et al., 2015).

2.4. Genotypic characterization of LAB at species and strain level

All Gram positive and catalase negative isolates were subjected to genotypic investigation. The DNA from the presumptive LAB was extracted using the InstaGene Matrix kit (Bio-Rad Laboratories, Hercules, CA, USA) (Martorana et al., 2015).