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An innovative method to produce green table olives based on "pied de cuve" technology

Alessandra Martorana [a], Antonio Alfonzo [a], Luca Settanni [a], Onofrio Corona [a], Francesco La Croce [b], Tiziano Caruso [a], Giancarlo Moschetti [a], Nicola Francesca [a, *]

[a] Department of Agricultural and Forest Science, University of Palermo, Viale delle Scienze 4, 90128 Palermo, Italy
[b] Geolive Belice S.A.S. Olive da tavola Castelvetrano, TP, Italy

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ABSTRACT

The technology of "pied de cuve" (PdC) is applied in food process only to produce wines with an enriched community of pro-technological yeasts. PdC promotes the growth of the desirable microbial strains in a small volume of grape must acting as a starter inoculums for higher volumes. The aim of the present work was to investigate the use of partially fermented brines, a technology known as PdC, developed with lactic acid bacteria (LAB) on the microbiological, chemical and sensory characteristics of green fermented table olives during two consecutive campaigns. The experimental plan included two trials based on different PdCs: trial A, PdC obtained with *Lactobacillus pentosus* OM13; trial B, PdC obtained through a spontaneous fermentation. Two control additional trials without PdC were included for comparison: trial C, spontaneous fermentation; trial D, direct inoculation of *L. pentosus* OM13. The use of PdCs favoured the rapid increase of LAB concentrations in both trials A and B. These trials showed levels of LAB higher than trial C and almost superimposable to that of trial D. Trial B was characterized by a certain diversity of *L. pentosus* strains and some of them dominated the manufacturing process. These results indicated PdC as a valuable method to favour the growth of autochthonous *L. pentosus* strains. Hierarchical cluster analysis (HCA) and principal component analysis (PCA) visibly discriminated olive processes fermented with the two experimental PdCs. Interestingly, on the basis of microbial and pH variables, both approaches showed that the olives produced with PdC technology are closely related to those of trial D, with the advantages of reducing the amount of starter to inoculate (trial A) and a higher LAB biodiversity (trial B). Volatile organic compound (VOC) composition and sensory analysis showed trials A and B different from the trials with no PdC added, in both years. Furthermore, the trial B showed the highest scores of green olive aroma and taste complexity. Spoilage microorganisms were estimated at very low levels in all trials. Undesired off-odours and off-flavours were not revealed at the end of the process.

1. Introduction

The 95% of world cultivation of olive trees (*Olea europaea* L.) is concentrated in the Mediterranean area. The International Olive Council (IOC) estimated a total world production of 2.5 million tons for the 2012/2013 campaign, and about 76.000 tons of this production is obtained in Italy (IOC, 2013). Olive drupes, just after harvest, cannot be eaten due to the presence of oleuropein that is a bitter phenolic glucoside consisting of glucose, elenolic acid and *o*-diphenol hydroxytyrosol compounds (Servili et al., 2006). A variety of technological methods are commonly applied during transformation to reduce the bitterness of table olives. "Spanish" (also known as "Sevillan") and "Greek" (also known as "natural") processing styles are the methods most quantitatively employed in Italy for table olive production (Catania et al., 2014). Most table olive fermentation processes start spontaneously (Silvestri et al., 2009; Tofalo et al., 2013), whereas several productions at industrial level are driven by *Lactobacillus plantarum* and *Lactobacillus pentosus* (Lu et al., 2003; Servili et al., 2006; Hurtado et al., 2012).

Several studies (Guzzon et al., 2011; Tofalo et al., 2014, 2012a; Sannino et al., 2013) showed that the foods produced through spontaneous fermentations are often characterized by marked

* Corresponding author. Tel.: +39 091 23896050; fax: +39 091 6515531.
E-mail address: nicola.francesca@unipa.it (N. Francesca).

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sensory profiles. However, the risk of off-odour and off-flavour generation, due to the development of spoilage microorganisms during the uncontrolled biological processes, has to be considered, because they may seriously affect the quality of the final products.

Generally, the direct inoculums of commercial starter cultures into olive brine assure a rapid increase of LAB concentration and the corresponding decrease of pH. This procedure limits the risk of undesired aromatic notes (Servili et al., 2006; Peres et al., 2008; Sabatini et al., 2008; Aponte et al., 2012). However, when a given starter preparation, composed of a limited number of strains, is used to ferment different varieties of olives of different geographical areas a flattening of the taste of table olives may occur, with the risk that the final products may no longer be distinguishable by production technology and/or geographical origin. This because the starter strains prevail over the native microflora (Aponte et al., 2012). For this reason, the use of selected autochthonous strains is becoming a common practise for table olive fermentation (Aponte et al., 2010, 2012; Bautista-Gallego et al., 2013; Di Cagno et al., 2013), but the microbial diversity that contributes to the expression of the varietal notes in the final products is extremely reduced.

The technology of "pied de cuve" (PdC), largely used for wine production (Ubeda Iranzo et al., 2000; Clavijo et al., 2011; Li et al., 2012), limits the reduction of the microbial complexity of the driven processes. This method promote the growth of the desirable microbial strains in a small volume of grape must which act as a starter inoculums for higher volumes, but does not exclude the risk of development of unwanted microorganisms. Usually, the ratio PdC/bulk for wine application is 1/10 (Li et al., 2012).

The present work was aimed to produce green table olives applying PdC technology. PdCs were obtained differently to evaluate the dominance of the LAB population: A, direct inoculum of commercial LAB starter; B, by spontaneous fermentation. Microbiological, chemical and sensory parameters were evaluated during two consecutive years.

2. Materials and methods

2.1. Experimental olive production and sample collection

Table olive production process was carried out with the cultivar "Nocellara del Belice" and performed at the company "Geolive Belice S.A.S." located in Castelvetrano (Trapani, Sicily, Italy). The olives were manually harvested from fields within Trapani province (37°37′11.29″N/12°50′33.27″E). The experimental plan included two technological steps: (i) preparation of different PdCs and 10 days of fermentation; (ii) *addition of* PdCs into fresh brines and olive production. The experimentation was carried out directly in brine in two consecutive years (2012 and 2013) adopting three replicates (three fermentation vessels per trial) in both years.

2.2. Preparation of PdC

Bulk olive fruits were transferred in two steel vats (180 l-volume) representing two different PdCs (A and B). Each vat contained 150 kg of olives and 30 l of brine composed of NaCl 9% (w/v). PdC A was inoculated with 0.15 g/kg of the autochthonous strain *L. pentosus* OM13, previously used to produce commercial Nocellara del Belice table olives in the Trapani province (Aponte et al., 2012), and kept freeze–dried (about 8.00×10^{12} CFU/g). PdC B was spontaneously fermented. The fermentation of both PdCs lasted 10 days and was carried out at room temperature (about 20–22 °C). Brine salt concentration was constantly maintained at the initial level by periodical addition of coarse salt.

2.3. Production of table olives

The two PdCs were transferred in six steel vats containing the same weight of olives and volume of brine as reported above and represented two distinct trials (A and B; preparing three replicates vats for each trial). The partially fermented brine of PdCs were added to a final ratio of 1:10 in each vat. In addition, two control trials without PdC (trials C and D) were included in the experimental plan. Trials C and D represented the control production for table olives obtained through spontaneous fermentation and with starter addition, respectively. Vats of trial C were spontaneously fermented. Vats of trial D were inoculated (0.1 g/l of brine) with the strain *L. pentosus* OM13. The fermentation of all trials was performed at room temperature for 200 d and was periodically monitored. Samples of brine (about 50 ml) were collected before inoculum of PdC and, then, soon after addition and at 3, 6, 9, 15, 25, 35, 65, 85, 115, 150 and 200 days of fermentation.

2.4. Physico-chemical and microbiological analyses

The values of pH of brine samples were determined by a pH meter (BASIC 20+; Crison Instrument S.A., Barcelona, Spain). Salt concentration was routinely analyzed as reported by Garrido Fernández et al. (1997).

Decimal dilutions of brines were prepared in Ringer's solution (Sigma–Aldrich, Milan, Italy). Different microbial groups were enumerated as follows: mesophilic rod LAB on de Man-Rogosa-Sharpe (MRS) agar, incubated anaerobically at 30 °C for 48 h; total yeasts (TY) and filamentous fungi on dichloran rose bengal chloramphenicol (DRBC) agar, incubated aerobically at 25 °C for 5 days; *Enterobacteriaceae* on violet red bile glucose agar (VRBGA), incubated aerobically at 37 °C for 24 h; pseudomonads on *Pseudomonas* agar base (PAB) supplemented with CFC supplement, incubated aerobically at 20 °C for 48 h; staphylococci on Baird Parker (BP) and coagulase positive staphylococci (CPS) on BP added with RPF supplement, incubated aerobically at 37 °C for 48 h. Analyses were performed in triplicate. All media and the supplements used were supplied from Oxoid (Thermofisher, Basingstoke UK).

2.5. Isolation and phenotypic grouping of LAB

Presumptive LAB (at least 4 colonies characterized by the same colour, morphology, edge, surface and elevation) were collected from the highest plated dilution following their growth on MRS agar. The isolates were purified by successive sub-culturing and the purity of the isolates were checked microscopically. Gram-positive (Gregersen KOH method) and catalase negative (determined in presence of H_2O_2 5%, v/v) were stored in broth containing 20% (v/v) glycerol at −80 °C until further experimentations.

LAB were initially subjected to a phenotypic grouping based on cell morphology and disposition, determined by an optical microscope, growth at 15 and 45 °C and metabolism type, testing the ability to produce CO_2 from glucose. The last assay was carried out with the same growth media used for isolation, without citrate from which certain LAB produce gas. The obligate homofermentative metabolism was determined by the absence of growth in presence of a mixture of pentose carbohydrates (xylose, arabinose, and ribose; 8 g/l each) in place of glucose. Sub-grouping of cocci included also the growth at pH 9.6 and in the presence of 6.5% (w/v) NaCl.

2.6. Identification of LAB at strain and species level

DNA from LAB isolates was extracted using the InstaGene Matrix kit (Bio-Rad Laboratories, Hercules, CA) according to the