Food Control 34 (2013) 96–105

Contents lists available at SciVerse ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Table olive fermentation with multifunctional *Lactobacillus pentosus* strains

F. Rodríguez-Gómez [a], J. Bautista-Gallego [a,b], F.N. Arroyo-López [a], V. Romero-Gil [a], R. Jiménez-Díaz [a], A. Garrido-Fernández [a], P. García-García [a,*]

[a] Food Biotechnology Department, Instituto de la Grasa (CSIC), Avda. Padre García Tejero 4, 41012 Seville, Spain
[b] DIVAPRA, Agricultural Microbiology and Food Technology Sector, Faculty of Agriculture, University of Turin, Via Leonardo da Vinci 44, 10095 Grugliasco, Torino, Italy

ARTICLE INFO

Article history:
Received 24 January 2013
Received in revised form
2 April 2013
Accepted 9 April 2013

Keywords:
Table olives
Fermentation
Lactobacillus pentosus
Probiotic
RAPD-PCR
Multifunctional starter

ABSTRACT

This study assesses the technological characteristics and the dominance in brines of four selected potential probiotic *Lactobacillus pentosus* strains during green table olive Spanish-style fermentations. When compared with the spontaneous process, the use of these microorganisms as starter led to higher lactic acid bacteria than yeast populations, a decrease in *Enterobacteriaceae* levels and a faster acidification of the brines. Microbial changes were properly modelled with equations including both growth and death phases. Diverse genetic profiles among the lactic acid bacteria populations were found at the end of fermentation. Hence, inoculation using the current green olive processing technology guaranteed the development of the typical Spanish-style with, as shown by discriminant function analysis, some distinctive characteristic processes, according to strains, but did not assure *per se* the imposition of the selected strain in the cover brines.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

The consumption of table olives is an ancient tradition in the countries around the Mediterranean Basin, which later extended worldwide. Their production reached 2,562,000 tons in 2011/2012 season (IOC, 2013), with approximately 60% of them processed as Spanish-style green table olives. The elaboration process for this type of table olive mainly consists of treating the olives with a diluted sodium hydroxide solution (lye) (1.8–2.5% NaOH, w/v) until the alkali reaches 2/3 of the flesh; then the fruits are washed to remove the excess alkali and covered with an initial 10–11% (w/v) NaCl brine solution (5% at equilibrium), where they undergo a lactic acid fermentation.

Traditionally, the fermentation process has been carried out spontaneously (Sánchez-Gómez, García-García, & Rejano-Navarro, 2006). However, the use of starter cultures in the elaboration of Spanish green table olives could be effective in order to control the process, so diverse studies have evaluated their application. Thereby, Rodríguez de la Borbolla y Alcalá, Fernández Díez, and González Cancho (1964) were the first to use pure starter cultures in the wooden barrels ("bocoyes") used at that time for the Spanish type elaboration. These authors concluded that the application was not necessary because of the rapid implantation of autochthonous lactobacilli. Sánchez, Rejano, Montaño, and de Castro (2001) studied the inoculation of *Lactobacillus pentosus* CECT 5138 at different time points and pH values ranging from 9 to 10 during the elaboration of Spanish style green table olives. They observed a 1–2 decimal log reduction in the initial population. However, depending on inoculation time, cultures grew and initiated an accelerated fermentation. De Castro, Montaño, Casado, Sánchez, and Rejano (2002) used a mixed culture of *Enterococcus casseliflavus* and *L. pentosus* as starter to ferment Spanish green table olives, reporting the good technological performance of both strains. The inoculation of *Lactobacillus plantarum* LPC10 (a bacteriocin producer strain) also led to a faster acidification and pH decrease than the spontaneous process (Vega Leal-Sánchez, et al., 2003). The use of starter cultures composed of three potential probiotic strains of *L. plantarum*, in combination with glucose, in "Bella di Cerignola" table olives, decreased the pH to 4.3–4.5 and controlled the growth of yeasts (Perricone, Bevilacqua, Corbo, & Sinigaglia, 2010). In general, authors agree that inoculation leads to a rapid acidification, a fast pH decrease, and a rapid carbohydrate consumption as well as the partial inhibition of the *Enterobacteriaceae* population.

However, the above mentioned works did not control the survival of the specific inoculated strain and the results could not be

* Corresponding author. Tel.: +34 954 690850; fax: +34 954 691262.
E-mail address: pedrog@cica.es (P. García-García).

0956-7135/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodcont.2013.04.010

attributed with total certainty to its activity. Furthermore, it is likely that the lactic acid bacteria (LAB) populations in such experiments were a mixture of diverse strains or even species. A molecular phylogenetic approach for LAB identification was assayed to allow for the definition of products from distinct regions and environments (Da Silva, Pereira, Oliveira, Peres, & Rocheta, 2002); the homology between *L. plantarum* strains from different olive varieties and geographic regions was studied by Microsatellite-Primed PCR, finding that those from the same olive variety were homogeneous within the same geographical region but vary from region to region. De Bellis, Valerio, Sisto, Lonigro, and Lavermicocca (2010) used Repetitive Extragenic Palindromic-PCR (REP-PCR) and 16S rRNA sequencing to study the genetic diversity of the microbiota adhered to the surface of the debittered green olives *cv.* Bella di Cerignola inoculated with *Lactobacillus paracasei* IPMC2.1. Random Amplification of Polymorphic DNA (RAPD-PCR) analysis is also a very common method applied for LAB strain differentiation (Arroyo-López et al., 2012; Rantsiou et al., 2006; Rossi, Torriani, & Dellaglio, 1998; Urso, Comi, & Cocolin, 2006).

The aim of the present survey is to assess the technological properties such as inhibition of spoilage microorganisms, yeast control, acidification rate, sugar consumption and dominance in the cover brines, of four potentially probiotic *L. pentosus* strains in the Spanish-style green table olive fermentation. This type of study is essential to assess the efficiency of using multifunctional (with both technological and probiotic properties) starter cultures for the production of table olives with a higher value added.

2. Materials and methods

2.1. Fruits and experimental design

The olives used in this research were of the Manzanilla variety (*Olea europaea pomiformis*), picked by hand at the green maturation stage (season 2009/2010), and supplied by JOLCA S.L. (Huevar del Aljarafe, Seville, Spain). Manzanilla cv. was chosen because it is widely used worldwide in manufacturing Spanish style green table olives.

The olives followed the standard debittering (2.2% NaOH for 6 h) and washing (20 h) processes used for the elaboration of Spanish style green table olives carried out at industry scale. Then, fruits (5.2 kg) were placed into ten cylindrical polypropylene fermentation vessels and covered with 3.5 L of 10% NaCl solution supplemented with 0.5% acetic acid (time 0) to partial neutralization of the excess alkali. After 2 days in brine, CO_2 was bubbled through the fermentation vessels (olives and brine) to produce a pH decrease from approximately 9.0 to 6.5 units.

2.2. Inoculation and fermentation

After pH adjustment, the fermentation systems were inoculated with overnight cultures (MRS at 37° for 18 h) of the four strains described below. These cultures were grown till early stationary phase. The inocula sizes were calculated to reach approximately 6 $\log_{10}$ cfu/mL in the cover brines, adding a volume of 0.03% of inoculum to the different fermentation vessels. Four *L. pentosus* strains isolated from diverse table olive processing (TOMC LAB2, TOMC LAB3, TOMC LAB4 and TOMC LAB5), belonging to the table olive microorganism collection from the Instituto de la Grasa (CSIC), were selected according to diverse *in vitro* phenotypic tests related to probiotic potential, such as resistance to gastric and pancreatic digestion, auto-aggregation, hydrophobicity, bacteriocin production, haemolytic activity and ability to deconjugate bile salt (Bautista Gallego et al., 2013), and used in the present study as inocula. The experiment consisted of: treatment F1, spontaneous and un-inoculated process; treatment F2, inoculated with TOMC LAB2 strain; treatment F3, inoculated with TOMC LAB3 strain; treatment F4, inoculated with TOMC LAB4 strains; and treatment F5, inoculated with TOMC LAB5 strain. All experiences were performed in duplicate using the same lot of fruits (two fermentation vessels for each treatment).

The fermentation vessels were kept in the pilot plant of the Instituto de la Grasa (CSIC, Seville, Spain), where the room temperature decreased progressively from 28 °C to 16 °C during the 120 days that the containers were monitored (from October 2009 to January 2010). Cover brines were supplemented with 0.65 g glucose/100 mL on the 55th and 100th day of fermentation due to the low initial sugar content of the fruits (data not shown). This practice is common during Spanish style olive processing to achieve adequate final pH values (below 4.2) and ensure the safe storage of the fermented olives (Garrido-Fernández, Fernández-Díez, & Adams, 1997).

2.3. Physicochemical analysis

Analysis of the pH of the cover brines was carried out with a pH meter model micropH 2001 (Crison). Reducing sugars in the cover brines (glucose, fructose, sucrose and mannitol), organic acids (lactic and acetic) and ethanol were determined by HPLC according to the protocols described by Rodríguez-Gómez, Bautista-Gallego, Romero-Gil, Arroyo-López, Garrido-Fernández, & García-García (2012).

Surface colour of olives was measured using a BYKGardner Model 9000 Colour-view spectrophotometer. Interference by stray light was minimized by covering the samples with a box having a matt black interior. Colour was expressed in terms of the CIE L^* a^* b^* parameters and as colour index (*Ci*), calculated according to Sánchez, Rejano, and Montaño (1985):

$$Ci = \frac{-2^*R_{560} + R_{590} + 4^*R_{635}}{3} \quad (1)$$

where R are the reflectance values at 560, 590 and 635 nm, respectively. The data of each measurement were the average of twenty olives.

The firmness of olives was measured using a Kramer shear compression cell coupled to an Instron Universal Machine (Canton, MA, USA). The crosshead speed was 200 mm/min. The firmness, expressed as N/100 g flesh, was the mean of ten replicate measurements, each of which was performed on three pitted olives.

2.4. Microbiological analyses

Brine samples or their decimal dilutions were plated using a Spiral System model dwScientific (Don Whitley Sci. Ltd., Shipley, U.K) on the media described below. Then, plates were counted using a CounterMat v.3.10 (IUL, Barcelona, Spain) image analysis system, and results expressed as $\log_{10}$ cfu/mL. *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) supplemented with 0.02% (wt/vol) sodium azide (Sigma, St. Luis, USA), and yeasts on YM (yeast-malt-peptone-glucose) agar (Difco™, Becton and Dickinson Company, Sparks, MD, USA) supplemented with oxytetracycline and gentamicin sulphate as selective agents for yeasts. Plates were incubated at 30 °C for 24 h (*Enterobacteriaceae*) or 48 h (yeasts and LAB).

Changes in the microbial populations *versus* time were modelled using the Two-term Gompertz equation (Bello & Sánchez Fuertes, 1995), in the case of growth and death, or the model of

Download English Version:

https://daneshyari.com/en/article/6392708

Download Persian Version:

https://daneshyari.com/article/6392708

Daneshyari.com