Food Microbiology 53 (2016) 71–75

Contents lists available at ScienceDirect

Food Microbiology

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

Short communication

Survival of potential probiotic lactic acid bacteria on fermented green table olives during packaging in polyethylene pouches at 4 and 20 °C

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ARTICLE INFO

Article history:
Received 15 February 2015
Received in revised form
31 August 2015
Accepted 3 September 2015
Available online 5 September 2015

Keywords:
Table olives
Lactobacillus pentosus
Lactobacillus plantarum
Packaging
Probiotics

ABSTRACT

The survival of selected lactic acid bacteria (LAB) with *in vitro* probiotic potential was studied during storage of cv. Halkidiki green olives previously subjected to inoculated Spanish-style fermentation. After fermentation olives were packed in polyethylene pouches, covered with freshly prepared brine (9%, w/v, NaCl), acidified with 2‰ (w/v) citric acid and 1.5‰ (w/v) ascorbic acid, and stored at 4 and 20 °C for 357 days. Four packing treatments were studied, namely olives previously fermented by (i) the indigenous microbiota (control); (ii) *Lactobacillus pentosus* B281; (iii) *Lactobacillus plantarum* B282; and (iv) a co-culture of both LAB strains. Microbiological analyses were performed on the olives in parallel with physicochemical changes (pH, titratable acidity, salt content, a_w and colour) at the early (day 1), middle (day 197) and final stage (day 357) of storage, as well as sensory evaluation at the end of the storage. The survival of probiotic strains was confirmed by Pulsed Field Gel Electrophoresis (PFGE). LAB decreased throughout storage reaching a final population of *ca.* 3.5–4.0 log CFU/g and 4.5–5.0 log CFU/g at 4 and 20 °C, respectively. The pH values ranged between 3.90 and 4.61 during storage depending on packaging condition. PFGE analysis revealed that *L. pentosus* B281 and *L. plantarum* B282 showed a high survival rate with a recovery of 100 and 96%, respectively, at 4 °C, and less than 20% for both strains at 20 °C. Finally, in the packing treatment with a co-culture of both strains, *L. pentosus* dominated over *L. plantarum* throughout storage at both temperatures.

1. Introduction

Table olives have been explored as a vehicle for incorporating bacterial species with probiotic potential, in an effort to develop a new plant based functional food. In the last years the focus on table olive research has shifted from the spontaneous process controlled by the indigenous microbiota to inoculated fermentations with selected starter cultures of LAB with probiotic potential in order to transform a traditional agricultural commodity into a novel high added value functional food providing new perspectives for the table olive industry (Lavermicocca et al., 2005; De Bellis et al., 2010; Argyri et al., 2014; Blana et al., 2014; Rodríguez-Gómez et al., 2014a). It needs to be noted however that the focus has been given on the production of probiotic table olives whereas there is little information on the survival of probiotic LAB strains on olive drupes during storage of the final product in retail packages. It is thus important to ensure the presence of the inoculated probiotic starter in high numbers not only at the end of fermentation but also during the shelf life of the product. In a recent work (Rodríguez-Gómez et al., 2014b), fermented Spanish style green olives were fortified with a probiotic strain of *Lactobacillus pentosus* TOMC-LAB2 after being packed in glass jars with brine and stored for 200 days at ambient temperature. The authors reported that the added LAB culture was able to colonize the olive surface and presented high recovery rates at the end of the shelf life, providing thus the possibility of successful enrichment of the microbiota of olive drupes with selected multifunctional starters. In another work (Argyri et al., 2015), green olives subjected to inoculated Spanish-style fermentation with probiotic LAB strains (*L. pentosus* B281 and *Lactobacillus plantarum* B282), were packed in polyethylene pouches under modified atmospheres (70% N_2–30% CO_2) and stored at 4 and 20 °C for 12 months. The authors reported that both strains presented high survival rates during storage with *L. pentosus* B281 exhibiting higher survival rates (94.1%) after six months of

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http://dx.doi.org/10.1016/j.fm.2015.09.004
0740-0020/

storage.

However, the application of modified atmospheres in polyethylene pouches is not a common practice employed by the table olive industry today that still prefers to use brine as a covering liquid in the pouches or other containers. Consequently, the present study is a continuation of a previously published work (Argyri et al., 2015) aiming to evaluate the ability of two *Lactobacillus* strains, namely *L. pentosus* B281 and *L. plantarum* B282, to survive and retain adequate populations during storage of Spanish style fermented green olives packed in polyethylene pouches and covered with brine for an extended period of time (12 months). Both selected LAB strains were investigated for their *in vitro* probiotic potential (Argyri et al., 2013) and employed successfully as starters in Spanish-style green olive fermentation (Blana et al., 2014).

2. Materials and methods

2.1. Samples treatment

Samples of approximately 150 g of green olives cv. Halkidiki after the end of fermentation described in detail elsewhere (Blana et al., 2014) were packed in multi-laminated polyethylene pouches (OPA 15 μm/PE 85 μm), covered with 200 mL of freshly prepared brine 9% (w/v, NaCl), initially acidified with 2‰ (w/v) citric acid and 1.5‰ (w/v) ascorbic acid and heat sealed using an industrial scale packing machine at the facilities of Konstantopoulos S.A. table olive industry located in Northern Greece. The selected concentrations of salt and acids were based on common practice followed by the Greek table olive industry today. The pouches were maintained at controlled temperatures (4 and 20 °C) in thermostatic chambers (MIR-153, Sanyo Electric Co., Osaka, Japan) for approximately 12 months (357 days). The experiment consisted of four packing treatments with olives previously fermented by (i) the indigenous microbiota (control); (ii) *L. pentosus* B281; (iii) *L. plantarum* B282; and (iv) a co-culture of both strains (B281 and B282).

2.2. Microbiological analyses

Duplicate packages were randomly removed during storage and analysed at the beginning (day 1), middle (day 196) and end of storage (day 357). The selective enumeration of Enterobacteriaceae, LAB and yeasts on olive drupes during storage was undertaken according to Blana et al. (2014).

2.3. Isolation and characterization of LAB

A total of 364 LAB isolates were picked at the same sampling times as for microbiological analysis from the highest dilution of MRS medium. The survival of the selected probiotic strains (*L. pentosus* B281 and *L. plantarum* B282) on the olive drupes during storage was determined using Pulsed Field Gel Electrophoresis (PFGE) as described elsewhere (Blana et al., 2014).

2.4. Physicochemical analyses

Physicochemical analyses were performed at the same time points as for microbiological analysis to monitor the changes in pH, titratable acidity, NaCl concentration, and water activity according to standard methods described elsewhere (Panagou, 2004). All determinations were carried out in duplicate and results are expressed as mean value ± standard deviation. The surface colour of the olive drupes was measured using the Minolta CR-300 (Minolta Ltd., Osaka, Japan) colorimeter. The instrument was calibrated using a reference white tile and colour was expressed as L^* (lightness/brightness), a^* (redness/greenness) and b^* (yellowness/blueness) parameters. Results were reported as total colour difference (ΔE) (Valera-Santos et al., 2012):

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where ΔL^*, Δa^* and Δb^* are the differences in the L^*, a^* and b^* values between olive samples and the reference tile ($L^* = 96.98$, $a^* = -0.81$ and $b^* = 3.19$) of the instrument. Three measurements on five different olive drupes were performed per packing treatment and results were averaged.

2.5. Organoleptic assessment

Sensory evaluation of olive samples was performed at the end of the storage period (357 days) by a taste panel consisted of ten persons according to the method of sensory analysis of table olives established by the International Olive Council (IOC, 2011). The sensory attributes taken into account included the following descriptors: off-odour (abnormal fermentation), salty, bitter, acid, hardness and crunchiness. Also, an overall acceptability descriptor was evaluated as an indication of the overall quality of the sample considered. Sensory data were analyzed as described elsewhere (Blana et al., 2014).

3. Results and discussion

3.1. Microbiological changes during storage

The evolution of the microbial population during table olive storage at 4 and 20 °C on olive drupes is presented in Tables 1 and 2, respectively. The population of the microbiota at the beginning of storage was quite similar in all packages. Specifically, the dominant microbial group was LAB (5.9–6.7 log CFU/g) followed by yeasts (2.0–3.0 log CFU/g) reflecting a successful lactic fermentation of the olives prior to packing. These observations are in accordance with the microbial population reported elsewhere for packed fermented green olive fruits covered with/without brine (Argyri et al., 2015; Arroyo López et al., 2005; Panagou, 2004; Rodríguez-Gómez et al., 2014a). During storage, the population of LAB decreased in olive flesh and reached 4.0–5.2 and 4.5–4.8 log CFU/g after 196 days of storage at 4 and 20 °C, respectively. The final LAB population was maintained at 4.3–4.6 log CFU/g at the end of storage (357 days) at 20 °C, whereas the same bacterial group presented lower counts of *ca.* 3.2–3.8 log CFU/g at 4 °C, indicating that the final population of LAB was affected by storage temperature. This observation is in good agreement with a previous work (Argyri et al., 2015) in which the population of LAB on olive drupes presented lower counts at 4 °C compared to 20 °C, apparently due to the sensitivity of LAB at low temperatures. The lowest levels of LAB corresponded to olive drupes previously fermented by a co-culture of both *Lactobacillus* strains, followed by those previously fermented by *L. plantarum* B282, whereas the highest survival was observed in the long run for *L. pentosus* B281. Yeast population levels reached 3.5–4.1 and 2.7–3.9 log CFU/g after 196 days of storage at 4 and 20 °C, respectively. At the end of storage (357 days), yeasts were maintained at 3.0 and 2.6 log CFU/g at 4 and 20 °C, respectively, irrespective of packing treatment. In previous works, the survival of probiotic LAB has been reported in population above 5.0 log CFU/mL for a period of six months (Argyri et al., 2015), whereas olives previously fermented and fortified before packing with a putative probiotic *L. pentosus* strain TOMC-LAB2 reached 5.5 log CFU/cm^2 after 200 days of storage at room temperature (Rodríguez-Gómez et al., 2014b). In both works, no