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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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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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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

Table 1
Microbiological and physicochemical changes during storage of cv. Halkidiki green olives previously fermented by the indigenous microbiota (control), *Lactobacillus pentosus* B281, *L. plantarum* B282 and a co-culture of the two *Lactobacillus* strains, at the beginning (day 1), middle (day 196) and end of storage (day 357) at 4 °C.

Treatments	Storage time (days)	LAB (log CFU/g)	Yeasts (log CFU/g)	pH	TA	a_w	ΔE
Control	1	6.11 ± 0.35	2.69 ± 0.30	4.21 ± 0.00	0.31 ± 0.00	0.96 ± 0.00	49.82 ± 1.42
	196	4.90 ± 0.80	3.53 ± 0.04	4.23 ± 0.01	0.24 ± 0.00	0.93 ± 0.00	50.46 ± 1.55
	357	3.75 ± 0.10	3.88 ± 0.06	4.21 ± 0.00	0.23 ± 0.01	0.94 ± 0.00	50.15 ± 1.54
L. pentosus B281	1	6.34 ± 0.00	2.92 ± 0.11	4.03 ± 0.00	0.53 ± 0.00	0.96 ± 0.00	50.14 ± 1.24
	196	5.24 ± 0.08	3.82 ± 0.28	3.93 ± 0.01	0.21 ± 0.02	0.94 ± 0.00	50.27 ± 1.87
	357	3.43 ± 0.51	3.05 ± 1.49	3.90 ± 0.00	0.22 ± 0.01	0.94 ± 0.00	49.10 ± 1.34
L. plantarum B282	1	5.91 ± 0.00	2.45 ± 0.21	4.28 ± 0.00	0.31 ± 0.00	0.96 ± 0.00	51.21 ± 1.08
	196	4.54 ± 0.59	3.77 ± 0.18	4.23 ± 0.01	0.18 ± 0.02	0.94 ± 0.00	51.32 ± 3.47
	357	3.51 ± 0.30	3.04 ± 1.46	4.20 ± 0.00	0.18 ± 0.00	0.95 ± 0.00	50.41 ± 1.77
Mixed strains B281/B282	1	6.52 ± 0.22	2.30 ± 0.43	4.19 ± 0.00	0.47 ± 0.00	0.96 ± 0.00	49.52 ± 2.04
	196	3.97 ± 0.33	4.09 ± 0.09	3.90 ± 0.00	0.17 ± 0.00	0.95 ± 0.00	49.60 ± 1.27
	357	3.20 ± 0.23	2.93 ± 1.32	4.00 ± 0.00	0.21 ± 0.00	0.95 ± 0.00	49.13 ± 1.39

LAB: Lactic acid bacteria; TA: titratable acidity (%, lactic acid); a_w: water activity; ΔE: total colour difference; data points represent mean values ± standard deviation.

Table 2
Microbiological and physicochemical changes during storage of cv. Halkidiki green olives previously fermented by the indigenous microbiota (control), *Lactobacillus pentosus* B281, *L. plantarum* B282 and a co-culture of the two *Lactobacillus* strains, at the beginning (day 1), middle (day 196) and end of storage (day 357) at 20 °C.

Treatments	Storage time (days)	LAB (log CFU/g)	Yeasts (log CFU/g)	pH	TA	a_w	ΔE
Control	1	6.11 ± 0.35	2.69 ± 0.30	4.21 ± 0.00	0.31 ± 0.00	0.96 ± 0.00	49.82 ± 1.42
	196	4.46 ± 0.38	2.68 ± 0.28	4.32 ± 0.00	0.20 ± 0.00	0.95 ± 0.00	50.67 ± 2.50
	357	4.59 ± 0.04	2.61 ± 0.86	4.46 ± 0.00	0.20 ± 0.00	0.94 ± 0.00	51.22 ± 2.06
L. pentosus B281	1	6.34 ± 0.00	2.92 ± 0.11	4.03 ± 0.00	0.53 ± 0.00	0.96 ± 0.00	50.14 ± 1.24
	196	4.68 ± 0.01	3.86 ± 0.05	4.51 ± 0.01	0.12 ± 0.00	0.95 ± 0.00	50.94 ± 1.82
	357	4.62 ± 0.02	2.51 ± 0.72	4.16 ± 0.00	0.19 ± 0.01	0.94 ± 0.00	49.38 ± 1.83
L. plantarum B282	1	5.91 ± 0.00	2.45 ± 0.21	4.28 ± 0.00	0.31 ± 0.00	0.96 ± 0.00	51.21 ± 1.08
	196	4.76 ± 0.32	3.07 ± 0.12	4.50 ± 0.00	0.14 ± 0.01	0.95 ± 0.00	50.96 ± 1.26
	357	4.38 ± 0.06	2.75 ± 1.05	4.61 ± 0.00	0.12 ± 0.00	0.94 ± 0.00	51.18 ± 1.61
Mixed strains B281/B282	1	6.52 ± 0.22	2.30 ± 0.43	4.19 ± 0.00	0.47 ± 0.00	0.96 ± 0.00	49.52 ± 2.04
	196	4.73 ± 0.11	3.19 ± 0.13	4.17 ± 0.00	0.17 ± 0.00	0.95 ± 0.00	50.38 ± 2.20
	357	4.24 ± 0.24	2.67 ± 0.95	4.32 ± 0.00	0.20 ± 0.00	0.94 ± 0.00	49.69 ± 2.00

LAB: Lactic acid bacteria; TA: titratable acidity (%, lactic acid); a_w: water activity; ΔE: total colour difference; data points represent mean values ± standard deviation.

Enterobacteriaceae could be detected throughout storage regardless of temperature indicating that a prior successful fermentation can ensure the microbiological stability of the final product as far as this spoilage microbial group is concerned. The absence of enterobacteria has been also confirmed during the post-fermentation storage of Spanish-style green Manzanilla olives (Rodríguez-Gómez et al., 2014a). The final fermented product cannot be subjected to any thermal treatment due to the presence of beneficial microorganisms, so a successful lactic acid fermentation is a vital step in product stabilization to avoid secondary fermentation into the package that would result in spoilage (Sánchez-Gómez et al., 2013). Moreoever, the absence of thermal treatment justifies the use of high salt concentration in the preparation of the packing brine (9%, w/v) employed by the Greek table olive industry to minimize the risk of spoilage during storage.

3.2. Survival of LAB on olives

The survival rates of the selected strains of LAB on packed olive drupes during storage were determined by PFGE analysis (Table 3). At the beginning of storage, *L. pentosus* B281 and *L. plantarum* B282 were recovered at a percentage of 90% and 87.5% respectively, at both storage temperatures, whereas in the case of fermented olives with a co-culture of *L. pentosus* B281 and *L. plantarum* B282, only the former strain was able to be recovered at a high percentage (90%). The obtained results are in agreement with the survival rates of the specific strains at the end of Spanish style fermentation published previously (Blana et al., 2014). In a previous work (Argyri et al., 2015), the same *L. pentosus* B281 strain showed high survival rates (100%) on green olives packed under modified atmospheres in contrast to *L. plantarum* B282 that was recovered at low levels (46.7%) under the same storage time.

The storage time and temperature affected differently the survival rates of the inoculated LAB strains. Specifically, after 196 days *L. pentosus* B281 and *L. plantarum* B282 showed high survival with a recovery rate of 100 and 96%, respectively at 4 °C. However, for the same time at 20 °C, *L. pentosus* B281 was recovered in lower rates (20%) and *L. plantarum* B282 could not be recovered at all. At the end of storage period (357 days), only *L. pentosus* B281 showed high survival rate (>70%), while *L. plantarum* B282 failed to survive during storage at both temperatures. Finally, in the packing treatment that contained olives fermented by the co-culture of both LAB strains, *L. pentosus* B281 dominated over *L. plantarum* B282 throughout storage at both temperatures with recovery rates of approximately 96% and 50% at 4 °C and 20 °C, respectively. Considering all of the above, it can be concluded that storage at 4 °C would be an appropriate condition for high survival of *L. pentosus* B281 for a time period of six months (*ca.* 196 days) maintaining a population above 5.0 log CFU/g. The same was observed for *L. plantarum* B282 that presented a high survival rate (96%) with a comparable population over the same storage period. It is noteworthy that at 20 °C the variation in the LAB population was negligible from a practical point of view and although the recovery rate of *L. pentosus* B281 was reduced to 70% it could be maintained at high population after 12 months of storage. Thus this temperature would be more preferable for an extended storage of the final product. In a recent study (Argyri et al., 2015) the survival of the same strains was monitored in inoculated fermentations of heat shocked green olives of the same variety followed by packaging under modified atmospheres at the same temperatures. It was

Table 3
Survival rate (%) of the inoculated strains *Lactobacillus pentosus* B281, *L. plantarum* B282 and a co-culture of the two *Lactobacillus* strains on olive drupes during storage at 4 and 20 °C according to PFGE analysis.

Inoculated strains	Storage time (days)	Survival rate (%)	
		4 °C	20 °C
L. pentosus B281	1	90	90
	196	100	20
	357	93.8	70
L. plantarum B282	1	87.5	87.5
	196	96	0
	357	0	0
Mixed strains B281/B282	1	90 B281/0 B282	90 B281/0 B282
	196	100 B281/0 B282	60 B281/0 B282
	357	95.6 B281/0 B282	50 B281/0 B282

reported that the strain *L. plantarum* B282 presented higher survival rate (94.1%) compared to *L. pentosus* B281 (64.7%) after 6 months of storage at 4 °C, which was selected as the most suitable storage condition. Rodríguez-Gómez et al. (2014b) reported a recovery frequency of 57.9% of a probiotic *L. pentosus* strain after 200 days of storage, when green table olives were previously fermented with the same LAB as starter and the packing brine was also fortified with the same strain. A recovery frequency of 100% was reported when olive fortification was undertaken after thermal treatment of the drupes. Additionally, lactobacilli (3 *Lactobacillus rhamnosus* and 2 *Lactobacillus paracasei* strains) and bifidobacteria (*Bifidobacterium bifidum* and *Bifidobacterium longum*) inoculated in the brine of already fermented and thermally treated olive drupes showed good survival rates after 3 months of storage at room temperature (Lavermicocca et al., 2005).

3.3. Physicochemical changes during storage

The evolution of pH on olive drupes during storage at 4 and 20 °C is presented in Tables 1 and 2, respectively. At the onset of storage the average pH of olives was 4.18 ± 0.11. At both storage temperatures, olives previously fermented by *L. pentosus* B281 and a co-culture of both LAB strains presented lower pH values compared to *L. plantarum* B282 and control treatments and this difference was more pronounced at 4 °C (Table 1). The final pH of olive flesh ranged between 3.90–4.21 and 4.16–4.61 at 4 and 20 °C, respectively. After 357 days, the lowest pH in olives were recorded in pouches containing olives previously fermented by *L. pentosus* B281 (4.03 ± 0.18) followed by those previously fermented by a co-culture of both strains (4.16 ± 0.23), at both temperatures. The evolution of titratable acidity was monitored at the initial, middle and final stage of storage at 4 and 20 °C (Tables 1 and 2). At the beginning of storage the average acidity was 0.404 ± 0.105 g of lactic acid/100 g of olive flesh. The acidity values at 4 °C were higher than those at 20 °C. In particular, the highest acidity in olive flesh was observed in pouches containing olives previously fermented by the indigenous microbiota followed by those previously fermented by *L. pentosus* B281 at 4 °C and a co-culture of both strains at 20 °C; *L. plantarum* B282 packing treatment presented the lowest acidity throughout storage, at both temperatures. At the end of the storage (357 days), acidity ranged between 0.210–0.233 and 0.188–0.195 g of lactic acid/100 g of olive flesh for samples previously fermented by *L. pentosus* B281, a co-culture of both strains and the indigenous microbiota at 4 and 20 °C, respectively. Regarding *L. plantarum* B282, acidity was 0.180 and 0.120 g of lactic acid/100 g of olive flesh at 4 and 20 °C, respectively. Based on the specifications of the trade standard applying to table olives of the IOC (2004), for natural fermentations the maximum limit of pH should be 4.3 and the minimum acidity 0.3 g of lactic acid/100 mL of brine for olives after equilibrium for olives preserved with their specific chemical characteristics. It is obvious that many packages after one year of storage, especially at ambient temperature, do not meet these specifications. It can thus be concluded that the amount of citric and ascorbic acid commonly employed by the Greek industry today during packaging is not adequate to reach the desired values of pH and acidity at equilibrium and consequently further optimization is needed.

The addition of salt in the packing brine increased the salinity in olives on average from 8.17 (±0.73) to 9.78 (±1.04) g NaCl/100 g of olive flesh in all pouches at the beginning and end of storage. The increased salt level in the packing brine had an impact on the attained values of water activity (a_w) measured on olive drupes. Thus, the average value of a_w in all packages at the beginning of storage was 0.961 (±0.003) and decreased to 0.942 (±0.004) at the end of storage reflecting the increased salt content in the olives.

In order to reduce the browning of green olives during storage, the packing brine was supplied with ascorbic and citric acids. Both acids are included in the list of permitted additives in the trade standard of table olives (IOC, 2004), the former as antioxidant and the latter as acidity regulator. The ΔE values, which are an indicator of total colour difference, illustrated that there were no significant differences ($P > 0.05$) in colour between control treatment and treated (i.e., previously fermented by *Lactobacillus* strains B281 and B282) olive drupes as shown in Tables 1 and 2. The average ΔE values ranged from 50.17 (±0.68) at the beginning of storage to 49.70 (±0.68) and 50.37 (±0.97) after 357 days of storage at 4 and 20 °C, respectively. These results are in line with a recent study (Sánchez-Gómez et al., 2013) in which ascorbic acid had been added in the brine of pasteurized green olives that were subsequently stored for 3 years. The authors reported that the colour remained stable throughout the long-term storage of olives, making thus the use of this antioxidant a widely known practice by the table olive industry to prevent colour degradation.

3.4. Sensory analysis

The results of the organoleptic assessment after 12 months of storage at 4 and 20 °C are presented in Table 4. No off-odours were detected in any packing treatment as inferred by the low scores of the taste panel for this organoleptic perception. The median values of the scores for 'abnormal fermentation' were below the threshold value of 3.0 and therefore olive samples were categorized as 'extra'. The scores for saltiness were lower for treatments stored at 4 °C than those at 20 °C. Most of the treatments received low scores (2.6–3.5) for bitterness with the exception of olives previously fermented by *L. plantarum* B282 stored at both temperatures and the co-culture of both strains stored at 20 °C which were characterized as more bitter and scores exceeded 4.2. Olives received

Table 4
Sensory profile (median value ± robust standard deviation) of packed cv. Halkidiki green olives after 357 days of storage at 4 and 20 °C; olives previously fermented by the indigenous microbiota (control), *Lactobacillus pentosus* B281, *L. plantarum* B282 and a co-culture of the two *Lactobacillus* strains.

Sensory attribute	Control	*L. pentosus* B281	*L. plantarum* B282	Mixed strains B281/B282
4 °C				
Abnormal fermentation	1.1 ± 0.3^{A}	1.0 ± 1.0^{A}	1.2 ± 0.8^{A}	1.1 ± 0.6^{A}
Salty	7.5 ± 1.3^{A}	6.5 ± 1.8^{A}	7.8 ± 1.2^{A}	7.9 ± 1.3^{A}
Bitter	2.6 ± 1.5^{A}	2.7 ± 1.4^{A}	4.8 ± 2.1^{A}	3.5 ± 2.4^{A}
Acid	5.2 ± 1.7^{A}	5.8 ± 1.5^{A}	6.0 ± 2.2^{A}	4.6 ± 1.8^{A}
Hardness	7.0 ± 1.5^{A}	7.6 ± 0.9^{A}	4.0 ± 1.9^{B}	7.2 ± 1.2^{A}
Crunchiness	7.5 ± 2.1^{A}	8.0 ± 1.2^{A}	3.6 ± 2.7^{A}	6.0 ± 1.7^{A}
Overall acceptability	5.1 ± 1.5^{A}	6.5 ± 1.6^{A}	6.0 ± 1.4^{A}	6.0 ± 1.6^{A}
20 °C				
Abnormal fermentation	1.2 ± 1.1^{A}	1.8 ± 0.4^{A}	1.3 ± 0.6^{A}	1.7 ± 0.4^{A}
Salty	8.4 ± 0.8^{A}	8.8 ± 0.5^{A}	8.5 ± 0.7^{A}	8.5 ± 0.7^{A}
Bitter	3.1 ± 1.8^{A}	3.5 ± 1.9^{A}	4.2 ± 2.2^{A}	4.9 ± 2.1^{A}
Acid	5.1 ± 1.8^{A}	5.4 ± 1.6^{A}	5.1 ± 1.6^{A}	5.8 ± 1.3^{A}
Hardness	7.5 ± 1.4^{A}	6.8 ± 1.7^{A}	5.0 ± 2.3^{A}	6.9 ± 1.2^{A}
Crunchiness	7.9 ± 1.6^{A}	6.5 ± 1.6^{A}	4.7 ± 1.9^{A}	6.4 ± 1.3^{A}
Overall acceptability	5.7 ± 1.6^{A}	5.9 ± 1.8^{A}	4.8 ± 1.2^{A}	4.6 ± 1.5^{A}

Different letters (A, B) within the same row indicate statistical significant difference at $P < 0.05$ according to Kruskal–Wallis test.

similar and moderate values in acidity for all packing treatments and storage temperatures. Concerning the kinaesthetic characteristics of hardness and crunchiness, there was a general tendency to score lower values for olives previously fermented by *L. plantarum* B282, stored at both temperatures. It is also noteworthy that olive samples that came from inoculated fermentations received higher scores for the descriptor 'overall acceptability' at 4 °C compared to 20 °C. Finally, olives previously fermented by *L. pentosus* B281 and stored at 4 °C was the most accepted treatment by the taste panel as it received the highest median score for this descriptor.

4. Conclusion

This research was mainly focused on monitoring the survival of selected LAB strains with *in vitro* probiotic potential, used as starter cultures, during the storage of green table olives in polyethylene pouches filled with brine at 4 and 20 °C for approximately 12 months. After 6 months of storage at chill temperature, both *Lactobacillus* strains were recovered at high percentages exceeding 96%, whereas only *L. pentosus* B281 managed to survive in high rates after 12 months. On the contrary, lower survival rates were observed after storage at 20 °C for both strains. Overall, table olives could be characterized as a good substrate for developing a novel functional food product, which can be handled better at chill temperatures for a period of time of 6 months whereas for extended storage (12 months), room temperature is recommended.

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