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Application of technological protocols on an industrial scale to improve Seville-style table olive production in Italy and Spain

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ABSTRACT

Improving the fermentation performance of starter strains used in the fermentation of table olives is a biotechnological solution of current interest to improve the quality characteristics of the final product. The aim of this study was to evaluate the use of *Lactiplantibacillus pentosus* OM13 as a starter culture for the fermentation of Seville-type table olives in two different production areas: Italy and Spain. The starter strain L. *pentosus OM13* was inoculated into two different table olive varieties: Nocellara del Belice in Italy and Manzanilla in Spain. *Lactiplantibacillus plantarum* Vege-Start 60 was used as a commercial control, while an additional control production was carried out by spontaneous fermentation. The industrial productions consisted of three different protocols, differing in the type of nutrient and the presence/absence of acclimatisation of the starter strain. All trials were subjected to microbiological monitoring, evaluation of acidification dynamics and sensory analysis of the final product. After 90 days, the pH reached values below 5 in the different treatments. The LAB reached microbial loads varying between 6.5 and 8.7 log CFU/mL throughout the monitoring period. The microbial populations of spoilage and/or potential pathogenic microorganisms were variable depending on the microbial group monitored. However, after 12 days of fermentation, Enterobacteriaceae showed values below the detection limit. In contrast, a fluctuating trend was observed for yeasts, Pseudomonadaceae and Staphylococcaceae. Sensory analyses showed variable differences depending on the technological protocol used. Table olives obtained with L. *pentosus* OM13 in the presence of nutrient, activator and acclimatisation period achieved higher overall acceptability values compared to the other trials. The use of adjuvants (nutrients and activators) is a strategy used in the production of table olives fermented with L. *pentosus* OM13 to improve the sensory characteristics of table olives.

1. Introduction

The production of table olives is an important economic activity in the Mediterranean countries, particularly in Italy and Spain Portilha--Cunha et al. (2020). The world production of table olives in the year 2022–2023 was 3.1 million t against a consumption of 2.95 million t (IOC, 2022a). The production of table olives in Spain in the last year was 449,000 t, which was higher than the Italian production of 62,000 t However, according to the report published by the IOC (2022b), Italy consumes far more table olives (116,000 t) than it produces when compared to Spain (210,000 t). Increased market demand for nutraceutical or probiotic products has led to significant technological developments that justify the increase in the price of table olives (Guiné et al., 2020). The quality of this product depends on many factors, mainly related to the parameters that limit the metabolic activity of the indigenous lactic acid bacteria (LAB) during the fermentation process, the variety of olives used, the ripeness of the drupes, the production area and the technological protocol used (Delgado et al., 2017). In recent years, there has been a decisive shift, especially at the industrial level, towards the use of selected microorganisms that can positively influence fermentation dynamics and reduce losses due to undesirable fermentations that can compromise the quality of the final product (Perpetuini et al., 2020). The selection of LAB strains with high technological performance was a decisive step in this direction, with the aim of improving

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the sensory qualities of this product and satisfying the most demanding consumers (Conte et al., 2020). *Lactiplantibacillus plantarum* and *Lactiplantibacillus pentosus* are the most frequently isolated LAB species in spontaneous fermentations of table olives (Zotta et al., 2022). Several studies aimed at the technological selection of different strains belonging to these two species mentioned above have led to the development of commercial starter cultures, which are now used in the controlled fermentation of industrially produced table olives (Nain et al., 2020). The next step was the use of adjuvants to improve the fermentation performance of the starter strains and thus further enhance the quality of the table olives produced (Alfonzo et al., 2018, 2023a, 2023b; Portilha-Cunha et al., 2020). The use of commercial starter strains concerns the fermentation of table olives by the two most common processing methods: Natural or Greek (Martorana et al., 2016) and Seville or Spanish (Aponte et al., 2012; Martorana et al., 2017). In this work, two commercial strains belonging to two different LAB species (L. *plantarum* and L. *pentosus*) were used in the controlled fermentation of Seville-style table olives. The use of technological protocols involving the addition of adjuvants to improve fermentation efficiency was applied to the production of Nocellara del Belice olives in Italy and Manzanilla olives in Spain. The use of these olive varieties in industrial productions is justified by the fact that they are the most widely cultivated varieties in their respective countries (Altrouz et al., 2021). The monitoring of chemical-physical and microbiological parameters, supported by sensory analysis, made it possible to establish the suitability of the different technological protocols for use at industrial level.

2. Materials and methods

2.1. Experimental design, table olive manufacturing and sample collection

The experiments were carried out in two different table olive processing companies: Geolive Belice srl (Castelvetrano, Italy) and S.A.T. Oliva de Barros (Almendralejo, Spain). The olive varieties used for fermentation were: Nocellara del Belice in Italy and Manzanilla in Spain. Once the drupes reached commercial maturity, they were selected, calibrated and processed according to the Sevillian style. The drupes were subjected to a debittering treatment with lye (2.6 Be) for 8 h. The olives were then washed three times to remove residual lye and transferred to 13 tonne fibreglass containers according to the experimental design shown in Fig. 1.

For each production site (Italy and Spain), five trials were performed on three different batches. The spontaneous fermentation control trials were indicated with C-ITA (produced in Italy) and C-SPA (produced in Spain). The trials inoculated with the commercial strain *Lactiplantibacillus plantarum* Vege-Start 60 were designated S1-ITA and S1-SPA (commercial control) for the Italian and Spanish productions respectively. The experimental productions included 3 conditions: (i) inoculation of *Lactiplantibacillus pentosus* OM13 with the addition of M.C.L.−15 Nutrient (S2-ITA and S2-SPA); (ii) inoculation of L. *pentosus* OM13 with the addition of Lal'Olive Nutrient (S3-ITA and S3-SPA); (iii) acclimatisation of the L. *pentosus* OM13 strain in the presence of Lal'Olive Activator followed by inoculation with the addition of Lal'Olive Nutrient (S4-ITA and S4-SPA).

In detail, the initial pH of the C-ITA, C-SPA, S2-ITA, S2-SPA, S3-ITA, S3-SPA, S4-ITA and S4-SPA trials was adjusted to ~ 8.0 by the addition of lactic acid (90% w/v, Proquiex, Alemendralejo, Spain). Whereas, the initial pH of the commercial controls (S1-ITA and S1-SPA) was acidified to ~ 5.5 units as recommended by the manufacturer. Except for the control trials (C-ITA and C-SPA), the S2-ITA, S2-SPA, S3-ITA, S3-SPA, S4-ITA and S4-SPA experimental productions were inoculated with Lal'Olive Crispy *Lactiplantibacillus pentosus* OM13 (8.3 g/t olive) in freeze-dried form (Lallemand, Inc., Montreal, Canada) and food-grade maltodextrin as carrier, containing approximately 1.1×10^9 colony-forming units CFU/g. The commercial controls S1-ITA and S1-SPA were inoculated with Bactoferm® *Lactiplantibacillus plantarum* Vege-Start 60 (8.3 g/t olive) in freeze-dried form (Chr. Hansen, Hørsholm, Denmark) containing approximately 1.0×10^8 colony-forming units CFU/g. In order to verify the fermentation performance of the strains, two different nutrients were used as follows: M.C.L. – 15 Nutrient

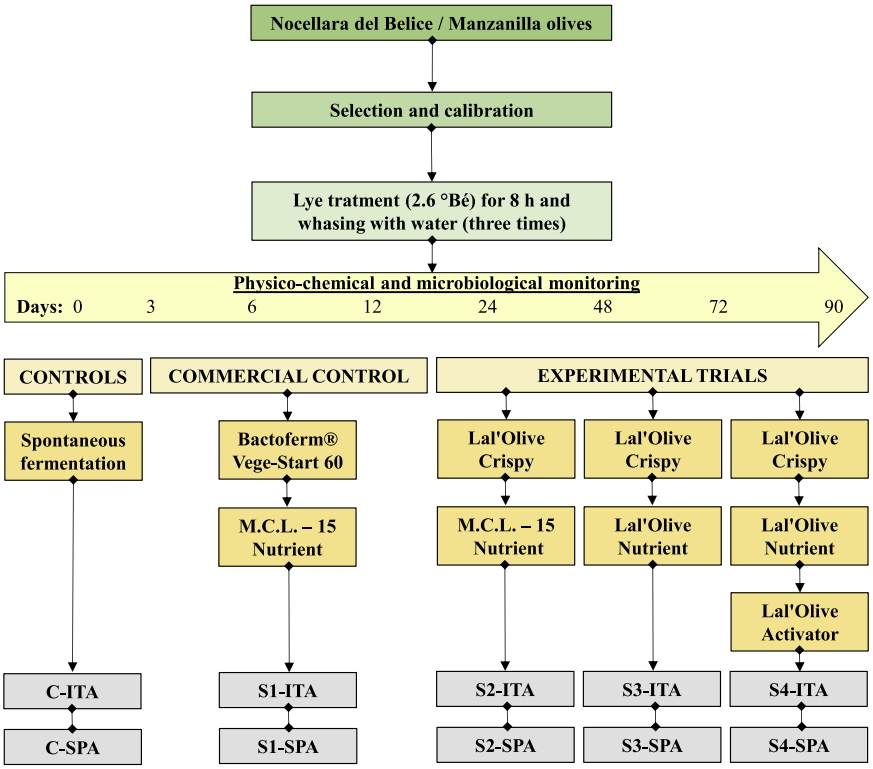

Fig. 1. Experimental plan of Seville-style table olives produced in two different countries: Italy and Spain.

(Serproquim Food S.L.U., Sevilla, Spain; 3 kg/t of olives) was added to the S1-ITA, S1-SPA, S2-ITA and S2-SPA trials; Lal'Olive Nutrient (1 kg/t of olives) was used for the S3-ITA, S3-SPA, S4-ITA and S4-SPA trials. The C-ITA and C-SPA control production did not include any nutrient addition. Lal'Olive Activator was used for the S4-ITA/SPA trials. In detail, the starter strain L. *pentosus* OM13 was acclimated for 12 h in 6 % NaCl (w/v) brine supplemented with Lal'Olive Activator (0.033 kg/t olive) at room temperature (20 ± 2 °C) before inoculation. M.C.L.−15 Nutrient consists of sugars (glucose), sodium chloride and amino acids. Lal'Olive Nutrient is composed of sugars (glucose), maltodextrins and inactivated yeast (Alfonzo et al., 2023a). Lal'Olive Activator has a similar composition to Lal'Olive Nutrient but does not contain maltodextrins (Alfonzo et al., 2023b).

All fermentations were carried out at room temperature (22±2 °C in Italy, 23±1 °C in Spain) throughout the process and were monitored periodically. Brine samples for physico-chemical and microbiological analysis were collected immediately after the addition of adjuvants and starter cultures (day 0) and after 3, 6, 12, 24, 48, 72 and 90 days of fermentation.

2.2. Monitoring of physico-chemical and microbiological parameters

Samples were taken in aliquots of approximately 200 mL of brine and then transferred to sterile plastic containers, appropriately labelled and sealed. The Spanish samples were stored at 4 ± 1 °C and transported under refrigerated conditions to the Agricultural Microbiology laboratory of the Department of Agricultural, Food and Forest Sciences (SAAF) of the University of Palermo. The Italian samples were transported and stored at 4 ± 1 °C. The analyses were carried out at the same time as the spanish samples. The pH of the brine was measured with a Russell RL060P pH metre (Thermo Fisher Scientific, Beverly, MA) by direct immersion (Fernàndez et al., 1997). All measurements were performed in triplicate for each sample.

All samples were serially diluted in Ringer's solution (Sigma-Aldrich, Milan, Italy) and subjected to microbiological analysis to determine the levels of mesophilic rod LAB, yeast, Enterobacteriaceae, Pseudomonadaceae and Staphylococcaceae. The culture media and incubation conditions used were the same as those reported by Martorana et al. (2015). The results of the concentrations of each monitored microbial group were obtained by direct plate counting and expressed as log (colony--forming units) CFU/mL of brine. All analyses were performed in triplicate.

2.3. Dominance of starter strains

During microbiological monitoring of LAB populations, at least five colonies with the same colour, edge, elevation shape and surface grown on De Man, Rogosa and Sharpe (MRS) agar at the highest dilutions were collected for each production and sampling point. For each isolate, after purification in the same growth medium, microscopic analysis was performed to determine cell morphology, Gram reaction (Gregersen, 1978) and the presence/absence of the catalase enzyme (Reniner, 2010). Isolates that were rod-shaped, Gram+ and catalase negative were considered presumptive LAB. The metabolism type for each presumptive LAB isolate was determined as described by Martorana et al. (2015). To genotypically characterise the inoculated starter strains (L. *plantarum* Vege-Start 60 and Lal'Olive Crispy L. *pentosus* OM13) in the different technological protocols used, DNA was extracted for each isolate using the method described by Alfonzo et al. (2013). Random amplification polymorphic DNA-PCR (RAPD-PCR) with primer M13 was used to assess the dominance of the starter strains using the same conditions as described by Rosetti and Giraffa (2005). The visualisation and band analysis of the RAPD patterns were performed according to Alfonzo et al. (2017).

2.4. Sensory analysis

Twenty panelists (10 women and 10 men, aged between 24 and 57) fully trained at the Department of Agricultural, Food and Forest Sciences (SAAF) participated in the tasting of the 10 experimental products. The subject of the study was explained to each panellist prior to the sensory analysis. Verbal consent to participate in the sensory analysis session was also obtained. The study was exempt from ethics committee review. The samples were served in plastic plates at room temperature. Each sample was given an alphanumeric code. To cleanse the palate, the panellists were offered unsalted crackers and mineral water between each sample. The attributes that defined the sensory profiles of table olives included: visual characteristics (brightness andgreen colour intensity), odour (green olive aroma and off-flavours), rheological characteristics (crispness), taste (acidity, astringency, bitterness, juiciness, saltiness, sweetness, off-flavours and overall acceptability). The intensity of each attribute was evaluated using a numerical scale (Saúde et al., 2017), ranging from 1 (no perception) to 7 (high perception).

2.5. Statistical analysis

Data on brine acidification (pH), microbiological counts and sensory analysis were statistically analysed using ANOVA. Pairwise comparisons of data were analysed using Tukey's post-hoc procedure. The level of statistical significance was set at $p \leq 0.001$. Principal component analysis (PCA) was used to visualise the differences between treatments in relation to sensory attributes by biplot graphs. All analyses were performed using XLStat software version 2019.2.2 (Addinsoft, New York, NY, USA).

3. Results

3.1. Acidification kinetics of brine and lactic acid bacteria monitoring

Fig. 2 shows the pH changes and LAB concentrations measured during 90 days of fermentation in table olives produced in Italy (Fig. 2A) and Spain (Fig. 2B).

In relation to the table olive production area, brine acidification showed a different trend. In Italy (Fig. 2A), the pH values at the beginning (day 0) of the process ranged from 5.75 to 8.18. After three days, the lowest brine pH values were observed in the S1-ITA (5.55). After 12 days, all trials except C-ITA (6.41) showed pH values lower than 6. After 48 days of fermentation, pH values of less than 5 units were observed in S1-ITA (4.66) and S4-ITA (4.84). In this case, the acclimatisation period of the strain with the activator and nutrient for L. *pentosus* OM13 resulted in pH values comparable to those of the commercial control. At day 90, the S1-ITA and S4-ITA treatments showed the lowest pH values (~ 4.5). As a result, the acclimatisation of the OM13 strain and the use of adjuvants (activator and nutrient) allowed for an acidification dynamic similar to that of the commercial control, avoiding the initial acidification of the brine with lactic acid.

In Spain (Fig. 2B), the acidification of the brine occurred faster than the trend observed in the Italian production. Immediately after inoculation (0 days), the pH of the brine in the different treatments ranged from 5.56 to 8.25. After 3 days, only the C-SPA and S1-SPA treatments showed pH values below 6. On the 6th day of fermentation, the S1-SPA treatment showed a lower pH (4.68). Up to day 12, with the exception of S1-SPA and S2-SPA, the other treatments showed pH values slightly above 5. From day 48 to day 72 of fermentation, the lowest pH values were observed for the S1-SPA treatment (4.38 and 4.37 respectively). At the end of the process, significantly lower brine pH values (4.33–4.37) were observed in the S1-SPA, S2-SPA and S4-SPA treatments than in the other trials (C-SPA = 4.50 and S3-SPA = 4.47).

Overall, the growth dynamics of the LAB populations were overall similar in the two production sites. In the Italian trials, immediately after the inoculation of the starter strains (day 0), except the C-ITA

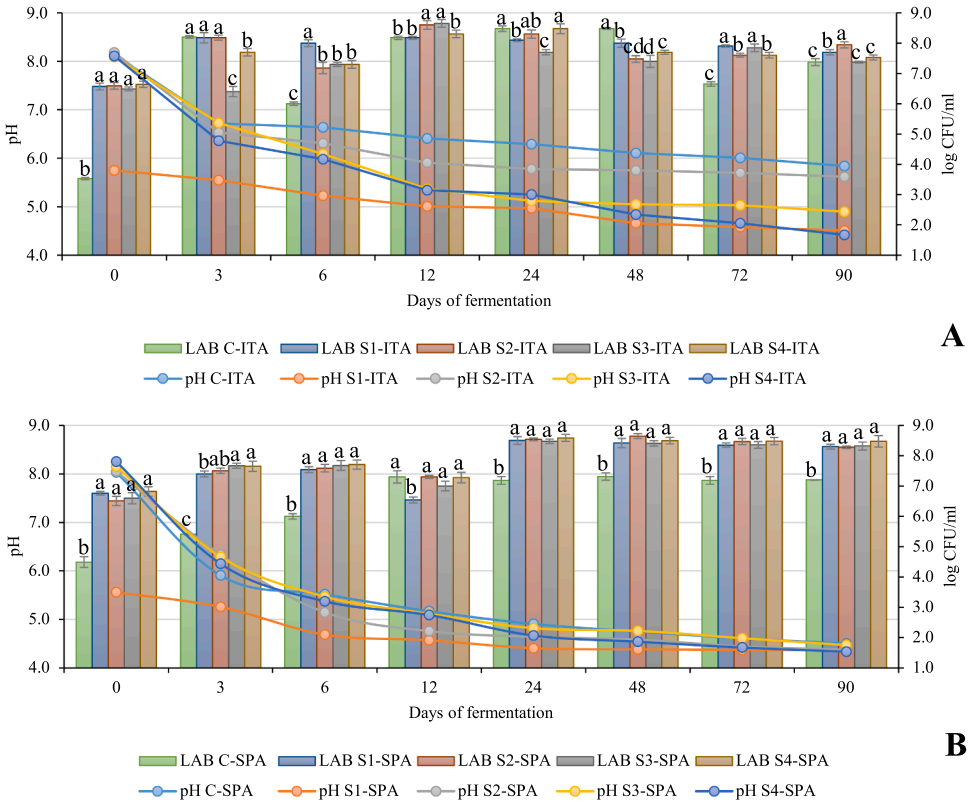

Fig. 2. Trends in pH and lactic acid bacteria populations monitored during fermentation in Italian (A) and Spanish (B) productions.

control, all trials (S1-ITA, S2-ITA, S3-ITA and S4-ITA) reached LAB counts in the range of 6.5–6.6 Log CFU/mL. This trend was observed until day 6 of fermentation. On day 12, counts above 8 Log cycles were recorded in all treatments. Between day 24 and day 90, the LAB count fluctuated slightly, but overall the values observed were always above 7 Log CFU/mL. In Spain, at the beginning of the fermentation (day 0), counts above 6 Log CFU/mL were observed in all trials inoculated with the starter strains, but in the C-SPA control, the values obtained were 4.5 Log CFU/mL. Throughout the fermentation process, the LAB population in the inoculated treatments (S1-ITA, S2-ITA, S3-ITA and S4-SPA) was in the range of 7.0 to 8.7 Log CFU/mL. C-SPA, however, did not exceed 7 Log CFU/mL until day 12 of fermentation.

3.2. LAB dominance

Of a total of 1363 rod-shaped, Gram+, catalase-negative isolates, 1119 showed facultative heterofermentative metabolism. RAPD-PCR analysis distinguished the 1119 isolates into 19 LAB strains. The dominance of the LAB starters was assessed by comparing the polymorphic profiles of the inoculated starter strains with the collected isolates (Fig. 3). Bactoferm® L. *plantarum* Vege-Start 60 in commercial controls achieved a dominance% ranging from 80.3 % (S1-ITA) to 87.7 % (S1-SPA). On the other hand, the dominance% of Lal'Olive Crispy L. *pentosus* OM13 varied between 82.3 % (S2-ITA) and 92.3 % (S4-SPA). In the trials (S4-ITA and S4-SPA) where acclimatisation of the OM13 strain was foreseen, the dominance was slightly higher than 90 % compared to the other trials, regardless of the production area. In the spontaneous fermentation trials, no RAPD profiles similar to those of the inoculated starter strains were found. However, 9 different polymorphic profiles were observed in C-ITA and 8 in C-SPA. The RAPD profiles detected in the Italian samples were not found in any of the Spanish samples. In both the commercial controls and the productions inoculated with L. *pentosus* OM13, in addition to the polymorphic profiles of the starter strains, the presence of other strains was detected, ranging from 2 [S1-SPA (12.3 %) and S3-ITA (14.5 %)] to 6 [S2-SPA (16.4 %)] was detected. RAPD profiles that did not have the same polymorphic profile as the starter strains in inoculated experimental productions were comparable to those observed in the respective spontaneously fermented productions.

3.3. Yeast population trends

During the fermentation process, yeast concentrations varied significantly between trials (Table 1). At the beginning of the fermentation process (day 0), the highest yeast concentrations were found in the S1-ITA (7.3 Log CFU/mL) and S4-ITA (7.2 Log CFU/mL) trials, while concentrations were below the detection limit in the C-SPA, S1-SPA and S3-SPA treatments. There was a fluctuation in yeast counts during the fermentation process. The highest value was detected in C-ITA (7.5 Log CFU/mL) at day 48 and the lowest value in S2-ITA (2.7 Log CFU/mL) on day 24. The count values at the end of the process ranged between 5.2–7.0 Log CFU/mL. The highest average values observed over the entire monitoring period (90 d) were found in C-ITA, while the lowest values were detected in S3-SPA.

3.4. Population kinetics of spoilage and/or potential pathogenic bacteria

The microbial counts for the Enterobacteriaceae, Pseudomonadaceae and Staphylococcaceae populations are shown in Table 1.

During the 90 days of fermentation, the highest levels of Enterobacteriaceae were observed in C-ITA (3.2 Log CFU/mL) on day 0 and S1-SPA (3.3 Log CFU/mL) on day 6. In the other treatments, they ranged from 1.7 to 2.7 Log CFU/mL. No Enterobacteriaceae were observed in S2-SPA and S4-ITA throughout the monitoring period. From day 12 of fermentation until the end of the process, Enterobacteriaceae counts were below the detection limit in all treatments.

Pseudomonas counts fluctuated between 2 and 4 logarithmic cycles throughout the fermentation period. The highest pseudomonad counts were found in S3-ITA on days 0 (4.1 Log CFU/mL) and 24 (4.0 Log CFU/

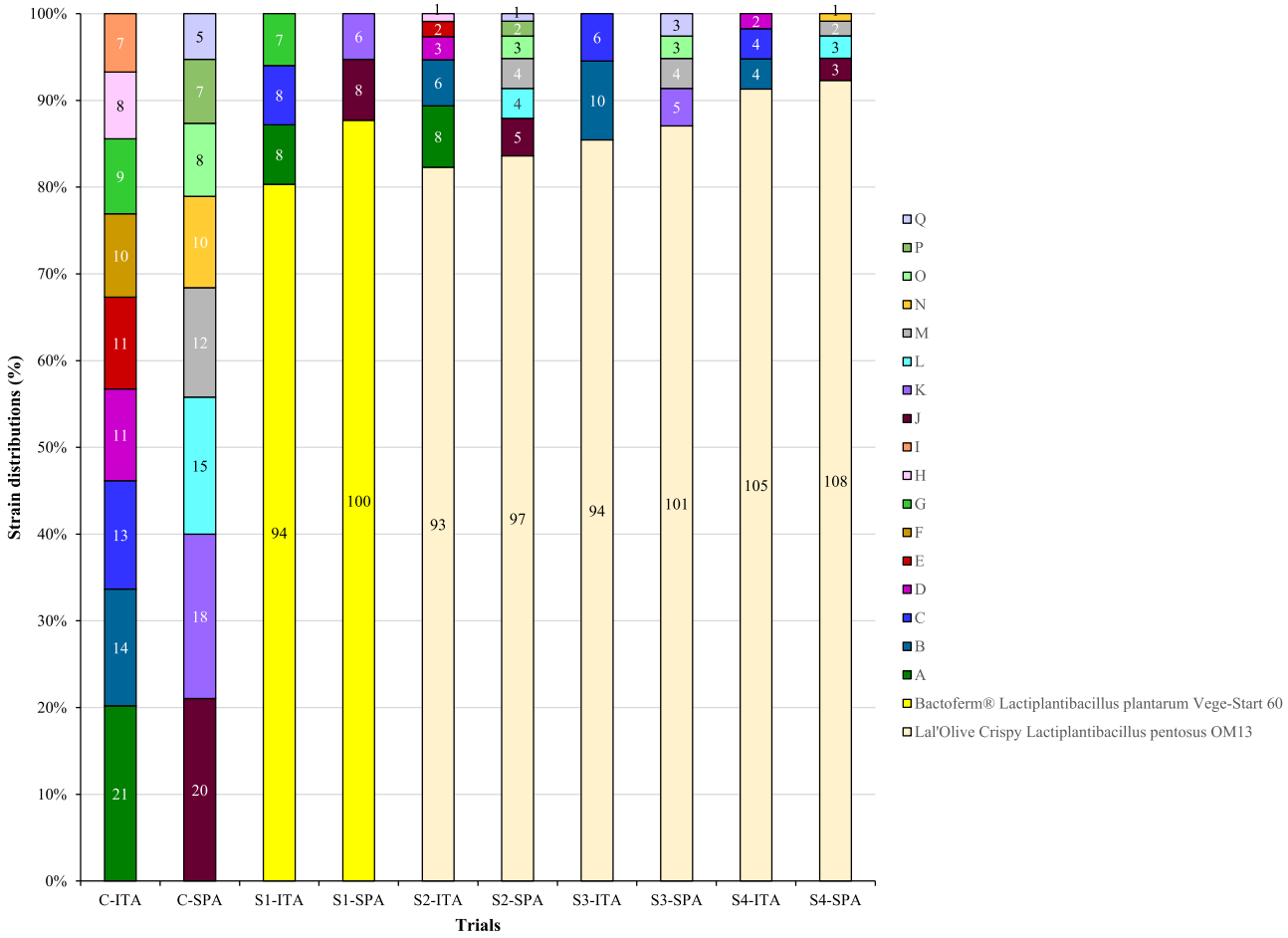

Fig. 3. Distribution of LAB strains isolated during fermentation of Seville-style table olives in Italy and Spain. The number within each histogram indicates the number of isolates with the same RAPD profile. Histograms with different colours represent different groups of strains.

mL). From day 72 to the end of monitoring, pseudomonad levels were below the detection limit in all treatments except S4-ITA on day 90 (2.5 Log CFU/mL).

Staphylococcaceae during the fermentation process showed variations in the range of 2.2–4.1 Log CFU/mL. The highest value was observed in S1-SPA (4.8 Log CFU/mL) on day 6 of fermentation. In the Italian production, Staphylococcaceae counts were below the detection limit (< 2 Log CFU/mL) for up to 48 days of fermentation. Conversely, in the Spanish production, no Staphylococcaceae were detected from day 24 to the end of fermentation, with the exception of S1-SPA (2.9 Log CFU/mL) on day 72. The presence of coagulase-positive staphylococci was not observed.

3.5. *Sensory profiles of fermented table olives*

The values of each sensory attribute recorded for each treatment are shown in Table 2. In general, different sensory profiles of the product were found in each trial, mainly related to the variety of olives used and the technological protocol applied. The spontaneous fermentation controls (C-ITA and C-SPA) showed the highest scores for off-odour and off-flavour attributes. However, the Italian control production (C-ITA) achieved the highest values for salt (6.19) and the Spanish control production (C-SPA) for astringent (1.88) compared to all other trials. The trials fermented with the starter strains showed off-flavour and off-odour scores slightly above the minimum, ranging from 1.02 to 1.09. For the attributes describing visual characteristics, S1-ITA had the highest scores for brightness (4.65) and green colour intensity (6.71). In the treatments S3-ITA, S4-ITA and S4-SPA, the highest scores were observed for the green olive aroma (6.58, 6.46 and 6.78 respectively). Crispness was perceived with scores ranging from 3.89 (C-ITA) to 6.95 (S3-ITA), with other productions averaging ~5. In terms of taste, the juicy attribute obtained high scores in four treatments: S1-SPA (6.26), S3-ITA (6.63), S4-ITA (6.48) and S4-SPA (6.13), while the lowest values were determined in C-ITA (5.42) and S3-SPA (5.45). The highest score for sweet was achieved by the S2-SPA trial (3.76). However, in terms of overall acceptability, the highest scores was achieved by S1-ITA (5.95) and S4-ITA (5.86).

In order to correlate each sensory attribute with the different trials, the Principal Component Analysis (PCA) is shown in Fig. 4 by means of a biplot graph for both the productions obtained in Italy (Fig. 4A) and in Spain (Fig. 4B). The cumulative variability explained by the F1 and F2 factors was 70.86 % for the Italian trials and 66.93 % for the Spanish trials. The PCA analysis of the Italian production allowed the classification of the evidence into three clusters: (i) C-ITA; (ii) S1-ITA, S3-ITA and S4-ITA; (iii) S2-ITA. C-ITA was correlated with astringency, off-flavours, off-odours and salt, S2-ITA with bitterness and the second group with the remaining sensory attributes. The Spanish productions were divided into four clusters: (i) C-SPA; (ii) S1-SPA; (iii) S2-SPA and S3-SPA; (iv) S4-SPA. The first cluster was most highly correlated with astringent, bitter, green colour intensity, off-odours and off-flavours. The second cluster correlated with juicy and brightness. The third cluster differed from the others by a close correlation with crispness, sweet and acid. Finally, cluster 4 was related to green olive aroma, salt and overall acceptability.

Table 1
Levels of spoilage and/or potentially pathogenic microorganism populations (Log CFU/mL) detected during the fermentation process in Seville-style table olives produced in Italy and Spain.

Microbial groups	Days of fermentation							
	0	3	6	12	24	48	72	90
Yeasts								
C-ITA	6.9 ± 0.1^{b}	6.5 ± 0.2^{ab}	3.7 ± 0.1^{e}	6.1 ± 0.1^{bc}	4.7 ± 0.1^{ef}	7.5 ± 0.1^{a}	6.2 ± 0.1^{b}	6.2 ± 0.1^{b}
C-SPA	$< 2.0^{g}$	5.4 ± 0.1^{c}	5.5 ± 0.1^{b}	4.8 ± 0.1^{d}	4.9 ± 0.1^{de}	6.2 ± 0.2^{b}	6.5 ± 0.1^{a}	7.0 ± 0.1^{a}
S1-ITA	7.3 ± 0.1^{a}	4.7 ± 0.1^{d}	3.7 ± 0.1^{e}	6.3 ± 0.1^{ab}	6.2 ± 0.1^{a}	6.3 ± 0.1^{b}	6.4 ± 0.2^{a}	5.2 ± 0.1^{d}
S1-SPA	$< 2.0^{g}$	6.6 ± 0.1^{ab}	6.0 ± 0.1^{a}	6.0 ± 0.2^{c}	5.6 ± 0.1^{c}	5.9 ± 0.2^{cd}	6.0 ± 0.2^{c}	5.2 ± 0.1^{d}
S2-ITA	5.5 ± 0.1^{c}	6.4 ± 0.1^{b}	4.2 ± 0.2^{d}	6.4 ± 0.1^{a}	2.7 ± 0.2^{h}	5.9 ± 0.2^{cd}	6.0 ± 0.1^{c}	6.1 ± 0.2^{b}
S2-SPA	2.7 ± 0.1^{f}	4.4 ± 0.1^{e}	5.7 ± 0.1^{b}	4.9 ± 0.1^{d}	5.0 ± 0.1^{d}	5.2 ± 0.1^{f}	5.3 ± 0.1^{e}	5.7 ± 0.1^{c}
S3-ITA	4.7 ± 0.1^{d}	6.7 ± 0.2^{a}	4.2 ± 0.1^{d}	6.5 ± 0.1^{a}	4.7 ± 0.1^{f}	5.7 ± 0.1^{de}	5.8 ± 0.1^{d}	6.3 ± 0.1^{d}
S3-SPA	$< 2.0^{g}$	5.3 ± 0.1^{c}	3.7 ± 0.2^{e}	6.4 ± 0.1^{a}	5.9 ± 0.1^{b}	5.1 ± 0.1^{f}	5.2 ± 0.2^{e}	5.3 ± 0.1^{b}
S4-ITA	7.2 ± 0.1^{a}	6.7 ± 0.1^{a}	5.0 ± 0.1^{c}	6.5 ± 0.1^{a}	3.0 ± 0.1^{g}	5.5 ± 0.1^{e}	6.2 ± 0.1^{b}	6.3 ± 0.1^{b}
S4-SPA	3.0 ± 0.1^{e}	4.4 ± 0.1^{e}	5.1 ± 0.2^{c}	4.6 ± 0.1^{e}	6.3 ± 0.1^{a}	6.1 ± 0.1^{bc}	5.8 ± 0.1^{d}	5.4 ± 0.1^{d}
Statistical significance	***	***	***	***	***	***	***	***
Enterobacteriaceae								
C-ITA	3.2 ± 0.2^{a}	$< 1.0^{b}$	$< 1.0^{c}$	$< 1.0^{a}$	$< 1.0^{a}$	$< 1.0^{a}$	$< 1.0^{a}$	$< 1.0^{a}$
C-SPA	2.0 ± 0.2^{c}	2.4 ± 0.1^{a}	$< 1.0^{c}$	$< 1.0^{a}$	$< 1.0^{a}$	$< 1.0^{a}$	$< 1.0^{a}$	$< 1.0^{a}$
S1-ITA	2.2 ± 0.1^{c}	$< 1.0^{b}$	$< 1.0^{c}$	$< 1.0^{a}$	$< 1.0^{a}$	$< 1.0^{a}$	$< 1.0^{a}$	$< 1.0^{a}$
S1-SPA	2.2 ± 0.1^{c}	$< 1.0^{b}$	3.3 ± 0.1^{a}	$< 1.0^{a}$	$< 1.0^{a}$	$< 1.0^{a}$	$< 1.0^{a}$	$< 1.0^{a}$
S2-ITA	2.1 ± 0.1^{c}	2.3 ± 0.2^{a}	$< 1.0^{c}$	$< 1.0^{a}$	$< 1.0^{a}$	$< 1.0^{a}$	$< 1.0^{a}$	$< 1.0^{a}$
S2-SPA	$< 1.0^{e}$	$< 1.0^{b}$	$< 1.0^{c}$	$< 1.0^{a}$	$< 1.0^{a}$	$< 1.0^{a}$	$< 1.0^{a}$	$< 1.0^{a}$
S3-ITA	2.5 ± 0.2^{b}	$< 1.0^{b}$	$< 1.0^{c}$	$< 1.0^{a}$	$< 1.0^{a}$	$< 1.0^{a}$	$< 1.0^{a}$	$< 1.0^{a}$
S3-SPA	$< 1.0^{e}$	$< 1.0^{b}$	2.7 ± 0.1^{b}	$< 1.0^{a}$	$< 1.0^{a}$	$< 1.0^{a}$	$< 1.0^{a}$	$< 1.0^{a}$
S4-ITA	$< 1.0e$	$< 1.0^{b}$	$< 1.0c$	$< 1.0a$	$< 1.0^{a}$	$< 1.0^{a}$	$< 1.0^{a}$	$< 1.0^{a}$
S4-SPA	1.7 ± 0.1^{d}	$< 1.0^{b}$	$< 1.0^{c}$	$< 1.0^{a}$	$< 1.0^{a}$	$< 1.0^{a}$	$< 1.0^{a}$	$< 1.0^{a}$
Statistical significance	***	***	***	N.S.	N.S.	N.S.	N.S.	N.S.
Pseudomonadaceae								
C-ITA	3.9 ± 0.1^{b}	$< 2.0^{c}$	$< 2.0^{e}$	$< 2.0^{e}$	3.9 ± 0.2^{a}	3.6 ± 0.1^{a}	$< 2.0^{a}$	$< 2.0^{b}$
C-SPA	$< 2.0^{e}$	2.5 ± 0.1^{a}	2.3 ± 0.1^{d}	2.2 ± 0.1^{d}	$< 2.0^{e}$	$< 2.0^{c}$	$< 2.0^{a}$	$< 2.0^{b}$
S1-ITA	$< 2.0^{e}$	$< 2.0^{c}$	$< 2.0^{e}$	$< 2.0^{e}$	2.8 ± 0.1^{c}	3.6 ± 0.1^{a}	$< 2.0^{a}$	$< 2.0^{b}$
S1-SPA	$< 2.0^{e}$	$< 2.0^{c}$	2.5 ± 0.1^{c}	2.5 ± 0.1^{c}	2.7 ± 0.1^{c}	$< 2.0^{c}$	$< 2.0^{a}$	$< 2.0^{b}$
S2-ITA	$< 2.0^{e}$	$< 2.0^{c}$	$< 2.0^{e}$	$< 2.0^{e}$	3.5 ± 0.1^{b}	3.5 ± 0.2^{a}	$< 2.0^{a}$	$< 2.0^{b}$
S2-SPA	$< 2.0^{e}$	$< 2.0^{c}$	3.3 ± 0.2^{b}	2.3 ± 0.2^{d}	2.7 ± 0.1^{c}	$< 2.0^{c}$	$< 2.0^{a}$	$< 2.0^{b}$
S3-ITA	4.1 ± 0.1^{a}	$< 2.0^{c}$	$< 2.0^{e}$	$< 2.0^{e}$	4.0 ± 0.1^{a}	2.7 ± 0.2^{b}	$< 2.0^{a}$	$< 2.0^{b}$
S3-SPA	$< 2.0^{e}$	2.4 ± 0.1^{b}	2.2 ± 0.1^{d}	3.1 ± 0.1^{b}	$< 2.0^{e}$	$< 2.0^{c}$	$< 2.0^{a}$	$< 2.0^{b}$
S4-ITA	3.6 ± 0.1^{c}	$< 2.0^{c}$	$< 2.0^{e}$	$< 2.0^{e}$	2.5 ± 0.1^{d}	3.6 ± 0.1^{a}	$< 2.0^{a}$	2.5 ± 0.1^{a}
S4-SPA	2.7 ± 0.2^{d}	$< 2.0^{c}$	3.5 ± 0.1^{a}	3.7 ± 0.1^{a}	$< 2.0^{e}$	$< 2.0^{c}$	$< 2.0^{a}$	$< 2.0^{b}$
Statistical significance	***	***	***	***	***	***	N.S.	***
Staphylococcaceae								
C-ITA	$< 2.0^{c}$	$< 2.0^{b}$	$< 2.0^{d}$	$< 2.0^{d}$	$< 2.0^{a}$	$< 2.0^{a}$	3.2 ± 0.1^{a}	3.0 ± 0.1^{a}
C-SPA	2.7 ± 0.1^{b}	2.4 ± 0.2^{a}	4.2 ± 0.2^{b}	2.9 ± 0.1^{b}	$< 2.0^{a}$	$< 2.0^{a}$	$< 2.0^{e}$	$< 2.0^{b}$
S1-ITA	$< 2.0^{c}$	$< 2.0^{b}$	$< 2.0^{d}$	$< 2.0^{d}$	$< 2.0^{a}$	$< 2.0^{a}$	$< 2.0^{e}$	$< 2.0^{b}$
S1-SPA	2.7 ± 0.1^{b}	$< 2.0^{b}$	4.8 ± 0.2^{a}	2.8 ± 0.1^{b}	$< 2.0^{a}$	$< 2.0^{a}$	2.9 ± 0.1^{b}	$< 2.0^{b}$
S2-ITA	$< 2.0^{c}$	$< 2.0^{b}$	$< 2.0^{d}$	$< 2.0^{d}$	$< 2.0^{a}$	$< 2.0^{a}$	$< 2.0^{e}$	$< 2.0^{b}$
S2-SPA	$< 2.0^{c}$	$< 2.0^{b}$	2.7 ± 0.1^{c}	2.5 ± 0.1^{c}	$< 2.0^{a}$	$< 2.0^{a}$	$< 2.0^{e}$	$< 2.0^{b}$
S3-ITA	$< 2.0^{c}$	$< 2.0^{b}$	$< 2.0^{d}$	$< 2.0^{d}$	$< 2.0^{a}$	$< 2.0^{a}$	2.5 ± 0.1^{d}	$< 2.0^{b}$
S3-SPA	$< 2.0^{c}$	$< 2.0^{b}$	$< 2.0^{d}$	3.4 ± 0.1^{a}	$< 2.0^{a}$	$< 2.0^{a}$	$< 2.0^{e}$	$< 2.0^{b}$
S4-ITA	$< 2.0^{c}$	$< 2.0^{b}$	$< 2.0^{d}$	$< 2.0^{d}$	$< 2.0^{a}$	$< 2.0^{a}$	2.7 ± 0.1^{c}	$< 2.0^{b}$
S4-SPA	4.2 ± 0.2^{a}	$< 2.0^{b}$	$< 2.0^{d}$	$< 2.0^{d}$	$< 2.0^{a}$	$< 2.0^{a}$	$< 2.0^{e}$	$< 2.0^{b}$
Statistical significance	***	***	***	***	N.S.	N.S.	***	***

The results are expressed as the mean ± standard deviation of three repetitions.
Data in the same column with different letters are significantly different according to Tukey's test.
p value: ***, $p < 0.001$; N.S., not significant.
Abbreviations: C-ITA, spontaneously fermented Nocellara del Belice table olives; C-SPA, spontaneously fermented Manzanilla table olives; S1-ITA, Nocellara del Belice table olives fermented with Bactoferm® Vege-Start 60 + M.C.L.−15 nutrient; S1-SPA, Manzanilla table olives fermented with Bactoferm® Vege-Start 60 + M.C.L.−15 nutrient; S2-ITA, Nocellara del Belice table olives fermented with Lal'Olive Crispy + M.C.L.−15 nutrient; S2-SPA, Manzanilla table olives fermented with Lal'Olive Crispy + M.C.L.−15 nutrient; S3-ITA, Nocellara del Belice table olives fermented with Lal'Olive Crispy + Lal'Olive nutrient; S3-SPA, Manzanilla table olives fermented with Lal'Olive Crispy + Lal'Olive nutrient; S4- ITA, Nocellara del Belice table olives fermented with Lal'Olive Crispy + Lal'Olive activator, acclimatation (12 h) in brine (6 % NaCl) + Lal'Olive nutrient; S4-SPA, Nocellara del Belice table olives fermented with Lal'Olive Crispy + Lal'Olive activator, acclimatation (12 h) in brine (6 % NaCl) + Lal'Olive Nutrient.

4. Discussion

In this study, five different protocols were evaluated in two companies located in Italy and Spain. The industrial productions allowed the comparison of the technological performance of Lal'Olive Crispy L. *pentosus* OM13 under different conditions with Bactoferm® L. *plantarum* Vege-Start 60. In particular, the fermentation temperature seems to have an effect on the acidification rate (Sánchez et al., 1995), although the average values recorded at the two sites differed by 1–2 °C. In fact, pH values were lower in the Spanish trials than in the Italian trials, especially up to 24 days of fermentation. The combined use of specific nutrients (Lal'Olive and M.L.C.−15) and the acclimatisation procedure (S4-ITA and S4-SPA) influenced the levels of LAB populations with a different trend depending on the production site. This condition leads to a rapid lowering of the pH below 5 and inhibits the growth of spoilage microorganisms and/or potential pathogens (Alfonzo et al., 2018,

Table 2
Evaluation of the sensory characteristics of table olives produced in Italy and Spain.

Attributes	Trials										SEM	S.S.	
	C-ITA	C-SPA	S1-ITA	S1-SPA	S2-ITA	S2-SPA	S3-ITA	S3-SPA	S4-ITA	S4-SPA		Judge	Trials
Visual characteristics													
Brightness	4.09^{ab}	4.29^{ab}	4.65^{a}	4.39^{a}	3.82^{b}	4.01^{ab}	3.46^{b}	3.82^{b}	4.56^{a}	4.45^{a}	0.12	*	*
Green colour intensity	5.22^{b}	5.43^{b}	6.71^{a}	5.25^{b}	4.36^{c}	5.11^{b}	5.61^{b}	5.33^{b}	5.18^{b}	5.03^{b}	0.19	*	*
Odour													
Green olive aroma	4.14^{e}	6.05^{ab}	5.11^{c}	6.00^{ab}	4.03^{e}	5.89^{b}	6.58^{a}	6.04^{ab}	6.46^{a}	6.78^{a}	0.29	**	**
Off-odours	2.02^{a}	1.55^{ab}	1.06^{b}	1.08^{b}	1.08^{b}	1.05^{b}	1.02^{b}	1.04^{b}	1.05^{b}	1.03^{b}	0.10	*	*
Rheological characteristics													
Crispness	3.89^{d}	5.66^{b}	4.68^{c}	4.82^{c}	4.50^{c}	5.76^{b}	6.95^{a}	6.02^{b}	5.76^{b}	5.88^{b}	0.32	**	***
Taste													
Acid	2.49^{bc}	2.58^{b}	2.80^{b}	2.98^{b}	2.36^{c}	3.02^{b}	2.81^{b}	3.88^{a}	2.55^{bc}	2.39^{bc}	0.14	**	*
Astringent	1.22^{c}	1.88^{a}	1.03^{c}	1.05^{c}	1.13^{c}	1.21^{c}	1.13^{c}	1.44^{bc}	1.01^{c}	1.29^{c}	0.08	**	*
Bitter	2.28^{ab}	2.29^{ab}	2.36^{ab}	2.13^{ab}	2.53^{a}	1.88^{c}	2.16^{ab}	2.21^{ab}	2.18^{ab}	2.02^{c}	0.06	*	*
Juicy	5.42^{b}	6.01^{ab}	5.78^{ab}	6.26^{a}	5.76^{ab}	5.81^{ab}	6.63^{a}	5.45^{b}	6.48^{a}	6.13^{a}	0.14	*	*
Salt	6.19^{a}	3.51^{cd}	5.51^{b}	4.13^{c}	5.25^{b}	3.32^{d}	5.58^{b}	3.89^{cd}	3.56^{cd}	4.41^{c}	0.42	*	***
Sweet	2.17^{ab}	2.03^{ab}	2.02^{ab}	2.44^{ab}	1.43^{b}	3.76^{a}	2.31^{ab}	2.23^{ab}	2.25^{ab}	2.45^{ab}	0.19	*	*
Off-flavours	1.77^{a}	1.37^{a}	1.02^{b}	1.04^{b}	1.05^{b}	1.03^{b}	1.08^{b}	1.09^{b}	1.02^{b}	1.04^{b}	0.06	*	*
Overall acceptability	5.02^{c}	5.27^{c}	5.95^{a}	5.11^{c}	5.08^{c}	5.38^{bc}	5.35^{c}	5.35^{c}	5.86^{a}	5.49^{bc}	0.10	*	*

Result indicate mean value. Data within a line followed by the same letter are not significantly different according to Tukey's test. Abbreviations: *, $P < 0.5$; **, $P < 0.01$; ***, $P < 0.001$. Abbreviations: SEM, standard error of mean; S.S., statistical significance.

2023a, 2023b; Martorana et al., 2017).

The use of specific nutrients that can improve the fermentative performance of LAB starter represents a strategy that should find wide application within the fermented table olive supply chain. However, the nutrient combination specific to the starter strain seems to have a significant effect on the fermentative efficiency, in agreement with what was reported by Alfonzo et al. (2023a). In this study, the use of the nutrient M.C.L.−15 was evaluated to assess its effect also on the starter strain L. *pentosus* OM13, although it is used for the L. *plantarum* Vege-Start 60. In fact, the use of Lal'Olive Nutrient combined with Lal'Olive Activator and the acclimatisation period represent the ideal solution in terms of acidification dynamics, the evolution of the microbial populations monitored and the sensory characteristics of the final product, confirming what was reported by Alfonzo et al. (2018) and Martorana et al. (2017).

The levels of dominance of the inoculated starter strains were variable, with significant percentages depending on the protocol used. Undoubtedly, the protocol involving the acclimation period of the OM13 strain in the presence of the activator and the use of the nutrient resulted in higher percentage dominance than the other treatments. The dominance percentages found were slightly higher than those reported by Alfonzo et al. (2023a,b) on Nocellara del Belice table olives processed in the same style but on a pilot scale. The presence in the inoculated productions of polymorphic profiles different from the starter strains could be related to the presence of indigenous LAB present in the drupes that persisted during fermentation or to the olive processing environments of industrial olives (Rodríguez-Gómez et al., 2017).

The monitoring of Enterobacteriaceae showed that their presence was detected until the 6th day of fermentation. While Pseudomondaceae and Staphylococcaceae were present in some treatments until the end of the process, the microbial densities achieved were low and never exceeded 4 logarithmic cycles. The results obtained confirm the data already described in the literature; in fact, several authors have highlighted the efficiency of table olive fermentations through the use of starter cultures (Panagou et al., 2003) and how adjuvants are able to influence the fermentation performance of selected inoculated LAB strains (Alfonzo et al., 2023a, 2023b; Martorana et al. 2015). The sensory analysis of the olive samples made it possible to distinguish the different productions in terms of different attributes. The sensory expression was influenced both by the olive variety used (Nocellara del Belice in Italy and Manzanilla in Spain) and by the technological protocol applied according to Conte et al. (2020). Undoubtedly, spontaneous fermentation had a negative impact on the organoleptic characteristics of the final product compared to the experiments fermented with starter strains. This confirms the need for the industrial production of table olives to be fermented with selected cultures of lactic acid bacteria in order to minimise product losses (Corsetti et al., 2012; Perpetuini et al., 2020). However, the strain/nutrient combination and the acclimatisation period influenced the sensory profile of the different productions. In fact, the identification of 7 different sensory profiles (3 in Italy and 4 in Spain) demonstrates how the addition of fermentation aids affects the fermentation performance of the starter strain and, consequently, the sensory characteristics of the final product, as reported by Alfonzo et al. (2023a,b).

5. Conclusions

The table olive supply chain is currently making steady progress compared to other major agricultural supply chains, but important challenges are being addressed at various levels to improve the quality of the product. The agronomic management of olive cultivation has a significant impact on the quality of the olives produced and to be fermented. Moreover, at the fermentation level, the use of starter cultures represents the most important biotechnological innovation of the last decade. At the industrial level, there is now a widespread tendency to use selected starter strains to limit product losses and improve organoleptic characteristics. However, the use of nutrients capable of enhancing the fermentation performance of LAB starters represents a further step towards improving the quality of the final product. In this study, five industrial productions in two different production areas (Italy and Spain) were compared. Differences were observed at the microbiological and sensory levels, demonstrating the high risk of producing green table olives by spontaneous fermentation. Productions inoculated with the commercial strains Bactoferm® *Lactiplantibacillus plantarum* Vege-Start 60 and Lal'Olive Crispy *Lactiplantibacillus pentosus* OM13 showed improved sensory characteristics compared to the spontaneously fermented control, where off-odours and off-flavours were perceived. In addition, the use of two different nutrients and the acclimatisation of Lal'Olive Crispy *Lactiplantibacillus pentosus* OM13 further positively diversified the product, as shown by the sensory analysis. Further applications on table olive productions of different varieties and production methods will allow to evaluate the suitability of LAB starter in other industrial productions.

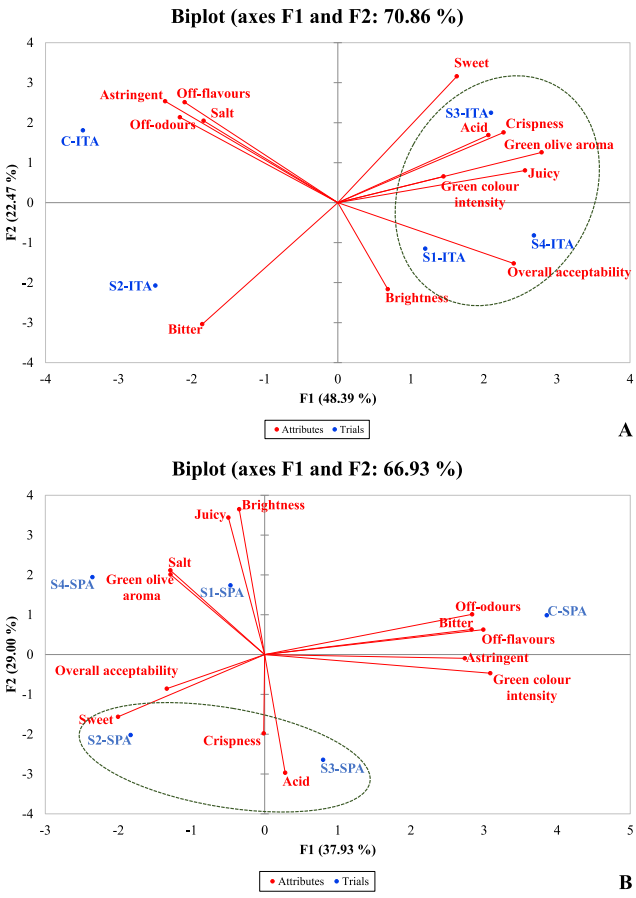

Fig. 4. PCA for sensory data of table olives produced in Italy (A) and Spain (B). Biplot graphs show the relationships between factors, attributes and trials. Abbreviations: C-ITA, spontaneously fermented Nocellara del Belice table olives; C-SPA, spontaneously fermented Manzanilla table olives; S1-ITA, Nocellara del Belice table olives fermented with Bactoferm® Vege-Start 60 + M.C.L.−15 nutrient; S1-SPA, Manzanilla table olives fermented with Bactoferm® Vege-Start 60 + M.C.L.−15 nutrient; S2-ITA, Nocellara del Belice table olives fermented with Lal'Olive Crispy + M.C.L.−15 nutrient; S2-SPA, Manzanilla table olives fermented with Lal'Olive Crispy + M.C.L.−15 nutrient; S3-ITA, Nocellara del Belice table olives fermented with Lal'Olive Crispy + Lal'Olive Nutrient; S3-SPA, Manzanilla table olives fermented with Lal'Olive Crispy + Lal'Olive Nutrient; S4- ITA, Nocellara del Belice table olives fermented with Lal'Olive Crispy + Lal'Olive Activator, acclimatisation (12 h) in brine (6 % NaCl) + Lal'Olive Nutrient; S4-SPA, Nocellara del Belice table olives fermented with Lal'Olive Crispy + Lal'Olive Activator, acclimatisation (12 h) in brine (6 % NaCl) + Lal'Olive Nutrient.

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Ethical statement

The authors declare that there was no animal or human study involved in this current research paper submitted.

Declaration of Competing Interest

Regarding the manuscript "Application of technological protocols on an industrial scale to improve Seville-style table olive production in Italy and Spain", the authors declare that we have no conflict of interest.

Data availability

The authors do not have permission to share data.

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References

Alfonzo, A., Ventimiglia, G., Corona, O., Di Gerlando, R., Gaglio, R., Francesca, N., et al. (2013). Diversity and technological potential of lactic acid bacteria of wheat flours. *Food Microbiology, 36*(2), 343–354. https://doi.org/10.1016/j.fm.2013.07.003

Alfonzo, A., Miceli, C., Nasca, A., Franciosi, E., Ventimiglia, G., Di Gerlando, R., et al. (2017). Monitoring of wheat lactic acid bacteria from the field until the first step of dough fermentation. *Food Microbiology, 62*, 256–269. https://doi.org/10.1016/j.fm.2016.10.014

Alfonzo, A., Martorana, A., Settanni, L., Matraxia, M., Corona, O., Vagnoli, P., et al. (2018). Approaches to improve the growth of the starter lactic acid bacterium OM13 during the early stages of green Spanish-style table olive production. *Grasas y Aceites, 69*(3). https://doi.org/10.3989/gya.0103181. e265–e265.

Alfonzo, A., Naselli, V., Gaglio, R., Settanni, L., Corona, O., La Croce, F., et al. (2023a). Use of different nutrients to improve the fermentation performances of *Lactiplantibacillus pentosus* OM13 during the production of Sevillian style green table olives. *Microorganisms, 11*(4), 825. https://doi.org/10.3390/microorganisms11040825

Alfonzo, A., Francesca, N., Naselli, V., Gaglio, R., Corona, O., Seminerio, V., et al. (2023b). Effect of glucose and inactivated yeast additions on the fermentation performances of *Lactiplantibacillus pentosus* OM13 during the production of nocellara del belice table olives. *Fermentation, 9*(7), 634. https://doi.org/10.3390/fermentation9070634

Aponte, M., Blaiotta, G., La Croce, F., Mazzaglia, A., Farina, V., Settanni, L., et al. (2012). Use of selected autochthonous lactic acid bacteria for Spanish-style table olive fermentation. *Food Microbiology, 30*(1), 8–16. https://doi.org/10.1016/j.fm.2011.10.005

Atrouz, K., Bousba, R., Marra, F. P., Marchese, A., Conforti, F. L., Perrone, B., et al. (2021). Algerian olive germplasm and its relationships with the Central-Western Mediterranean varieties contributes to clarify cultivated olive diversification. *Plants, 10*(4), 678. https://doi.org/10.3390/plants10040678

Conte, P., Fadda, C., Del Caro, A., Urgeghe, P. P., & Piga, A. (2020). Table olives: An overview on effects of processing on nutritional and sensory quality. *Foods, 9*(4), 514. https://doi.org/10.3390/foods9040514 (Basel, Switzerland).

Corsetti, A., Perpetuini, G., Schirone, M., Tofalo, R., & Suzzi, G. (2012). Application of starter cultures to table olive fermentation: An overview on the experimental studies. *Frontiers in Microbiology, 3*, Article 248. https://doi.org/10.3389/fmicb.2012.00248

Delgado, A. M., Parisi, S., Vaz Almeida, M. D., Delgado, A. M., Parisi, S., & Vaz Almeida, M. D. (2017). Olive oil and table olives. *Chemistry of the Mediterranean diet* (pp. 33–57). Cham: Springer. https://doi.org/10.1007/978-3-319-29370-7_4

Fernández, A. G., Adams, M. R., & Fernandez-Diez, M. J. (1997). *Table olives: Production and processing* (1st ed.). Springer Science & Business Media.

Gregersen, T. (1978). Rapid method for distinction of gram-negative from gram-positive bacteria. *European Journal of Applied Microbiology and Biotechnology, 5*, 123–127. https://doi.org/10.1007/BF00498806

Guiné, R. P., Florença, S. G., Barroca, M. J., & Anjos, O. (2020). The link between the consumer and the innovations in food product development. *Foods, 9*(9), 1317. https://doi.org/10.3390/foods9091317 (Basel, Switzerland).

IOC. (2022a). Production of table olive. Retrieved from https://www.internationaloliveoil.org/wp-content/uploads/2022/12/IOC-Table-Olive-Dashboard.html#production-2. Accessed September 19, 2023.

IOC. (2022b). Consumption of table olive. Retrieved from https://www.internationaloliveoil.org/wp-content/uploads/2022/12/IOC-Table-Olive-Dashboard.html#consumption-1. Accessed September 19, 2023.

Martorana, A., Alfonzo, A., Settanni, L., Corona, O., La Croce, F., Caruso, T., et al. (2015). An innovative method to produce green table olives based on "pied de cuve" technology. *Food Microbiology, 50*, 126–140. https://doi.org/10.1016/j.fm.2015.03.008

Martorana, A., Alfonzo, A., Settanni, L., Corona, O., La Croce, F., Caruso, T., et al. (2016). Effect of the mechanical harvest of drupes on the quality characteristics of green fermented table olives. *Journal of the Science of Food and Agriculture, 96*(6), 2004–2017. https://doi.org/10.1002/jsfa.7311

Martorana, A., Alfonzo, A., Gaglio, R., Settanni, L., Corona, O., La Croce, F., et al. (2017). Evaluation of different conditions to enhance the performances of *Lactobacillus pentosus* OM13 during industrial production of Spanish-style table olives. *Food Microbiology, 61*, 150–158. https://doi.org/10.1016/j.fm.2016.08.007

Nain, N., Kumari, K. G., Haridasan, H., Sharma, S. G., Sharma, S., Sharma, N., & Sharma, M. (2020). Microbes in food and beverage industry. *Microbial diversity, interventions and scope* (pp. 249–258). Springer. https://doi.org/10.1007/978-981-15-4099-8_15

Panagou, E. Z., Tassou, C. C., & Katsaboxakis, C. Z. (2003). Induced lactic acid fermentation of untreated green olives of the Conservolea cultivar by *Lactobacillus pentosus*. *Journal of the Science of Food and Agriculture, 83*(7), 667–674. https://doi.org/10.1002/jsfa.1336

Perpetuini, G., Prete, R., Garcia-Gonzalez, N., Khairul Alam, M., & Corsetti, A. (2020). Table olives more than a fermented food. *Foods, 9*(2), Article 178. https://doi.org/10.3390/foods9020178 (Basel, Switzerland).

Portilha-Cunha, M. F., Macedo, A. C., & Malcata, F. X. (2020). A review on adventitious lactic acid bacteria from table olives. *Foods, 9*(7), 948. https://doi.org/10.3390/foods9070948 (Basel, Switzerland).

Reiner K. (2010). *Catalase test protocol*. American society for microbiology. Retrieved from https://asm.org/Protocols/Catalase-Test-Protocol. Accessed April 13, 2023.

Rodríguez-Gómez, F., Romero-Gil, V., Arroyo-López, F. N., Roldán-Reyes, J. C., Torres-Gallardo, R., Bautista-Gallego, J., et al. (2017). Assessing the challenges in the application of potential probiotic lactic acid bacteria in the large-scale fermentation of Spanish-style table olives. *Frontiers in Microbiology, 8*, Article 915. https://doi.org/10.3389/fmicb.2017.00915

Rossetti, L., & Giraffa, G. (2005). Rapid identification of dairy lactic acid bacteria by M13-generated, RAPD-PCR fingerprint databases. *Journal of Microbiological Methods, 63*(2), 135–144. https://doi.org/10.1016/j.mimet.2005.03.001

Sánchez, A., García, P., Rejano, L., Brenes, M., & Garrido, A. (1995). The effects of acidification and temperature during washing of Spanish-style green olives on the fermentation process. *Journal of the Science of Food and Agriculture, 68*(2), 197–202. https://doi.org/10.1002/jsfa.2740680210

Saúde, C., Barros, T., Mateus, T., Quintas, C., & Pires-Cabral, P. (2017). Effect of chloride salts on the sensory and nutritional properties of cracked table olives of the Maçanilha Algarvia cultivar. *Food Bioscience, 19*, 73–79. https://doi.org/10.1016/j.fbio.2017.06.001

Zotta, T., Giavalisco, M., Parente, E., Picariello, G., Siano, F., & Ricciardi, A. (2022). Selection of *Lactiplantibacillus* strains for the production of fermented table olives. *Microorganisms, 10*(3), 625. https://doi.org/10.3390/microorganisms10030625