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Evaluation of different conditions to enhance the performances of *Lactobacillus pentosus* OM13 during industrial production of Spanish-style table olives

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ABSTRACT

The main objective was to set up a methodology to improve the high volume production of green table olives, cv. Nocellara del Belice. *Lactobacillus pentosus* OM13 was applied during three different industrial processes of table olives as follows: trial one (IOP1) was subjected to an addition of lactic acid until a brine level of pH 7.0 was reached; trial two (IOP2) subjected to same addition of lactic acid as in trial one plus nutrient adjuvant and trial three (IOP3) subjected to same addition as trial two, but with the strain *L. pentosus* OM13 acclimatized in brine for 12 h before inoculation. These trials were compared against two untreated controls (spontaneously fermented and addition of *L. pentosus* OM13 only).

Within the third day of fermentation, the pH of the brines decreased significantly, reaching pH 4.85 for trial three, pH 5.15 for trial two, and pH 5.92 for trial one. The pH of both controls decreased more slowly, and had values below pH 5.0 only after the fifteenth day of fermentation (control one) and the sixty-fifth day of fermentation (control two). Trial three reached the highest lactic acid bacteria (LAB) concentration on the third day of fermentation. After six days of fermentation, all trials showed similar values of LAB counts that were significantly higher compared to control number one. The result from genotypic identification showed that *L. pentosus* OM13 was the most frequently isolated in the inoculated trials. *Lactobacillus plantarum*, *Lactobacillus coryniformis* and *Pediococcus pentosaceous* were also detected at very low concentrations. Homoguaiacol, 2-butanol, 4-ethylphenol, phenylethyl alcohol and 4-ethylphenol were the volatile organic compounds detected at the highest levels in all experimental trials. Trial three showed a higher concentration of squalene that was not detected in other trials. The highest sensory scores of green olive aroma and overall satisfaction were found for all experimental olives, especially for those of trial one and trial two, that differed significantly from the untreated controls.

This study provides evidence that the addition of lactic acid, nutrient adjuvants and, most importantly, the acclimatization of LAB cells significantly shortens the acidification process of olive brine, and improves safety and sensory quality. Shorter acidification processes result in a more rapid transformation of table olives, with reduced commodity loss and lower costs of production compared to conventional manufacturing protocols.

1. Introduction

The use of starter cultures is widely applied in food fermentations (Corsetti et al., 2012), including table olive production (Roig and Hernández, 1991; Rodríguez-Gómez et al., 2013). Several

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studies have been performed to select the most adapted lactic acid bacteria (LAB) to be used as starters for table olive production (Servili et al., 2006; Aponte et al., 2012; Rodríguez-Gómez et al., 2013; Zago et al., 2013). The spontaneous fermentation, although still widely applied for table olive transformation, is an uncontrolled biological process.

The main purpose of using starter cultures during table olive manufacturing is to drive the fermentation and inhibit the development of spoilage microorganisms, such as pseudomonads, Enterobacteriaceae and staphylococci. In the absence of consistent levels of LAB, undesired microbial groups can rapidly increase and negatively affect the final product. Starter cultures may stabilize the manufacturing process in terms of chemico-physical, microbiological and sensory quality of table olives. The technological characteristics of starter cultures for table olive production include mainly the ability to grow at 15 °C, in presence of different concentrations of salt and phenolic compounds. Therefore, high levels of viability and efficiency of starter cultures are required to create the rapid drop of brine pH during the production of hydrogen sulphide; the β-glucosidase, lipolytic and proteolytic activities (Rodríguez-Gómez et al., 2010). Furthermore, the use of starter cultures may improve the food shelf-life by inhibition of spoilage microorganisms based on nutrient competition (Fernández-Díez et al., 1985; Garrido-Fernández et al., 1997; Durán Quintana et al., 1999; Holzapfel, 2002; Devlieghere et al., 2004; Silvestri et al., 2009), and should improve aroma and flavor of final products not only by inhibition of the spoilage microorganisms that interfere with the process of aroma generation, but also directly through their metabolism (Aponte et al., 2010). Until now, *Lactobacillus plantarum* (Lu et al., 2003) and *Lactobacillus pentosus* (Rodríguez-Gómez et al., 2013) have been the primary LAB species commonly applied to produce fermented table olives (Hurtado et al., 2012). However, some yeasts, such as *Wickerhamomyces anomalus*, *Saccharomyces cerevisiae* and *Pichia membranifaciens* have been selected as starters for this kind of production (Garrido-Fernández et al., 1997; Bautista-Gallego et al., 2011; Arroyo-López et al., 2012).

In order to enhance the activity of the starter cultures, the addition of some specific nutrient is suggested. Panagou et al. (2003) demonstrated that the death rate of Enterobacteriaceae and pseudomonads in presence of *L. pentosus* was more rapid in brines supplemented with glucose. Roig and Hernández (1991) suggested that enrichment of the brine with a nutritive supplement at the time of inoculation may increase the viability and efficiency of starter cultures, creating the rapid drop of brine pH. Within the published literature, however, little information is available on the use of supplements and adjuvants for fermenting table olives.

The main objective of this work was to set up a methodology to improve the high volume production of green table olives. To this end, the strain *L. pentosus* OM13 [selected previously (Aponte et al., 2012) and proved to be promising at pilot-plant scale (Martorana et al., 2015)], was applied during the industrial process, after an acclimatization procedure and in presence of supplements and adjuvants. The study was carried out with drupes of the cultivar Nocellara del Belice, and microbiological, chemical and sensory parameters were evaluated.

2. Materials and methods

2.1. Olive processing, experimental design and sample collection

A total of 250 tons of olives “Nocellara del Belice” were harvested at maturity. The drupes were selected, calibrated, washed by water and processed according to Sevillian technology, as follows: the drupes were supplemented with lye (2.6 °Bè) to remove the bitterness and, after 8 h, three sequential washings were performed with complete water replacement.

The bulk olives were transferred into 12.5 ton fiberglass vats [10 t of drupes and 2.5 t of brine (10% w/v NaCl)] and divided into three aliquots representing three experimental trials named IOP1, IOP2 and IOP3 (Fig. 1). Trial IOP1 was subjected to an addition of lactic acid (80% w/v, Purac, Biochem, Netherlands) until pH of the brine was 7.0. Trial IOP2 was added with lactic acid as in trial IOP1 plus 2 kg/t of the nutrient LBO2014 (Lallemand, Inc., Montreal, Canada) consisting of glucose, fructose and yeast autolysates for LAB nutritional requirements. Both trials IOP1 and IOP2 were inoculated with *L. pentosus* OM13 (150 g/t of olives) in freeze-dried form (Lallemand, Inc., Montreal, Canada) containing approximately 1.1×10^9 colony forming units CFU/g. This inoculum allowed a cell concentration of the starter strain of about 10^7 CFU/ml as confirmed by plate counts (Martorana et al., 2015). Trial IOP3 was subjected to the same additions as trial IOP2, but the starter culture *L. pentosus* OM13 was acclimatized in brine (6% w/v NaCl) for 12 h at room temperature before inoculation. Also for this trial the cell density reached in brine was approximately 10^7 CFU/ml.

Two untreated controls (C1 and C2) were also included in the experimental design. Both untreated controls did not receive any supplement. The fermentation of C1 was carried out by the indigenous drupe microorganisms. C2 was inoculated with the freeze-dried *L. pentosus* OM13 as reported above for trials IOP1 and IOP2.

The fermentation of all trials was performed at room temperature for 195 d and periodically monitored. Samples of brine (approximately 50 ml) were collected before addition of adjuvants and starter cultures, soon after their addition and after 3, 6, 9, 15, 35, 65, 143 and 195 d of fermentation. The experiment was performed in duplicate (two vats per trial). Two independent productions were performed in two consecutive weeks during October 2014 at the Geolive SAS company located in Castelvetrano (Trapani province, Siciliy, Italy) (37° 36′ 46″ N/12°50′ 52″ E).

2.2. Physico-chemical and microbiological analyses

Brine pH was measured using pH meter Russell RL060P (Thermo Fisher Scientific, Beverly, MA). The samples of brine were serially diluted in Ringer's solution (Sigma-Aldrich, Milan, Italy). Mesophilic rod LAB on de Man-Rogosa-Sharpe (MRS) agar added with cycloeximide (10 mg/ml), to avoid yeast growth. Yeasts, Enterobacteriaceae, pseudomonads, staphylococci and coagulase-positive staphylococci (CPS) were investigated as reported by Martorana et al. (2015). All analyses were performed in triplicate.

2.3. Isolation and phenotypic grouping of LAB

LAB were isolated after growth on MRS agar. At least five colonies per morphology were randomly collected from the agar plates and purified to homogeneity after several subculturing steps onto MRS agar. After microscopic inspection, three isolates (from each sample) sharing the same cellular morphology were stored at −80 °C. All isolates were subjected to Gregersen KOH method (Gregersen, 1978) and the test for the enzyme catalase (5%, w/v, H_2O_2). The isolates were grouped according to their cell morphology and disposition, growth at 15 and 45 °C and metabolism type (Martorana et al., 2015).

2.4. Genotypic characterization of LAB at species and strain level

All Gram positive and catalase negative isolates were subjected to genotypic investigation. The DNA from the presumptive LAB was extracted using the InstaGene Matrix kit (Bio-Rad Laboratories, Hercules, CA, USA) (Martorana et al., 2015).

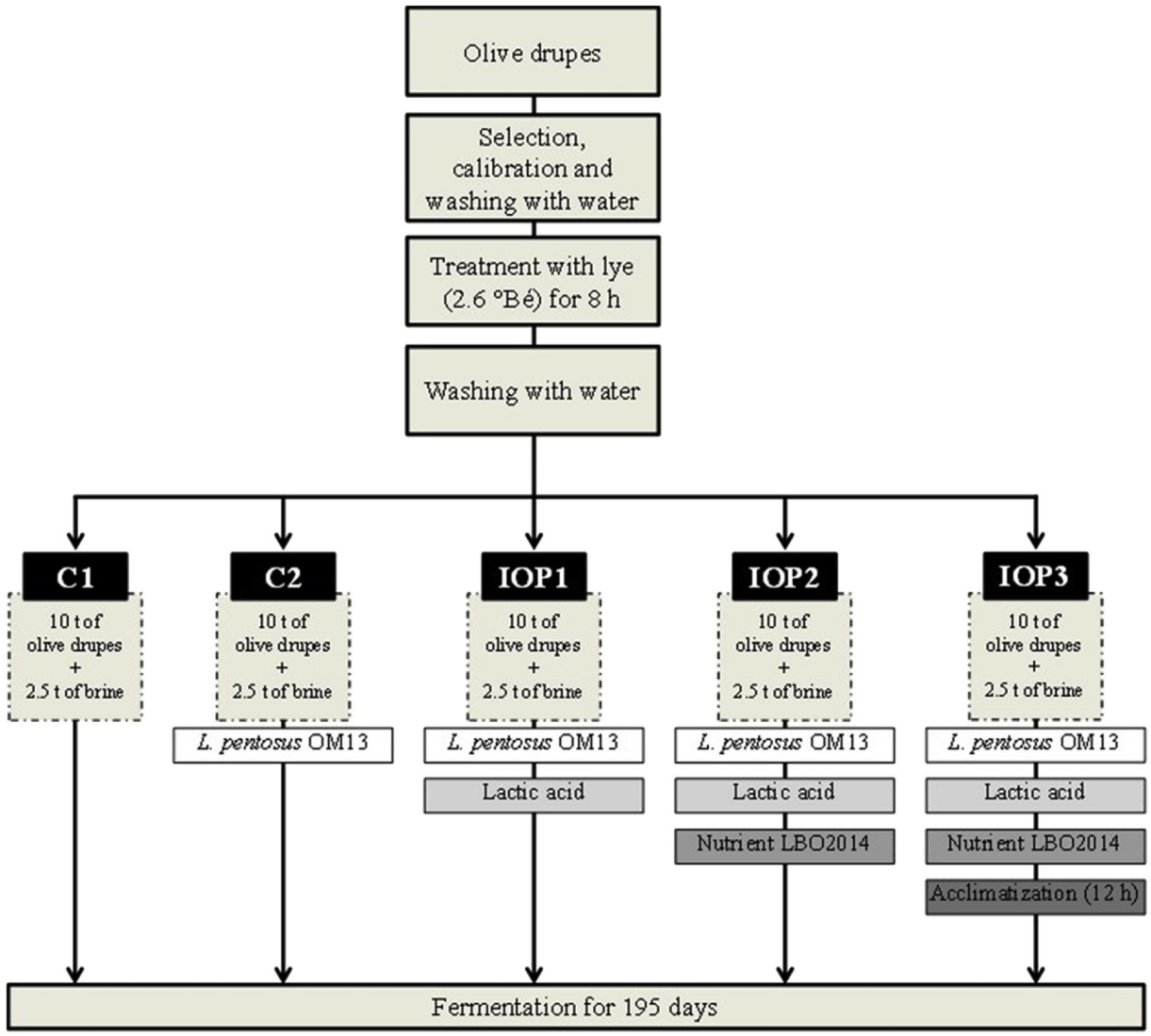

Fig. 1. Experimental design for manufacturing of green table olives.; Abbreviation: °Bé, Baumè degree; the codes C1 and C2 refer to the control trials; the codes from IOP1 to IOP3 refer to the experimental trials; *L.*, *Lactobacillus.*

All isolates were investigated at species level by multiplex PCR analysis of the *rec*A gene with the species-specific primers for *L. pentosus*, *L. plantarum* and *L. paraplantarum* (Torriani et al., 2001). One representative culture for each multiplex cluster and all strains that did not show amplification by multiplex PCR analysis were analysed by 16S rRNA gene sequencing as described by Weisburg et al. (1991). DNA sequencing reactions were performed at PrimmBiotech S.r.l. (Milan, Italy). The identities of the sequences were determined by BlastN search against the NCBI non-redundant sequence database located at http://www.ncbi.nlm.nih.gov and those available at EZTaxon located at http://www.ezbiocloud.net/eztaxon. In order to characterize LAB at strain level, the RAPD-PCR analysis was performed by using single primers M13, AB111 and AB106 (Settanni et al., 2012).

2.5. Determination of volatile organic compounds (VOCs) in fermented table olives

The analysis of VOCs was performed on table olives collected at the end of fermentation. VOCs were identified by Solid Phase Micro-Extraction technique in Head Space followed by Gas Chromatography/Mass Spectrometry (HS-SPMEGC/MS) (Pawliszyn, 1999; Aponte et al., 2010). The sample collection and the determination of VOCs were performed according to the methodologies reported by Martorana et al. (2015).

2.6. Sensory evaluation of fermented table olives

At the end of manufacturing, sensory analysis of processed olives was evaluated.

The descriptive panel consisted of twelve judges (6 females and 6 males, 23–31 years old) experts of green table olive evaluation. Preliminary sessions were arranged using commercial green table olives in order to train the judges with the descriptions of the sensory attributes, scales and procedures. The evaluation of the sensory profiles of the experimental olives was performed using a descriptive method (UNI 10957, 2003) as reported by Martorana et al. (2015). A total of 16 descriptors were included in the analysis and assessed into a 9-point quality scale [from 1.00 (absence of the descriptor) to 9.00 (extremely intense)].

The evaluations were carried out in individual booths under incandescent light. Samples were three-digit coded and the order

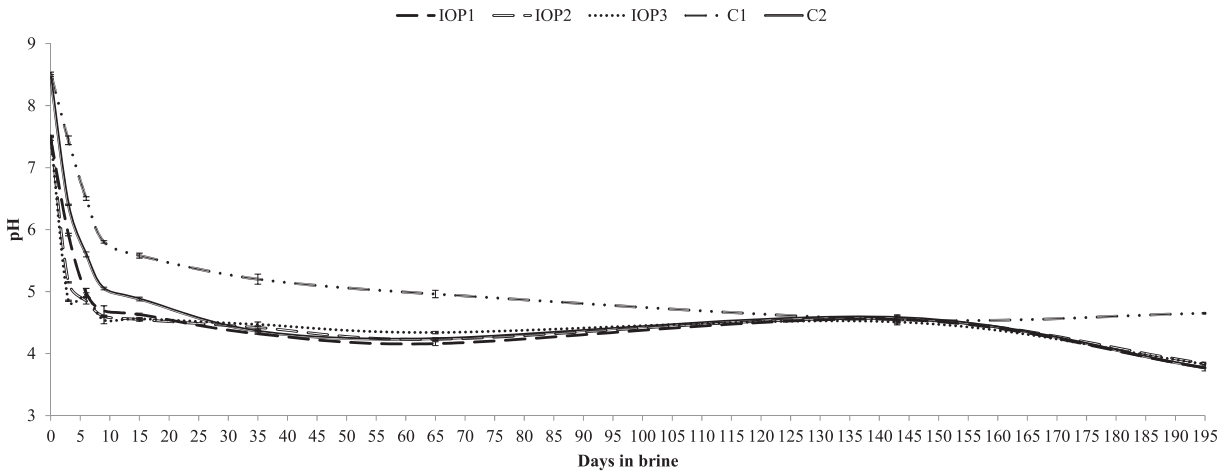

Fig. 2. Changes of pH during the manufacturing of green table olives. Results indicate the mean values of three measurements. Abbreviations: the codes IOP1, IOP2 and IOP3 refer to the experimental trials; the codes C1 and C2 refer to the control trials.

of serving was determined by random permutation. Two panel replications were carried out for each sample.

2.7. Statistical and explorative multivariate analyses

Data from physico-chemical, microbiological, VOCs and sensory analyses were investigated using a generalized linear model (GLM) based on ANOVA model that included effects of trials and week (1st and 2nd) of production, as well as the interaction between trials and week of production.

The post-hoc Tukey's method was applied for pairwise comparison. Statistical significance was attributed to $p < 0.05$.

Data from physico-chemical and microbiological analyses were subjected to an explorative multivariate analysis. The hierarchical cluster analysis (HCA) (joining, tree clustering) and principal component analysis (PCA) of data were performed in order to investigate relationships among samples as reported by (Martorana et al., 2015). All data were preliminary evaluated by using the Barlett's sphericity test (Dillon and Goldstein, 1984; Mazzei et al., 2010) in order to check the statistically significant difference among samples within each data set.

In order to graphically represent the values and distribution of VOC concentration among samples, a heat map clustered analysis (HMCA), based on double hierarchical dendrogram with heat map plot, was employed to represent the individual content values contained in the data matrix as colours. The relative values of concentration of VOCs were depicted by colour intensity from yellow (lowest concentration) to red (highest concentration). Heat map analysis of the VOC levels was performed using the autoscaled data.

The multivariate statistical analysis was concluded by using data of sensory analysis of olives. A principal component analysis by using the biplot graphical representation was constructed as reported by Martorana et al. (2015).

The STATISTICA software version 10 (StatSoft Inc., Tulsa, OK, USA) was achieved for Statistical data processing and graphic construction of HCA and PCA analyses. The XLStat software version 7.5.2 (Addinsoft, New York, USA) for HMCA and Biplot representation.

3. Results

3.1. Physico-chemical and microbiological analyses

Physico-chemical and microbiological data obtained from the analysis of brines collected during the entire olive manufacturing process are reported in Fig. 2 and Table S1, respectively.

Before fermentation took place, brine and olive drupes were characterized by average pH values of 7.1 and 5.8, respectively. Within the 3rd day of fermentation, the pH of the brines significantly decreased reaching 4.8 for trial IOP3, 5.1 for trial IOP2, and 5.9 for trial IOP1. The pHs of the untreated controls C1 and C2 decreased more slowly and values below 5.0 were registered only after the 15th and 65th day of fermention, respectively. From the 143rd day the differences among the pHs of the five trials were not significantly different, and the highest values were displayed by trial C2 until the end of the observation period.

With regard to the microbiological results, the olive drupes, before the fermentation process, showed concentrations of yeasts, LAB and pseudomonads of about 4.30, 1.9 and 2.7 Log CFU/g,

Table 1
Molecular identification of LAB species during the table olive production.

Species	Strain	Isolation source (day of sampling)	Size of multiplex- PCR[a] amplicon	% similarity[b] (accession no. of closest relative) by:		Sequence lenght (bp)	Acc. no.
				BLAST	EzTaxon		
Lactobacillus coryniformis	IOPBL-1	C2 (35d, 65d); C1 (3d, 15d)	n.a.	99 (KP893640.1)	98.71 (KCTC3535^T)	1478	KP893640.1
Lactobacillus pentosus	IOPBL-2	IOP1, IOP2, IOP3 (0–195 d)	218	99 (KU170095.1)	99.86 (JCM 1558^T)	1472	KU170095.1
Lactobacillus plantarum	IOPBL-3	IOP1 (143d); IOP2 (143, 195 d)	318	100(KT268296.1)	99.73 (ATCC14917^T)	1483	KT268296.1
Pediococcus pentosaceous	IOPBL-4	C2 (65, 143d); C1 (15, 35, 195d)	n.a.	99 (KT351717.1)	99.79 (DSM, 20336^T)	1437	KT351717.1

Abbreviations the codes IOP1, IOP2 and IOP3 refer to the experimental trials; the codes C1 and C2 refer to the control trials; n.a., not amplified.
[a] Results obtained by multiplex PCR analysis of the *rec*A gene with species-specific primers for *Lactobacillus pentosus*, *L. plantarum* and *L. paraplantarum*.
[b] Results obtained by the 16S rRNA sequence search.

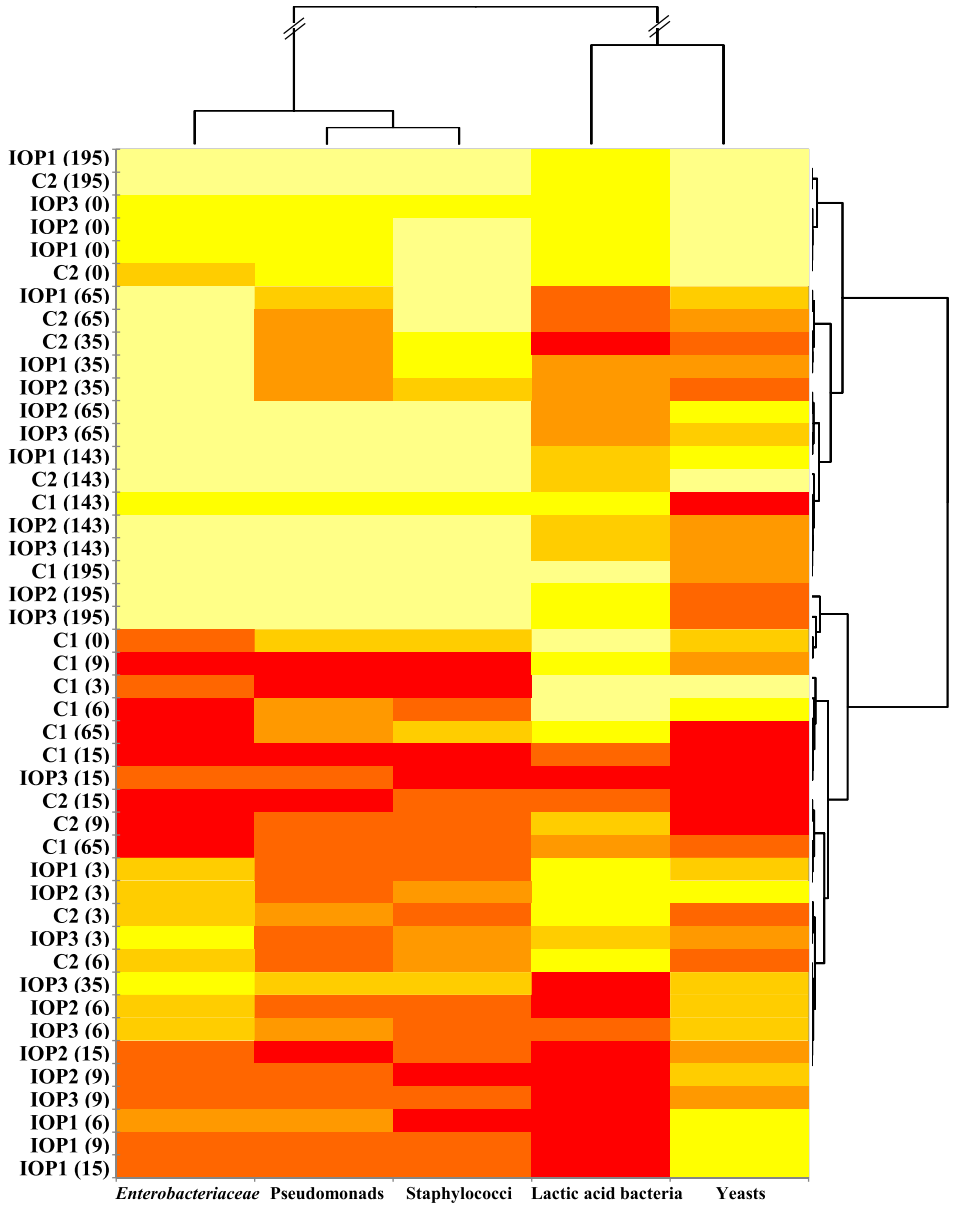

Fig. 3. Microbial population distribution among samples. The double hierarchical dendrogram is based on values of microbiological counts. The heat map plot depicts the relative percentage of each microbial groups (variables clustering on the Y-axis) within each sample (X-axis clustering). Results are expressed as relative percentage of mean values obtained from microbial loads of three plate counts. The values for microbial groups are depicted by colour intensity from yellow (lowest concentration) to red (highest concentration). Clusters based on the distance of the samples along the X-axis and the microbial groups along the Y-axis are indicated in the upper and left sections of the figure, respectively. The codes IOP1, IOP2 and IOP3 refer to the experimental trials; the codes C1 and C2 refer to the control trials. The numbers associated to each code, and reported between brackets, correspond to day of fermentation per each trials.

respectively; members of the Enterobactericeae family and staphylococci were not detected. All of these microbial groups were not detected into brine before the fermentation. Immediately after the addition of the starter strain OM13 and adjuvants, the concentration of LAB significantly increased in all experimental trials. The trial IOP3 reached the highest LAB concentration at day 3 of fermentation; from the 6th day onward, all IOP trials showed similar values of LAB counts that were significantly higher than those displayed by trial C1. Significant differences of LAB levels were also found between IOP trials and control C2 within day 9 of fermentation. With regard to yeast counts, control C1 showed the highest concentrations during the entire period of fermentation. Similar values were found for the experimental trials. The highest level of Enterobacteriaceae, pseudomonads and staphylococci were registered for control C1. From day 65, all these populations almost disappeared from IOP trials, and were detectable only in brines of control C1. At end of the monitoring process, only LAB and yeast populations were found in all trials.

3.2. Isolation, grouping, strain differentiation and identification of LAB

A total of 4147 colonies were collected from the highest plated dilutions of cell suspensions. The majority of the cultures showed cells with a rod shape morphology, typical of lactobacilli. Three thousand and two hundred seventy (3,270) isolates were

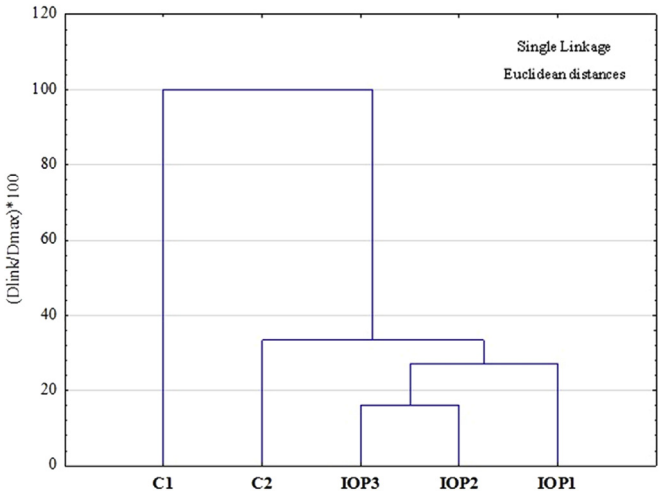

Fig. 4. Dendrogram resulting from HCA based on values of pH changes and microbiological data. The dissimilarity among samples was measured by Euclidean distance, whereas cluster aggregation was achieved by single linkage.

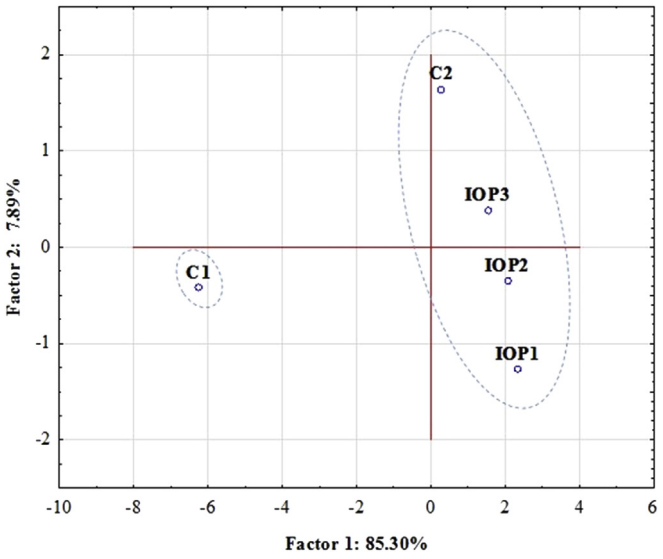

Fig. 5. PCA analysis based on the values of pH changes and microbiological data.

considered presumptive LAB and further characterized. All cultures were able to grow at 15 °C, unable to develop at 45 °C and facultatively homofermentative because they did not generate CO_2 from glucose and grew in presence of pentose carbohydrates. Due to the high number of isolates, about 40% of the cultures, selected to be representative of the isolation source, colony morphology, experimental trial and week of production, were genetically investigated. Based on the multiplex PCR amplicons, these bacteria formed two groups, indicating the presence of the species *L. pentosus* and *L. plantarum* (Table 1). To confirm the identification of LAB at species level, one strain representative of each multiplex PCR groups was subjected to the 16S rRNA gene sequencing, they belonged to the *L. pentosus/L. plantarum/L. paraplantarum* group. Moreover, the species *Lactobacillus coryniformis* and *Pediococcus pentosaceous* were detected among the bacteria that did non generated any amplicons when processed by the multiplex PCR (Table 1).

In order to verify the persistence of the starter OM13, all isolates characterized by a multiplex-PCR amplicon of 218 bp, corresponding to the species *L. pentosus*, were subjected to the RAPD-PCR analysis. Fourteen different RAPD profiles, indicated the presence of 14 *L. pentosus* strains isolated at high levels from the five trials. In particular, the direct comparison of the RAPD profiles showed that *L. pentosus* OM13 was the strain most frequently isolated in the inoculated trials, but was not recognized among the isolates from the control C1.

3.3. Analysis of VOCs and sensory profiles of fermented olives

The profiles of the VOCs emitted from the fermented olives are reported in Table S2. Homoguaiacol, 2-butanol, 4-ethylphenol, phenylethyl alcohol and 4-ethylphenol were the compounds detected at the highest levels in all experimental trials. Trial IOP3 also showed a high concentration of squalene that was not detected at in other samples. Benzyl alcohol, phenylethyl alcohol and, in particular, acetic acid were mainly found in olives from control C1. The experimental olives were also evaluated by expert panelists in sensory analysis (Table S3). All samples produced in accordance with IOP protocols significantly differed from both control trials. The main differences were estimated in terms of green olive aroma, sweet, bitter, astringent (taste) and, in particular, overall satisfaction. The highest scores of green olive aroma and overall satisfaction were found for olives of trials IOP1 and IOP2.

3.4. Statistical and explorative multivariate analysis

The analysis of variance over the two productions performed during the two consecutive weeks showed no significant effect between weeks and the dependent variables corresponding to physico-chemical and microbiological data, VOCs and sensory scores.

In contrast, the Barlett's sphericity test was applied to all data matrix inputs and significant differences ($p < 0.001$) were found among trials.

The graphical representation of the values obtained from the microbial counts are reported in Fig. 3. The double hierarchical dendrogram combined with heat map plot showed that IOP trials significantly affected the development of the microbial populations. LAB and yeasts were grouped into one mega-cluster characterized by the highest concentration of microorganisms in all experimental trials. The second mega-cluster included staphylococci, pseudomonads and Enterobactericeae that were present at the highest concentrations in samples of control C1.

The HCA (Fig. 4) clearly separated the olives of the IOP trials from those of the controls. Control C1 showed the highest level of dissimilarity among samples. On the other hand, all samples of control C2 were grouped into one mega-cluster that included all experimental trials.

In order to better evaluate better the differences among trials, and to condense all information from dependent variables into a restricted numbers of factors, the data matrix reported above were also subjected to PCA. The score plot (Fig. 5) confirmed the results obtained with HCA, since all IOP trials were significantly separated from the control C1 along the factor 1 that explained the majority of total variability (85.30%). The explorative multivariate analysis based on HCMA was performed to deeply investigate the composition and the concentrations of the VOCs (Fig. 6). The clustering dendrogram of heat map plot showed the effect of each transformation protocol on the VOCs. IOP trials and control C2 were grouped into one mega-cluster that significantly differed from the control C1 in terms of VOC distribution and concentration.

The multivariate statistical analysis was concluded with the analysis of the sensory scores (Fig. 7). The trials IOP2 and IOP3 were closely related based on green olive aroma, complexity (both odour

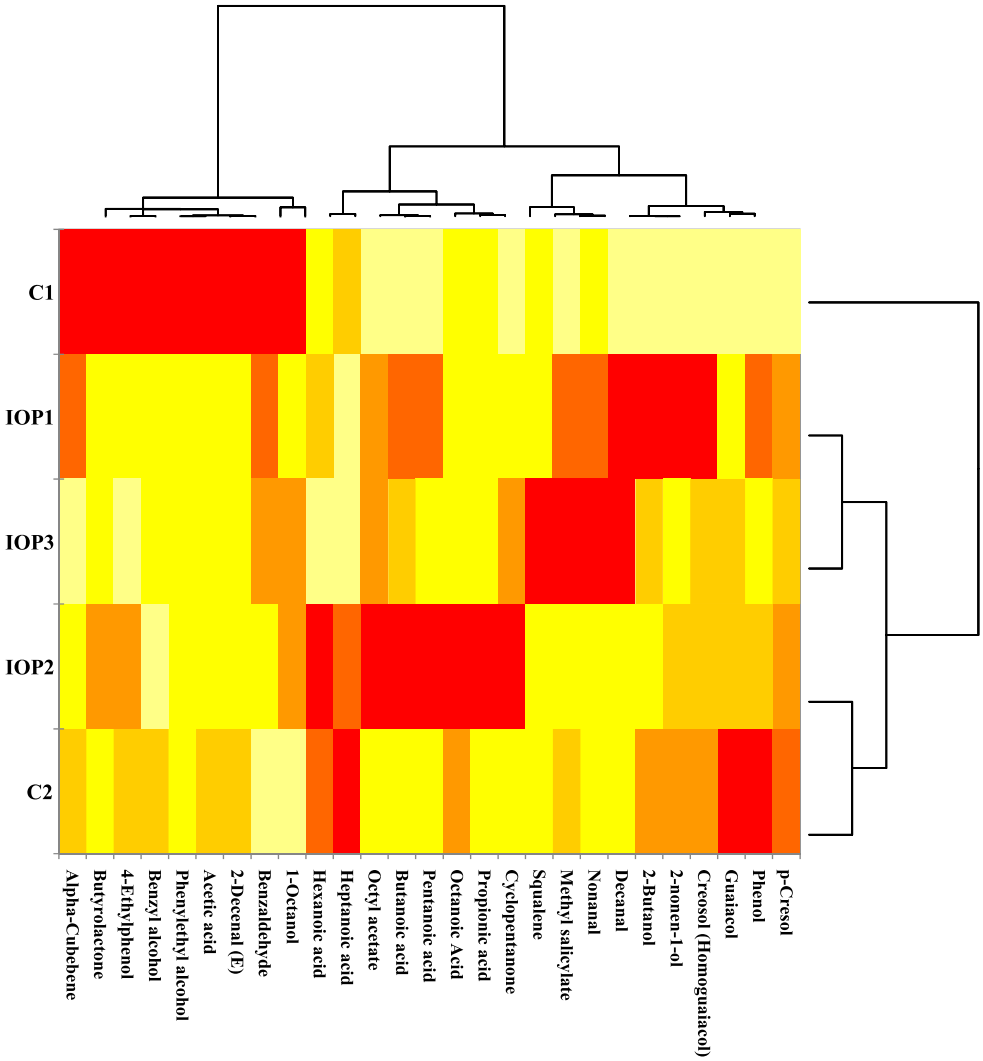

Fig. 6. Distribution of volatile organic compounds among trials at the end (day 195) of olive productions. The heat map plot depicts the relative percentage of each VOCs (variables clustering on the Y-axis) within each sample (X-axis clustering). The codes IOP1, IOP2 and IOP3 refer to the experimental trials; the codes C1 and C2 refer to the control trials.

and taste) and overall acceptability, descriptors that characterize positively the final products. In contrast, the control C1 was associated to the highest score of bitter, astringent and off-odors.

4. Discussion

In this study, innovative procedures to accelerate the growth of starter LAB cultures, and improve microbiological and final quality of table olives through shortening of the acidification period, were investigated. Although, the use of LAB starter in table olive fermentation has been widely investigated (Aponte et al., 2012; Martorana et al., 2015), to our knowledge, no study has been carried out on the use of acclimatization procedure of starter culture during table olive manufacturing. This operation is commonly followed in oenology to better rehydrate the microbial cells responsible for the fermentation process. As an example, Kontkanen et al. (2004), achieved a higher biomass and higher viable cell concentration of *S. cerevisiae* strain by acclimatization determining an acceleration of the alcoholic fermentation. Usually, the starter cultures are largely marketed in spray-dried and frozen form. Thus, rehydration is a critical step in the recovery of spray-dried cultures to be applied as starters during food fermentation. The solution and conditions of culture rehydration may affect the survival rate of dried microbial cells (Peighambardoust et al., 2011).

In order to inhibit the growth of spoilage and/or pathogenic microorganisms, a rapid decrease of pH to 5.0 is required. During the manufacturing of table olives, the most consistent drop of brine pH is commonly registered after the 20th day of olive manufacturing; a slow decrease of pH is, generally, associated to a slow growth of LAB populations (Romeo, 2012).

The results of this research show that the use of nutrients and adjuvants can also improve the microbiological quality of fermented olives by promoting the drop of pH and inhibiting growth of spoilage microorganisms. The use of nutrient adjuvants is commonly used in oenological practice (Gockowak and Henschke, 2003; Anonymous, 2008; Terrade and Mira de Orduña, 2009). Ruiz-Barba and Jiménez-Díaz (1994, 1995) stated that the starter cultures grow better in presence of reducing sugars, vitamins and amino acids and demonstrated that the addition of nutrients reduced the overall time required for wine fermentation from about 500 to 350 h stimulating the production of yeast biomass at a faster rate. On the other hand, very little information is available in literature on the use of nutrient adjuvants during table olive productions. Some authors (Garrido-Fernández et al., 1997; Poiana and

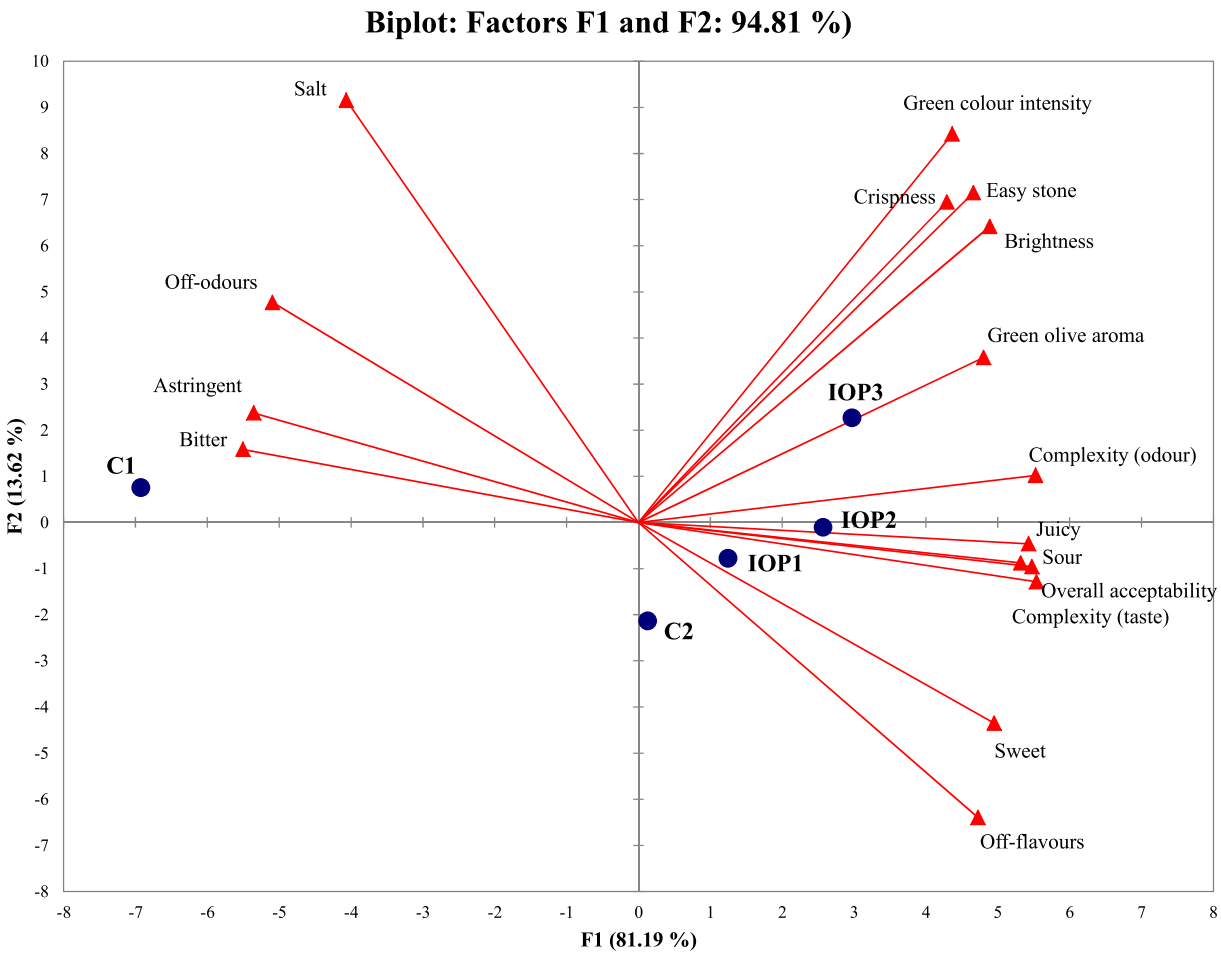

Fig. 7. PCA for sensory data of table olives at the end of process (195 day). Biplot graph show relationships among factors, variables and trials.

Romeo, 2006; Durán Quintana et al., 2005) tested the use of lactic acid to acidify brine during olive fermentation. Panagou et al. (2003) suggested that the enrichment of brine with nutritive supplements, at the time of inoculation, may increase the viability and efficiency of starter cultures. In particular, those authors inoculated untreated green olives with *L. pentosus* with and without glucose supplement and showed that the death rate of Enterobacteriaceae and pseudomonads was higher in brines inoculated with starter and supplemented with glucose.

In our study, the ability of *L. pentosus* OM13 to dominate the microbial population and to accelerate the drop of brine pH was registered for all IOP trials with the best results observed for trial IOP3 that included the acclimatization procedure. The dominance of *L. pentosus* OM13 during the entire olive fermentation in all experimental trials could represent a guarantee for the quality of the final products. In fact, the presence of the starter strain at dominating level during olive fermentation has been already reported to improve the safety and the complexity of the sensory profile of green table olives (Martorana et al., 2015, 2016), as well as of other fermented vegetables (Francesca et al., 2016). The monitoring of pH decrease and the evolution of the microbial populations clearly showed the positive effects of the starter OM13. Microbiological, chemical and sensory quality of olives at the end of the process improved consistently in comparison with those of the spontaneously fermented trial C2.

As reported by other authors (Martorana et al., 2015; Sabatini et al., 2008, 2009), alcohols and acids reached the highest concentrations in olives fermented by LAB starter. Furthermore, acetic acid is commonly represented by VOCs of table olives since it is representative of LAB and yeast metabolism (Bleve et al., 2015).

Thus, chemical and sensory quality of experimental olives at the end of the process improved consistently in comparison to those of the control C2 spontaneously fermented. These results suggest that the acclimatization of the starter culture is a valuable method to accelerate the pH decrease and represents a relevant innovation for green table olive manufacturing.

In conclusion, our study provided evidences that the acclimatization of LAB cells before their inoculation into brine, as well as the addition of lactic acid and nutrient adjuvants, shortened significantly the acidification process of olive brine and improved consistently the safety and the sensory quality of the final products. This study has a high potential for high volumes of table olive productions, since a shorter acidification period means a more rapid transformation of the drupes with lower losses of olives and lower costs for production than those manufactured following the traditional protocol. In particular, the production system carried out with IOP3 conditions determined an early presence of Nocellara del Belice green table olives for the year 2015, proving the economic relevance of this new system.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.fm.2016.08.007.

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