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An innovative method to produce green table olives based on "pied de cuve" technology

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ABSTRACT

The technology of "pied de cuve" (PdC) is applied in food process only to produce wines with an enriched community of pro-technological yeasts. PdC promotes the growth of the desirable microbial strains in a small volume of grape must acting as a starter inoculums for higher volumes. The aim of the present work was to investigate the use of partially fermented brines, a technology known as PdC, developed with lactic acid bacteria (LAB) on the microbiological, chemical and sensory characteristics of green fermented table olives during two consecutive campaigns. The experimental plan included two trials based on different PdCs: trial A, PdC obtained with *Lactobacillus pentosus* OM13; trial B, PdC obtained through a spontaneous fermentation. Two control additional trials without PdC were included for comparison: trial C, spontaneous fermentation; trial D, direct inoculation of *L. pentosus* OM13. The use of PdCs favoured the rapid increase of LAB concentrations in both trials A and B. These trials showed levels of LAB higher than trial C and almost superimposable to that of trial D. Trial B was characterized by a certain diversity of *L. pentosus* strains and some of them dominated the manufacturing process. These results indicated PdC as a valuable method to favour the growth of autochthonous *L. pentosus* strains. Hierarchical cluster analysis (HCA) and principal component analysis (PCA) visibly discriminated olive processes fermented with the two experimental PdCs. Interestingly, on the basis of microbial and pH variables, both approaches showed that the olives produced with PdC technology are closely related to those of trial D, with the advantages of reducing the amount of starter to inoculate (trial A) and a higher LAB biodiversity (trial B). Volatile organic compound (VOC) composition and sensory analysis showed trials A and B different from the trials with no PdC added, in both years. Furthermore, the trial B showed the highest scores of green olive aroma and taste complexity. Spoilage microorganisms were estimated at very low levels in all trials. Undesired off-odours and off-flavours were not revealed at the end of the process.

1. Introduction

The 95% of world cultivation of olive trees (*Olea europaea* L.) is concentrated in the Mediterranean area. The International Olive Council (IOC) estimated a total world production of 2.5 million tons for the 2012/2013 campaign, and about 76.000 tons of this production is obtained in Italy (IOC, 2013). Olive drupes, just after harvest, cannot be eaten due to the presence of oleuropein that is a bitter phenolic glucoside consisting of glucose, elenolic acid and *o*-diphenol hydroxytyrosol compounds (Servili et al., 2006). A variety of technological methods are commonly applied during transformation to reduce the bitterness of table olives. "Spanish" (also known as "Sevillan") and "Greek" (also known as "natural") processing styles are the methods most quantitatively employed in Italy for table olive production (Catania et al., 2014). Most table olive fermentation processes start spontaneously (Silvestri et al., 2009; Tofalo et al., 2013), whereas several productions at industrial level are driven by *Lactobacillus plantarum* and *Lactobacillus pentosus* (Lu et al., 2003; Servili et al., 2006; Hurtado et al., 2012).

Several studies (Guzzon et al., 2011; Tofalo et al., 2014, 2012a; Sannino et al., 2013) showed that the foods produced through spontaneous fermentations are often characterized by marked

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sensory profiles. However, the risk of off-odour and off-flavour generation, due to the development of spoilage microorganisms during the uncontrolled biological processes, has to be considered, because they may seriously affect the quality of the final products.

Generally, the direct inoculums of commercial starter cultures into olive brine assure a rapid increase of LAB concentration and the corresponding decrease of pH. This procedure limits the risk of undesired aromatic notes (Servili et al., 2006; Peres et al., 2008; Sabatini et al., 2008; Aponte et al., 2012). However, when a given starter preparation, composed of a limited number of strains, is used to ferment different varieties of olives of different geographical areas a flattening of the taste of table olives may occur, with the risk that the final products may no longer be distinguishable by production technology and/or geographical origin. This because the starter strains prevail over the native microflora (Aponte et al., 2012). For this reason, the use of selected autochthonous strains is becoming a common practise for table olive fermentation (Aponte et al., 2010, 2012; Bautista-Gallego et al., 2013; Di Cagno et al., 2013), but the microbial diversity that contributes to the expression of the varietal notes in the final products is extremely reduced.

The technology of "pied de cuve" (PdC), largely used for wine production (Ubeda Iranzo et al., 2000; Clavijo et al., 2011; Li et al., 2012), limits the reduction of the microbial complexity of the driven processes. This method promote the growth of the desirable microbial strains in a small volume of grape must which act as a starter inoculums for higher volumes, but does not exclude the risk of development of unwanted microorganisms. Usually, the ratio PdC/bulk for wine application is 1/10 (Li et al., 2012).

The present work was aimed to produce green table olives applying PdC technology. PdCs were obtained differently to evaluate the dominance of the LAB population: A, direct inoculum of commercial LAB starter; B, by spontaneous fermentation. Microbiological, chemical and sensory parameters were evaluated during two consecutive years.

2. Materials and methods

2.1. Experimental olive production and sample collection

Table olive production process was carried out with the cultivar "Nocellara del Belice" and performed at the company "Geolive Belice S.A.S." located in Castelvetrano (Trapani, Sicily, Italy). The olives were manually harvested from fields within Trapani province (37°37′11.29″N/12°50′33.27″E). The experimental plan included two technological steps: (i) preparation of different PdCs and 10 days of fermentation; (ii) *addition of* PdCs into fresh brines and olive production. The experimentation was carried out directly in brine in two consecutive years (2012 and 2013) adopting three replicates (three fermentation vessels per trial) in both years.

2.2. Preparation of PdC

Bulk olive fruits were transferred in two steel vats (180 l-volume) representing two different PdCs (A and B). Each vat contained 150 kg of olives and 30 l of brine composed of NaCl 9% (w/v). PdC A was inoculated with 0.15 g/kg of the autochthonous strain *L. pentosus* OM13, previously used to produce commercial Nocellara del Belice table olives in the Trapani province (Aponte et al., 2012), and kept freeze–dried (about 8.00×10^{12} CFU/g). PdC B was spontaneously fermented. The fermentation of both PdCs lasted 10 days and was carried out at room temperature (about 20–22 °C). Brine salt concentration was constantly maintained at the initial level by periodical addition of coarse salt.

2.3. Production of table olives

The two PdCs were transferred in six steel vats containing the same weight of olives and volume of brine as reported above and represented two distinct trials (A and B; preparing three replicates vats for each trial). The partially fermented brine of PdCs were added to a final ratio of 1:10 in each vat. In addition, two control trials without PdC (trials C and D) were included in the experimental plan. Trials C and D represented the control production for table olives obtained through spontaneous fermentation and with starter addition, respectively. Vats of trial C were spontaneously fermented. Vats of trial D were inoculated (0.1 g/l of brine) with the strain *L. pentosus* OM13. The fermentation of all trials was performed at room temperature for 200 d and was periodically monitored. Samples of brine (about 50 ml) were collected before inoculum of PdC and, then, soon after addition and at 3, 6, 9, 15, 25, 35, 65, 85, 115, 150 and 200 days of fermentation.

2.4. Physico-chemical and microbiological analyses

The values of pH of brine samples were determined by a pH meter (BASIC 20+; Crison Instrument S.A., Barcelona, Spain). Salt concentration was routinely analyzed as reported by Garrido Fernández et al. (1997).

Decimal dilutions of brines were prepared in Ringer's solution (Sigma–Aldrich, Milan, Italy). Different microbial groups were enumerated as follows: mesophilic rod LAB on de Man-Rogosa-Sharpe (MRS) agar, incubated anaerobically at 30 °C for 48 h; total yeasts (TY) and filamentous fungi on dichloran rose bengal chloramphenicol (DRBC) agar, incubated aerobically at 25 °C for 5 days; *Enterobacteriaceae* on violet red bile glucose agar (VRBGA), incubated aerobically at 37 °C for 24 h; pseudomonads on *Pseudomonas* agar base (PAB) supplemented with CFC supplement, incubated aerobically at 20 °C for 48 h; staphylococci on Baird Parker (BP) and coagulase positive staphylococci (CPS) on BP added with RPF supplement, incubated aerobically at 37 °C for 48 h. Analyses were performed in triplicate. All media and the supplements used were supplied from Oxoid (Thermofisher, Basingstoke UK).

2.5. Isolation and phenotypic grouping of LAB

Presumptive LAB (at least 4 colonies characterized by the same colour, morphology, edge, surface and elevation) were collected from the highest plated dilution following their growth on MRS agar. The isolates were purified by successive sub-culturing and the purity of the isolates were checked microscopically. Gram-positive (Gregersen KOH method) and catalase negative (determined in presence of H_2O_2 5%, v/v) were stored in broth containing 20% (v/v) glycerol at −80 °C until further experimentations.

LAB were initially subjected to a phenotypic grouping based on cell morphology and disposition, determined by an optical microscope, growth at 15 and 45 °C and metabolism type, testing the ability to produce CO_2 from glucose. The last assay was carried out with the same growth media used for isolation, without citrate from which certain LAB produce gas. The obligate homofermentative metabolism was determined by the absence of growth in presence of a mixture of pentose carbohydrates (xylose, arabinose, and ribose; 8 g/l each) in place of glucose. Sub-grouping of cocci included also the growth at pH 9.6 and in the presence of 6.5% (w/v) NaCl.

2.6. Identification of LAB at strain and species level

DNA from LAB isolates was extracted using the InstaGene Matrix kit (Bio-Rad Laboratories, Hercules, CA) according to the

manufacturer's instructions. Strain differentiation was performed by random amplification of polymorphic DNA-PCR (RAPD-PCR) analysis in a 25-μl reaction mix using single primers M13, AB111 and AB106 as previously described by Settanni et al. (2012a). The identification at species level was performed by multiplex PCR analysis of the *rec*A gene with species-specific primers for *L. pentosus*, *L. plantarum* and *Lactobacillus paraplantarum*, according to the protocol described by Torriani et al. (2001).

One representative cultures 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.

2.7. Isolation, grouping and identification of yeasts

Yeasts were collected from DRBC medium. At least five colonies per morphology were randomly collected from the agar plates, purified to homogeneity after several sub-culturing steps onto DRBC medium and subjected to genetic characterization.

DNA extraction was performed as reported above. All selected isolates were preliminary grouped by restriction fragment length polymorphism (RFLP) analysis of the region spanning the internal transcribed spacers (ITS1 and ITS2) and the 5.8S rRNA gene as reported by Esteve-Zarzoso et al. (1999). One isolate per group was identified at species level by sequencing the D1/D2 region of the 26S rRNA gene to confirm the preliminary identification obtained by RFLP analysis. D1/D2 region was amplified and PCR products were visualized as described by Settanni et al. (2012b). The reaction of DNA sequencing and the identities of sequences were determined as reported above.

2.8. Volatile organic compounds (VOCs)

VOCs at 200 days of fermentation 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). Analysis of samples was carried out by homogenizing 0.50 g of drupes and transferring it into 2 ml vials with pierceable silicone rubber septa coated with polytetrafluoroethylene (PTFE) film. Fifty microlitres of 2-Pentanol-4-methyl methanol solution (0.981 μg/ml) were used as an internal standard. A SUPELCO SPME (Bellefonte, PA) fibre holder and fibre used was coated with divinylbenzene/carboxen/polydimethylsiloxane. The vials were heated at controlled temperature (40 ± 0.5 °C) in order to reach equilibrium and 30 min exposure time. Collected data were processed with the instrument data system. Olive volatile compounds were identified by comparison of the retention times with those of the reference compounds (NIST/EPA/MSDC Mass Spectral Database, T.G. House, Cambridge, UK). Semi-quantitative determination was carried out by the method of internal standard. The calibration curve was constructed with readings on five 2-Pentanol-4-methyl methanol solutions with concentrations ranging from 1.5 μg/ml to 8 μg/ml (R^2 0.994). All analyses were performed in duplicate.

2.9. Sensory evaluation

The evaluation of the sensory profiles of the experimental olives was performed applying the descriptive method UNI 10957 (2003) as reported by Aponte et al. (2012). The analysis was applied on olives at the end of fermentation (day 200).

Twelve judges (6 females and 6 males, 22–35 years old) were trained in preliminary sessions using different samples of commercial table olives belonging to the Nocellara del Belice cultivar, in order to develop a common vocabulary for the description of the sensory attributes of the experimental samples, as well as to familiarize them with scales and procedures. Each attribute was extensively described and explained to avoid any doubt about the relevant meaning.

The sensory attributes cited with a frequency higher than 60% by panelists when selected for sample evaluation. Thus, 9 descriptors were included in the analysis for the odour (green olive aroma), rheological characteristics (crunchiness), taste (sweet, acid, bitter, salty and complexity). The descriptors off-odour and off-flavour were also included in the analyses and they were described to judges as the perception of negative sensations due to abnormal fermentation and/or other defects as listed by IOC (2013). The olive samples were randomly evaluated by assigning a score between 1.00 (absence of sensation) and 9.00 (extremely intense) in individual booths under incandescent white light.

2.10. Statistical and explorative multivariate analysis

Data of pH, microbiological investigation and sensory evaluation were analysed using a generalised linear model (GLM) that included the effects of samples; the Student "*t*" test was used for mean comparison. The post-hoc Tukey method was applied for pairwise comparison. Statistical significance was attributed to p values of <0.05. The resulting scores from the sensory analysis were averaged and compared. The ANOVA test was applied to identify significant differences among olive attributes.

In addition, explorative multivariate analysis was employed to investigate relationship among data obtained from the different experimentations. A hierarchical cluster analysis (HCA) (joining, tree clustering) was carried out for grouping the experimental trials according to their similarity, measured by Euclidean distances, whereas cluster aggregation was based on the single linkage method (Todeschini, 1998). Furthermore, the principal component analysis (PCA) was employed to investigate relationships among samples. The input matrix used for HCA and PCA consisted of the total area under growth/decline curves of LAB, yeasts, enterobacteria, pseudomonads, CPS, as well as pH values (Bautista-Gallego et al., 2011; Blana et al., 2014). Areas were calculated by integration using the OriginPro 7.5 software (OriginLab Corporation, Northampton, USA). In addition, other relevant indexes of pH and microbial changes were taken into account as follows: maximum and minimum values of pH, maximum and minimum values of microbial populations (Bautista-Gallego et al., 2011).

PCA was also employed to investigate relationship among olive samples based on VOC profile, as well as sensory analysis (Rodríguez-Gómez et al., 2014).

The number of principal factors was selected according to the Kaiser criterion (Jolliffe, 1986) and only factors with eigen-values higher than 1.00 were retained. 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.

Statistical data processing and graphic construction were achieved by using STATISTICA software version 10 (StatSoft Inc., Tulsa, OK, USA) and XLStat software version 7.5.2 (Addinsoft, New York, USA) for excel.

Table 1
Values of pH and microbial concentrations of samples collected during the table olive production during the 2012 year.

2012	Pied de cuve				Days of fermentation											
	0	3	6	10	0*	3	6	9	15	25	35	65	85	115	150	200
pH																
Trial A	7.06 ± 0.11^{a}	5.04 ± 0.16^{a}	4.94 ± 0.33^{a}	4.19 ± 0.07^{a}	7.17 ± 0.10^{a}	5.06 ± 0.13^{a}	4.00 ± 0.12^{a}	4.13 ± 0.09^{a}	3.85 ± 0.11^{a}	3.51 ± 0.28^{a}	3.85 ± 0.80^{a}	3.82 ± 0.13^{a}	4.15 ± 0.16^{a}	4.24 ± 0.21^{a}	4.11 ± 0.09^{a}	4.25 ± 0.06^{a}
Trial B	7.01 ± 0.01^{a}	6.15 ± 0.28^{b}	5.97 ± 0.23^{b}	5.23 ± 0.10^{a}	7.28 ± 0.12^{a}	5.43 ± 0.12^{a}	4.77 ± 0.12^{b}	4.44 ± 0.16^{b}	4.36 ± 0.04^{b}	4.28 ± 0.14^{b}	4.38 ± 0.13^{a}	4.23 ± 0.11^{a}	4.43 ± 0.10^{a}	4.41 ± 0.09^{a}	4.48 ± 0.14^{a}	4.48 ± 0.12^{a}
Trial C	n.s.	n.s.	n.s.	n.s.	7.10 ± 0.10^{a}	6.48 ± 0.15^{b}	6.31 ± 0.33^{c}	5.71 ± 0.10^{c}	5.05 ± 0.16^{c}	4.22 ± 0.12^{b}	4.41 ± 0.10^{a}	4.20 ± 0.08^{a}	4.38 ± 0.28^{a}	4.48 ± 0.19^{a}	4.44 ± 0.21^{a}	4.39 ± 0.17^{a}
Trial D	n.s.	n.s.	n.s.	n.s.	7.21 ± 0.10^{a}	5.14 ± 0.11^{a}	4.09 ± 0.17^{a}	4.04 ± 0.07^{a}	3.89 ± 0.20^{a}	3.82 ± 0.10^{a}	3.81 ± 0.09^{a}	3.82 ± 0.08^{a}	4.07 ± 0.08^{a}	4.27 ± 0.10^{a}	4.19 ± 0.08^{a}	4.17 ± 0.14^{a}
MRS																
Trial A	7.82 ± 0.13^{a}	7.39 ± 0.24^{d}	7.27 ± 0.15^{a}	7.69 ± 0.02^{a}	7.22 ± 0.08^{c}	6.79 ± 0.05^{c}	6.75 ± 0.13^{c}	7.11 ± 0.11^{c}	6.99 ± 0.22^{b}	7.16 ± 0.16^{c}	7.31 ± 0.10^{c}	6.51 ± 0.09^{b}	6.10 ± 0.28^{b}	5.37 ± 0.06^{a}	5.34 ± 0.09^{a}	5.51 ± 0.18^{a}
Trial B	1.11 ± 0.09^{b}	2.41 ± 0.10^{b}	3.27 ± 0.10^{b}	4.21 ± 0.03^{a}	3.85 ± 0.06^{b}	4.39 ± 0.15^{b}	5.99 ± 0.11^{b}	6.40 ± 0.23^{b}	6.59 ± 0.41^{b}	6.76 ± 0.08^{b}	6.53 ± 0.23^{b}	6.38 ± 0.29^{b}	5.90 ± 0.10^{b}	5.68 ± 0.18^{a}	6.60 ± 0.18^{b}	6.20 ± 0.20^{b}
Trial C	n.s.	n.s.	n.s.	n.s.	0.41 ± 0.14^{a}	1.32 ± 0.12^{a}	4.08 ± 0.25^{a}	2.86 ± 0.07^{a}	3.55 ± 0.29^{a}	3.87 ± 0.35^{a}	5.60 ± 0.20^{a}	5.32 ± 0.11^{a}	4.49 ± 0.17^{a}	5.23 ± 0.13^{a}	5.04 ± 0.05^{a}	5.24 ± 0.31^{a}
Trial D	n.s.	n.s.	n.s.	n.s.	7.09 ± 0.12^{c}	6.56 ± 0.33^{c}	7.02 ± 0.27^{d}	7.11 ± 0.18^{c}	7.04 ± 0.07^{c}	7.10 ± 0.20^{c}	7.25 ± 0.28^{c}	6.68 ± 0.31^{b}	5.89 ± 0.09^{b}	5.60 ± 0.26^{a}	5.60 ± 0.42^{a}	5.52 ± 0.28^{a}
DRBC																
Trial A	3.98 ± 0.17^{a}	4.28 ± 0.29^{a}	3.52 ± 0.07^{a}	6.04 ± 0.14^{a}	5.20 ± 0.27^{a}	3.98 ± 0.12^{a}	4.91 ± 0.21^{a}	6.19 ± 0.28^{b}	5.30 ± 0.42^{a}	6.53 ± 0.34^{b}	6.38 ± 0.15^{b}	5.81 ± 0.26^{b}	5.25 ± 0.14^{a}	5.38 ± 0.34^{a}	5.15 ± 0.15^{a}	3.76 ± 0.27^{a}
Trial B	4.25 ± 0.12^{a}	3.91 ± 0.14^{a}	5.74 ± 0.16^{b}	6.72 ± 0.21^{b}	5.93 ± 0.23^{b}	4.01 ± 0.18^{a}	4.82 ± 0.08^{a}	6.73 ± 0.10^{c}	6.15 ± 0.05^{b}	6.70 ± 0.09^{b}	6.47 ± 0.20^{b}	5.53 ± 0.35^{a}	5.23 ± 0.25^{a}	5.44 ± 0.36^{a}	4.79 ± 0.27^{a}	4.55 ± 0.38^{b}
Trial C	n.s.	n.s.	n.s.	n.s.	4.79 ± 0.17^{a}	3.70 ± 0.28^{a}	5.74 ± 0.10^{b}	5.26 ± 0.23^{a}	5.00 ± 0.30^{a}	5.15 ± 0.16^{a}	5.74 ± 0.13^{a}	5.53 ± 0.38^{a}	4.95 ± 0.11^{a}	5.23 ± 0.16^{a}	5.01 ± 0.31^{a}	5.48 ± 0.28^{c}
Trial D	n.s.	n.s.	n.s.	n.s.	5.11 ± 0.12^{a}	3.43 ± 0.27^{a}	4.75 ± 0.22^{a}	6.04 ± 0.07^{b}	6.02 ± 0.08^{b}	6.19 ± 0.37^{b}	6.15 ± 0.27^{b}	5.96 ± 0.35^{b}	5.69 ± 0.23^{b}	5.44 ± 0.46^{a}	5.01 ± 0.26^{a}	4.19 ± 0.11^{b}
VRBGA																
Trial A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Trial B	n.d.	n.d.	1.07 ± 0.09	1.20 ± 0.14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Trial C	n.s.	n.s.	n.s.	n.s.	n.d.	1.09 ± 0.19	1.16 ± 0.28	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Trial D	n.s.	n.s.	n.s.	n.s.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PAB																
Trial A	1.31 ± 0.11^{a}	0.74 ± 0.21^{a}	1.15 ± 0.28^{a}	0.67 ± 0.21^{a}	n.d.	1.44 ± 0.10^{a}	1.07 ± 0.18^{a}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Trial B	1.92 ± 0.25^{a}	2.54 ± 0.12^{b}	2.06 ± 0.07^{b}	1.08 ± 0.17^{a}	n.d.	1.98 ± 0.13^{a}	2.73 ± 0.49^{b}	1.47 ± 0.42^{a}	1.17 ± 0.45^{a}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Trial C	n.s.	n.s.	n.s.	n.s.	2.40 ± 0.15	3.94 ± 0.22^{b}	2.37 ± 0.65^{b}	3.84 ± 0.31^{b}	4.64 ± 0.53^{b}	5.56 ± 0.34	5.73 ± 0.66	4.05 ± 0.52	n.d.	n.d.	n.d.	n.d.
Trial D	n.s.	n.s.	n.s.	n.s.	n.d.	1.67 ± 0.16^{a}	1.23 ± 0.56^{a}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BP																
Trial A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Trial B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Trial C	n.s.	n.s.	n.s.	n.s.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Trial D	n.s.	n.s.	n.s.	n.s.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Results of microbial loads are expressed as Log CFU/ml and indicate the mean values ± standard deviation of three plate counts.
Symbol:*, brine samples just after the inoculum of pied de cuve (for both trial A and trial B) and starter *L. pentosus* OM13 (for trial D).
Abbreviations: MRS, Man-Rogosa-Sharpe agar for mesophilic rod LAB; DRBC, dichloran rose bengal chloramphenicol agar for total yeasts and filamentous fungi; VRBGA, violet red bile glucose agar for *Enterobacteriaceae*; PAB, *Pseudomonas* agar base for pseudomonads; BP, Baird Parker only for staphylococci; n.d., not detected (value < detection limit of method); n.s. not sampled.
a–c: different letters indicate significant differences among experimental trials for the same sample and the same medium ($p < 0.05$).

Table 2
Values of pH and microbial concentrations of samples collected during the table olive production during the 2013 year.

2013	Pied de cuve				Days of fermentation											
	0	3	6	10	0*	3	6	9	15	25	35	65	85	115	150	200
pH																
Trial A	7.31 ± 0.18^{a}	5.20 ± 0.07^{a}	3.97 ± 0.25^{a}	4.13 ± 0.10^{a}	7.36 ± 0.17^{a}	5.17 ± 0.12^{a}	4.90 ± 0.27^{a}	4.30 ± 0.17^{a}	4.10 ± 0.15^{a}	4.20 ± 0.18^{a}	4.10 ± 0.17^{a}	4.20 ± 0.32^{a}	4.01 ± 0.16^{a}	4.10 ± 0.16^{a}	4.06 ± 0.27^{a}	3.78 ± 0.17^{a}
Trial B	7.18 ± 0.16^{a}	6.70 ± 0.13^{c}	6.22 ± 0.21^{b}	5.26 ± 0.08^{a}	7.21 ± 0.24^{a}	5.22 ± 0.14^{a}	4.81 ± 0.27^{a}	4.43 ± 0.18^{a}	4.22 ± 0.25^{a}	4.43 ± 0.24^{a}	4.33 ± 0.24^{a}	4.36 ± 0.21^{a}	4.10 ± 0.33^{a}	4.04 ± 0.17^{a}	4.18 ± 0.22^{a}	3.80 ± 0.13^{a}
Trial C	n.s.	n.s.	n.s.	n.s.	7.22 ± 0.13^{a}	5.50 ± 0.25^{a}	5.30 ± 0.18^{a}	5.10 ± 0.32^{b}	4.50 ± 0.27^{a}	4.40 ± 0.11^{a}	4.30 ± 0.19^{a}	4.30 ± 0.17^{a}	4.00 ± 0.30^{a}	4.00 ± 0.21^{a}	4.15 ± 0.08^{a}	4.00 ± 0.14^{a}
Trial D	n.s.	n.s.	n.s.	n.s.	7.31 ± 0.12^{a}	5.10 ± 0.08^{a}	4.81 ± 0.23^{a}	4.25 ± 0.20^{a}	4.17 ± 0.08^{a}	4.00 ± 0.14^{a}	4.06 ± 0.13^{a}	4.08 ± 0.18^{a}	4.10 ± 0.15^{a}	4.04 ± 0.05^{a}	4.10 ± 0.22^{a}	3.79 ± 0.21^{a}
MRS																
Trial A	7.73 ± 0.15^{a}	7.29 ± 0.24^{a}	7.38 ± 0.07^{a}	7.82 ± 0.03^{a}	7.08 ± 0.17^{a}	6.80 ± 0.24^{c}	6.55 ± 0.16^{c}	7.21 ± 0.12^{b}	7.10 ± 0.16^{b}	7.23 ± 0.11^{b}	7.09 ± 0.21^{b}	7.01 ± 0.13^{b}	6.64 ± 0.35^{c}	6.88 ± 0.21^{c}	6.21 ± 0.35^{b}	6.30 ± 0.22^{b}
Trial B	1.12 ± 0.10^{b}	2.09 ± 0.15^{b}	3.61 ± 0.25^{b}	4.22 ± 0.09^{b}	3.63 ± 0.17^{b}	4.73 ± 0.45^{b}	5.86 ± 0.41^{b}	6.97 ± 0.19^{b}	6.81 ± 0.13^{b}	7.31 ± 0.16^{b}	7.11 ± 0.11^{b}	6.98 ± 0.04^{b}	6.76 ± 0.36^{c}	6.77 ± 0.30^{c}	6.46 ± 0.37^{b}	6.40 ± 0.18^{b}
Trial C	n.s.	n.s.	n.s.	n.s.	1.07 ± 0.28^{c}	1.26 ± 0.10^{a}	3.32 ± 0.09^{a}	4.10 ± 0.12^{a}	4.50 ± 0.19^{a}	5.40 ± 0.41^{a}	5.83 ± 0.12^{a}	5.66 ± 0.22^{a}	4.57 ± 0.15^{a}	4.90 ± 0.26^{a}	5.80 ± 0.06^{a}	5.56 ± 0.33^{a}
Trial D	n.s.	n.s.	n.s.	n.s.	7.21 ± 0.09^{a}	6.97 ± 0.24^{c}	7.07 ± 0.19^{d}	7.38 ± 0.15^{b}	7.21 ± 0.26^{b}	7.00 ± 0.19^{b}	7.10 ± 0.21^{b}	6.97 ± 0.20^{b}	5.91 ± 0.29^{b}	6.01 ± 0.17^{b}	5.98 ± 0.13^{a}	5.86 ± 0.20^{a}
DRBC																
Trial A	3.11 ± 0.27^{a}	3.66 ± 0.29^{a}	5.00 ± 0.10^{a}	5.67 ± 0.10^{a}	4.94 ± 0.28^{b}	4.43 ± 0.13^{a}	4.53 ± 0.22^{a}	4.96 ± 0.09^{b}	6.08 ± 0.23^{b}	6.43 ± 0.19^{b}	5.43 ± 0.19^{a}	5.15 ± 0.27^{b}	5.22 ± 0.25^{b}	5.89 ± 0.37^{c}	5.79 ± 0.05^{c}	5.51 ± 0.15^{b}
Trial B	4.13 ± 0.15^{b}	3.21 ± 0.11^{a}	5.34 ± 0.17^{a}	6.26 ± 0.16^{b}	5.29 ± 0.32^{b}	4.26 ± 0.15^{a}	4.14 ± 0.10^{a}	4.44 ± 0.29^{a}	5.65 ± 0.15^{a}	5.39 ± 0.26^{a}	4.27 ± 0.16^{a}	4.34 ± 0.19^{a}	5.08 ± 0.17^{b}	5.27 ± 0.15^{a}	5.36 ± 0.21^{b}	4.57 ± 0.28^{b}
Trial C	n.s.	n.s.	n.s.	n.s.	4.47 ± 0.23^{a}	4.32 ± 0.26^{a}	4.41 ± 0.26^{a}	4.04 ± 0.20^{a}	5.54 ± 0.13^{a}	5.70 ± 0.14^{a}	5.04 ± 0.25^{a}	4.85 ± 0.11^{b}	3.48 ± 0.33^{a}	4.12 ± 0.25^{a}	3.85 ± 0.09^{a}	3.83 ± 0.14^{a}
Trial D	n.s.	n.s.	n.s.	n.s.	4.51 ± 0.26^{a}	4.10 ± 0.26^{a}	4.22 ± 0.34^{a}	4.11 ± 0.02^{a}	5.41 ± 0.39^{a}	5.97 ± 0.38^{a}	4.87 ± 0.33^{a}	5.00 ± 0.26^{b}	5.10 ± 0.40^{b}	4.87 ± 0.26^{b}	5.12 ± 0.07^{b}	4.12 ± 0.15^{a}
VRBGA																
Trial A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Trial B	n.d.	1.13 ± 0.05	1.48 ± 0.21	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Trial C	n.s.	n.s.	n.s.	n.s.	n.d.	1.14 ± 0.08	1.14 ± 0.19	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Trial D	n.s.	n.s.	n.s.	n.s.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PAB																
Trial A	2.12 ± 0.20^{a}	1.58 ± 0.21^{a}	1.97 ± 0.14^{a}	1.45 ± 0.11^{a}	0.88 ± 0.17^{a}	1.52 ± 0.11^{a}	1.04 ± 0.07^{a}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Trial B	1.78 ± 0.06^{b}	2.12 ± 0.09^{b}	2.38 ± 0.22^{a}	1.26 ± 0.06^{a}	n.d.	1.98 ± 0.07^{a}	2.79 ± 0.08^{b}	1.46 ± 0.10^{a}	1.01 ± 0.08^{a}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Trial C	n.s.	n.s.	n.s.	n.s.	1.73 ± 0.12^{b}	3.87 ± 0.16^{b}	2.48 ± 0.18^{b}	3.84 ± 0.13^{b}	4.72 ± 0.20^{b}	5.54 ± 0.20	5.79 ± 0.19	4.00 ± 0.20	n.d.	n.d.	n.d.	n.d.
Trial D	n.s.	n.s.	n.s.	n.s.	n.d.	1.74 ± 0.14^{a}	1.21 ± 0.09^{a}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BP																
Trial A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Trial B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Trial C	n.s.	n.s.	n.s.	n.s.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Trial D	n.s.	n.s.	n.s.	n.s.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Results of microbial loads are expressed as Log CFU/ml and indicate the mean values ± standard deviation of three plate counts.
Symbols: brine samples just after the inoculum of pied de cuve (for both trial A and trial B) and starter *L. pentosus* OM13 (for trial D).
Abbreviations: MRS, Man-Rogosa-Sharpe agar for mesophilic rod LAB; DRBC, dichloran rose bengal chloramphenicol agar for total yeasts and filamentous fungi; VRBGA, violet red bile glucose agar for *Enterobacteriaceae*; PAB, *Pseudomonas* agar base for pseudomonads; BP, Baird Parker only for staphylococci; n.d., not detected (value < detection limit of method); n.s. not sampled.
a–c: different letters indicate significant differences among experimental trials for the same sample and the same medium ($p < 0.05$).

3. Results and discussion

3.1. pH values and microbiological counts

Chemico-physical and microbiological characteristics of the brines collected during both the PdC preparation and the manufacturing process are reported in Table 1 (2012 year) and Table 2 (2013 year).

The pH values significantly decreased up to about 4.10 (trial A) and 5.20 (trial B) during the preparation of PdCs. LAB increased in concentration showing values of about 7.70 (trial A) and 4.20 (trial B) at end of PdC preparation (day 10), during both years. A significant increase in concentration was estimated also for yeast population.

During production, the pH ranged between values of about 7.20 (day 0) and 4.30 (day 200) in all experimental trials and for both years. Similar results were shown in other research (Bleve et al., 2015; Aponte et al., 2012). The use of selected LAB strains as starters determine a rapid acidification and decrease the growth of potential spoilage populations (Arroyo-López et al., 2008). However, except trial C, all samples showed a significant decrease of pH within the sixth day of fermentation, a result in agreement with that reported by Bleve et al. (2015); after that, the pH decreased approximately to 3.80 for the trials A and D at day 65. From day 85 onwards, pH reached constant values of about 4.30 in all trials. However, the most rapid decrease of pH values was registered for trials A and D.

The microbial groups mostly represented on "non PdC treated" olives were LAB (2.25 and 1.87 Log CFU/g for 2012 and 2013, respectively) and yeasts (3.01 and 3.15 Log CFU/g for 2012 and 2013, respectively). None of the microbial groups searched were at detectable levels in the brines added for fermentation.

Thanks to use of PdC, both experimentations (A and B) showed a higher concentration of LAB than controls and, at the same, the growth of potential spoilage microbial was inhibited.

In both PdCs, LAB were about 7.50 Log CFU/ml. This group was significantly higher than yeast group. After the addition of PdCs into new brines, trials A and B showed a significant increase of LAB and yeast levels. The cell densities of LAB and yeasts of trial D were comparable with those of trials A and B. On the contrary, trial C showed the lowest LAB and yeast concentrations. *Enterobacteriaceae*, pseudomonads and staphylococci were detected mainly in trial C. None CPS were detected in any samples.

During olive production, trials A and B showed LAB and yeasts at levels superimposable to that estimated for trial D, in both compaings. At the 25th day of olive transformation, LAB and yeasts reached the highest concentration in all trials. Trial C showed the lowest concentration of LAB during the entire experimentation in both campaigns. From day 65 onwards, LAB and yeast loads showed almost constant concentration levels until the end of the experimentation for all trials in both years.

Fermentative yeasts might contribute to the sensory profile of fermented olives. Those microorganisms may also oxidize lactic acid, thus an increase of pH might be estimated during table olive production (Nout and Rombouts, 2000). Once the fermentable substrates are exhausted, LAB populations tend to decrease. During this phase, the concentration of yeasts changes and exhibiting a wide variety of metabolic activities that affect chemical composition of final olives (Arroyo-López et al., 2008). The interrelationship between LAB and yeasts in table olives plays an essential role in product preservation. Furthermore, it has been clearly showed by other works on table olives (Arroyo-López et al., 2008) and/or in other food productions (Sannino et al., 2013; Francesca et al., 2014) that, although at very low concentrations, both yeasts and LAB could affect the

Table 3
Molecular identification of LAB and yeast species during the table olive production in both the 2012 and 2013 years.

Species	Strain	Isolation source (day of sampling)	Phenotypic group	Size of multiplex-PCR[a] amplicon	% similarity[b] (accession no. of closest relative) by:		Sequence lengths (bp)	Acc.no.	Distribution of species (day of sampling)			
									Pied de cuve		Fermentation	
					Blast	EzTaxon			2012[c]	2013	2012	2013
Lactobacillus coryniformis	1OCRBL452	Trial C (150d)	II	n.a.	97 (NR029018.1)	97.04 (AEOS01000123)	1497	KP256085	n.d.	n.d.	A[d](150d)	n.d.
Lactobacillus pentosus	2OCRBL104	Trial A (200d)	I	218	99 (KF923751.1)	99.13 (D79211)	1518	KP256079	A,B	A,B	A,B,D (0–200d) C (0,3,6,15,25–200d)	A,B,D (0–200d) C (6–200d)
Lactobacillus plantarum	1OCRBL277	Trial C (25d)	I	318	99 (AL935263.2)	99.20 (ACGZ01000098)	1513	KP256076	B	B	A (115,150d) B (35,65,115d) C (9,25d) D (115d)	A (85,115,150d) B (65,85,150d) C (0,3,15d) D (115d)
Pediococcus pentosaceus	1OCRBL365	Trial C (9d)	III	n.a.	99 (CP000422.1)	98.94 (AJ305321)	1513	KP256074	n.d.	n.d.	C (85d)	n.d.

Abbreviation: n.a., not amplified; n.d., not detected.
[a] Results obtained by multiplex PCR analysis of the *recA* gene with species-specific primers for *Lactobacillus pentosus*, *L. plantarum* and *L. paraplantarum* (Torriani et al., 2001).
[b] Results obtained by the 16S rRNA sequence search.
[c] Campaign during which the olive productions were carried out.
[d] Letters A, B, C and D refer to the experimental trials performed during the olive productions.

chemical composition of foods during the entire process of production.

3.2. Isolation, typing and identification of LAB

A total of 2417 colonies were collected from the highest plated dilutions of cell suspensions. After purification and grouping on colony appearance and microscopic inspection, the isolates were 2404 rods and only 13 cocci. After Gram and catalase tests, 2181 rods and 9 cocci were still considered presumptive LAB cultures, as being Gram-positive and catalase negative. They were separated into three main phenotypic groups, two for rods and one for cocci. The highest number of isolates (2174) was included in the group I as follows: rod-cell morphology, growth at 15 °C, in presence of pentose carbohydrates, and no growth at 45 °C, as well as no production of CO_2 from glucose. Only seven isolates clustered into group II that differed from the first one only for ability to grow in presence of pentose carbohydrates. The nine cultures of group III (coccus cell-morphology) showed growth at 15 °C, 45 °C, at pH 9.6, in presence of NaCl (6.5%) and pentose carbohydrates, as well as no production of CO_2 from glucose. Due to the high number of isolates, about 40% of group I was subjected to RAPD analysis. The isolates of groups II and III were all processed by RAPD. The unique patterns were used to construct a dendrogram revealing the presence of 65 strains (data not shown).

Multiplex PCR analysis of the *rec*A gene revealed the presence of a major group of *L. pentosus* (Table 3) composed of 60 strains (data not shown). Analysis of 16S rRNA gene confirmed the allotting of the strains positive at Multiplex PCR assay into *L. pentosus* and *L. plantarum*. The other strains were identified as *Lactobacillus coryniformis* and *Pediococcus pentosaceus*.

3.3. Distribution of LAB species and strains

The distribution of the LAB species among the samples collected during olive production is reported in Table 3. *L. pentosus* and *L. plantarum* dominated PdCs and all production trials. In details, *L. pentosus* dominated the LAB population during the entire period of monitoring in all trials and during both years of observation. *L. plantarum* was mainly isolated during the first days in the trials spontaneously fermented and at consistent levels in the trials inoculated with the starter culture only at the 115th day. *L. coryniformis* and *Pediococcus pentosaceous* were detected only in the 2012 campaign. *L. pentosus* species is commonly recognized as one of the main technological LAB due to its high aptitude to ferment olive. Thus, its dominance during olive production could represent a guarantee of quality of final product both in microbiological and chemical terms. Particularly, the presence of several *L. pentosus* strains during olive manufacturing is reported to improve the complexity of sensory profile of final product (Aponte et al., 2012; Blana et al., 2014). Although trial B was spontaneously fermented, very low species diversity was observed, which could be explained by the inoculum of PdC characterized by viable and fermenting LAB strain collected at day 10 of olive fermentation.

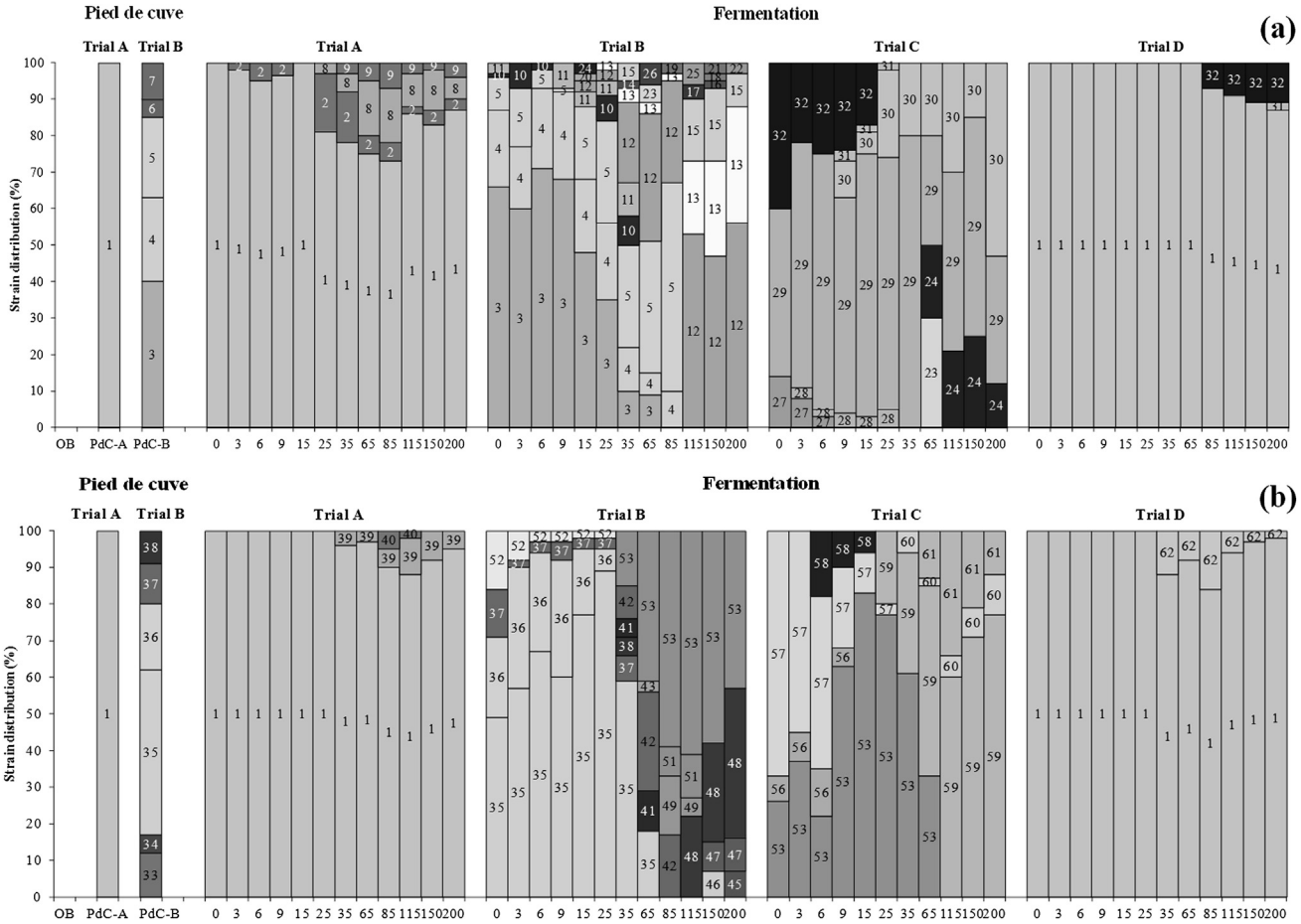

Fig. 1. Distribution of *Lactobacillus pentosus* strains during the table olive preparation during the 2012 (Fig. a) and 2013 (Fig. b) campaigns. Abbreviation: OB, olive bulk; PdC-A, pied de cuve of trial A; PdC-B, pied de cuve of trial B. The numbers reported inside the figure refer to strain codes.

With regards to the distribution of *L. pentosus* strains (Fig. 1), 32 strains were isolated during the 2012 campaign whereas 28 during that of 2013. In terms of *L. pentosus* strains, PdC B was richer than PdC A in both year. This behaviour was also observed after addition of PdCs. Some strains (i.e. 3, 4 and 5 collected during the 2012 year and 35, 36 and 37 collected during 2013 year) were first isolated from PdC B and dominated LAB populations during olive fermentation. As expected, the commercial starter OM13 inoculated in PdC A and used for direct inoculums of trial D were most frequently isolated during the entire olive fermentation process during both production campaings. The use of PdC resulted in a reduction of LAB species diversity but, at the same time, it did not negatively affect the strain variability of *L. pentosus* population. The number of strains found during the experimentations A and B was significantly higher than those commonly reported in literature for olive production (Cocolin et al., 2013). Thus, the use of PdC collected from partially fermented brine could represent an innovative strategy to start olive fermentation by high number of autochthonous *L. pentosus* strains. In addition, the use of PdC fermented by LAB starter could reduce the amount of starter concentration to be inoculated directly into brine to produce the fermented table olives. None of the *L. pentosus* strain was found in common between the two years of experimentation.

3.4. Isolation, identification and distribution of yeasts

A total of 4917 yeast colonies were collected from DRBC agar. Based on colony and cell morphology, 723 isolates were subjected to the molecular identification. After restriction analysis of 5.8S-ITS region, the isolates were clustered into 6 groups (Table 4). The sequencing of D1/D2 domain of the 26S rRNA gene identified six species: *Candida boidinii* (group I), *Candida diddensiae* (group II), *Candida membranifaciens* (group III), *Kluyveromyces marxianus* (group IV), *Pichia kudriavzevii* (group V) and *Wickerhamomyces anomalus* (group VI).

Table 4 also shows the distribution of the yeast species in the trials that followed. Members of *Candida* genus and *W. anomalus* were mainly found in PdCs. *Candida boindii* and *W. anomlus* were the species mostly isolated from trials A and B. The highest diversity of yeasts was detected in trial C. Yeast species isolated in the present work are commonly associated with olive environment (Tofalo et al., 2012a) mostly with the fermentation phase (Aponte et al., 2010; Tofalo et al., 2012b; Bleve et al., 2014).

3.5. VOCs and sensory analysis

The results of the VOCs emitted by the olives sampled at day 200 are reported in Table 5. Forty-nine compounds, comprising acids, alcohols, aldehydes, ketones, esters, phenol and aromatic hydrocarbons, were identified. Acids, alcohols and aldehydes were detected at the highest concentrations in both campaigns. In accordance to data published by Sabatini et al. (2008), acetic, hexanoic, pentanoic and propionic acids reached the highest values in the trials inoculated with LAB starter, such trial A. High concentrations of acetic acid, alcohols and aldehydes showed that table olives have undergone alcoholic and heterolactic fermentation (Sabatini et al., 2009). In fact, those compounds were found also in the Trial B, where LAB dominated microbial populations. Acetic acid is representative of yeast and lactic acid bacteria metabolism as clearly reported by Bleve et al. (2014). Trials A and B showed also high levels of alcohols, mainly represented by phenylethyl alcohol, cis-hexen-1-ol, 1-butanol-3-methyl and benzyl-alcohol. The presence in the table olives of cis-3-hexen-1-ol and 1-hexanol has been commonly associated to herbaceous flavours, a pleasant sensory descriptors of many fruit and vegetable fermented foods. The

Table 4
Molecular identification of yeasts isolated during the table olive production in both 2012 and 2013 years.

Species	Strain	Isolation source (day of sampling)	R. P.	5.8S-ITS PCR	Size of restriction fragments			% similarity[a] (accession no. of closest relative) by:	Acc. No.	Distribution of species (day of sampling)			
					CfoI	*HaeIII*	*HinfI*			Pied de cuve		Fermentation	
										2012[c]	2013	2012	2013
Candida boidinii	1OCRY365	Trial A (85d)	I	700	343 + 309 + 82	700	373 + 178 + 143	99 (KC442246.1)	KP256102	A,[b]B	A,B	A (0,3,9–200d) B (9,25,35d)	A (0,3,6,9,15–200d) B (3,6,15,25d) C (0,3,6,15,25,35d)
Candida diddensiae	1OCRY124	Trial C (6d)	II	630	299 + 182 + 140	400 + 120 + 80	324	99 (U45750.1)	KP256096	B	B	C (0,3,9,15,25,65,200d) D (6,9,65–200d) B (0,3,6,9,15d) C (6d) D (0.3d)	C (0,3,6,15,25,35d) D (0,3,25,65,150d) A (6d) B (0,3,6,9,15,25d) C (6d) D (6,9,15,35d)
Candida membranifaciens	1OCRY126	Trial C (6d)	III	650	314 + 297	420 + 150 + 80	334	99 (EF362752.1)	KP256097		B	C (6d) D (15,25,35d)	C (9d)
Kluyveromyces marxianus	1OCRY140	Trial A (6d)	IV	780	305 + 206+177 + 90	630 + 70	308 + 201+124 + 90	99 (FJ896140.1)	KP256108		B	A (6d) C (6d)	B (15d) C (15d)
Pichia kudriavzevii	2OCRY17	Trial B (3d)	V	560	225 + 195 + 83	415 + 104	237 + 167 + 155	99 (KM234441.1)	KP256093				B (3d)
Wickerhamomyces anomalus	1OCRY105	Trial B (150d)	VI	600	580	600	300	99 (JX049437.1)	KP256094	B	A,B	A (6,150d) B (0,3,6,9,15,35–200d) C (35,85,115,150d)	B (0,3,6,9,25–200d) C (65,85,115,150,200 d) D (85,115,200d)

Abbreviation: R.P., restriction profile.
All values for the 5.8S-ITS PCR, 26S PCR and restriction fragments are given in bp.
[a] According to BlastN search of D1/D2 26S rRNA gene sequences in NCBI database.
[b] Letters A, B, C and D refer to the experimental trials performed during the olive productions.
[c] Campaign during which the olive productions were carried out.

Table 5
Concentration of volatile organic compounds (μg/kg) at the end (day 200) of olive productions during both 2012 and 2013 years.

Compounds	2012 year				2013 year			
	Trial A	Trial B	Trial C	Trial D	Trial A	Trial B	Trial C	Trial D
2-ethylhexanoic acid	n.d.	n.d.	n.d.	n.d.	26.11 ± 3.74	51.41 ± 1.27	18.19 ± 4.60	22.44 ± 2.39
Acetic acid	13599.89 ± 362.60	12272.95 ± 955.16	5889.74 ± 549.47	7911.37 ± 918.32	5615.10 ± 376.85	10515.53 ± 516.97	7705.42 ± 1920.56	8696.03 ± 1299.78
Butanoic acid	259.28 ± 6.96	227.39 ± 22.26	216.01 ± 20.87	252.48 ± 28.69	78.91 ± 9.18	85.25 ± 2.29	33.57 ± 14.02	52.33 ± 6.62
Heptanoic acid	n.d.	n.d.	n.d.	n.d.	37.40 ± 0.81	57.15 ± 6.79	24.86 ± 6.25	36.44 ± 5.01
Hexadecanoic acid	13004.23 ± 797.27	877.80 ± 54.11	1757.29 ± 327.16	1392.40 ± 266.17	n.d.	n.d.	n.d.	n.d.
Hexanoic acid	625.49 ± 36.16	298.92 ± 19.84	170.25 ± 30.12	228.60 ± 31.37	58.31 ± 3.38	48.46 ± 3.53	28.53 ± 6.97	45.33 ± 6.51
Nonanoic acid	697.56 ± 37.93	1018.86 ± 142.84	200.75 ± 40.37	405.10 ± 44.48	n.d.	n.d.	n.d.	n.d.
Octanoic acid	656.04 ± 50.81	550.29 ± 44.79	250.27 ± 42.87	330.07 ± 45.37	27.69 ± 0.88	27.02 ± 1.30	78.96 ± 30.36	47.38 ± 4.42
Pentanoic acid	380.63 ± 9.23	348.77 ± 40.52	203.04 ± 40.40	247.45 ± 38.82	41.88 ± 6.64	38.29 ± 5.34	19.39 ± 7.43	26.32 ± 2.96
Propionic acid	678.39 ± 50.62	313.84 ± 51.85	54.65 ± 8.58	142.80 ± 17.54 ± 17.54 ± 17.54	120.28 ± 12.45	167.13 ± 7.39	112.00 ± 23.90	120.19 ± 14.50
1.4-butanediol	65.53 ± 10.46	6.81 ± 1.24	51.65 ± 12.36	46.10 ± 8.42	n.d.	n.d.	n.d.	n.d.
1-butanol-3-methyl	475.63 ± 10.75	779.87 ± 202.67	1099.53 ± 141.60	572.01 ± 101.70	430.07 ± 51.25	453.00 ± 61.30	284.82 ± 71.75	324.10 ± 49.54
1-hexanol	183.49 ± 12.02	149.11 ± 17.98	121.41 ± 31.55	115.78 ± 14.52	134.53 ± 21.59	176.05 ± 35.22	166.60 ± 29.14	142.06 ± 11.18
1-octanol	360.92 ± 17.47	320.72 ± 34.38	129.92 ± 17.67	238.39 ± 36.77	140.25 ± 14.64	186.48 ± 22.43	119.54 ± 16.77	110.70 ± 13.01
2-nonen-1-ol	586.15 ± 18.84	375.38 ± 74.20	317.46 ± 28.06	353.81 ± 48.94	16.10 ± 0.23	20.33 ± 4.07	34.75 ± 4.94	24.23 ± 2.76
Benzyl alcohol	1293.50 ± 94.70	618.40 ± 64.67	139.49 ± 13.43	569.07 ± 63.82	540.35 ± 20.47	713.22 ± 119.75	753.19 ± 102.35	653.35 ± 88.06
cis-hexen-1-ol	1001.91 ± 85.67	979.59 ± 48.11	32.54 ± 1.45	276.13 ± 31.59	450.46 ± 28.97	612.83 ± 87.62	437.16 ± 101.20	440.54 ± 66.33
Phenylethyl alcohol	2743.11 ± 146.90	1979.93 ± 341.19	1497.67 ± 353.99	1658.57 ± 225.77	867.19 ± 40.27	1113.10 ± 69.13	1109.44 ± 181.53	873.89 ± 128.59
2-butenal-2-methyl	55.67 ± 2.21	60.64 ± 14.18	60.50 ± 2.82	46.29 ± 3.49	41.22 ± 4.57	49.69 ± 12.12	14.62 ± 2.20	24.35 ± 3.14
2-decenal (E)	1577.52 ± 61.16	675.26 ± 157.23	403.84 ± 11.51	749.79 ± 51.10	143.11 ± 3.28	192.32 ± 58.24	129.96 ± 6.00	150.21 ± 8.84
Benzaldehyde	522.16 ± 23.79 ± 23.79	1134.82 ± 179.69 ± 179.69	275.19 ± 6.02 ± 6.02	338.32 ± 20.97	117.75 ± 5.10	274.96 ± 71.41	117.84 ± 4.13	94.98 ± 2.99
Benzaldehyde-2,5-dimethyl	34.97 ± 16.35	n.d.	53.96 ± 12.58	14.16 ± 0.41	n.d.	n.d.	n.d.	n.d.
Benzaldehyde-3-ethyl	n.d.	n.d.	39.45 ± 6.29	21.06 ± 0.95	n.d.	n.d.	n.d.	n.d.
Nonanal	481.37 ± 13.81	344.08 ± 83.26	395.24 ± 18.12	273.72 ± 7.38	155.11 ± 27.70	175.77 ± 21.57	203.98 ± 12.86	150.78 ± 3.82
Octanal	547.27 ± 38.53	272.34 ± 63.15	101.16 ± 1.75	191.70 ± 9.76	90.20 ± 2.65	121.61 ± 5.83	758.52 ± 229.84	244.55 ± 12.21
Phenylacetaldehyde	94.23 ± 2.68	n.d.	84.85 ± 14.12	70.72 ± 2.58	50.02 ± 0.98	88.86 ± 4.90	51.27 ± 7.32	39.66 ± 2.30
Vanillin	n.d.	n.d.	n.d.	n.d.	78.42 ± 15.30	n.d.	75.21 ± 0.34	69.41 ± 0.62
2-nonanone	436.21 ± 14.13	153.73 ± 36.43	75.31 ± 0.62	99.12 ± 1.40	n.d.	n.d.	n.d.	n.d.
3-hydroxybutanone	324.32 ± 46.71	205.96 ± 48.34	122.14 ± 1.07	144.35 ± 1.72	40.01 ± 3.61	51.23 ± 12.72	29.14 ± 0.74	33.00 ± 1.85
4-ethylacetophenone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	52.01 ± 0.43	11.84 ± 1.33
Butyrolactone	n.d.	n.d.	n.d.	n.d.	85.02 ± 6.70	90.93 ± 13.54	47.16 ± 8.28	42.42 ± 2.73
Cyclopentanone	441.76 ± 19.07	457.90 ± 105.98	225.58 ± 2.82	281.04 ± 38.28	159.82 ± 8.54	270.11 ± 44.30	89.34 ± 0.95	102.02 ± 3.28
Benzyl acetate	n.d.	n.d.	n.d.	n.d.	92.99 ± 18.78	95.28 ± 21.36	60.24 ± 0.97	65.89 ± 2.81
cis-3-hexenylacetate	n.d.	n.d.	n.d.	n.d.	33.68 ± 9.33	34.63 ± 8.36	32.45 ± 1.98	32.46 ± 4.18
Ethyl dihydrocinnamate	n.d.	n.d.	n.d.	n.d.	50.06 ± 2.71	55.59 ± 5.67	n.d.	24.75 ± 3.84
Ethyl lactate	n.d.	n.d.	n.d.	n.d.	62.60 ± 15.97	99.50 ± 14.92	14.48 ± 1.17	31.85 ± 3.24
Methyl hexadecanoate	n.d.	n.d.	n.d.	n.d.	189.20 ± 14.64	306.54 ± 68.03	141.62 ± 15.51	215.60 ± 31.22
Methyl hydrocinnamate	n.d.	n.d.	n.d.	n.d.	147.23 ± 13.87	110.41 ± 23.17	102.80 ± 15.34	12.57 ± 1.78
Methyl salicylate	n.d.	n.d.	n.d.	n.d.	33.27 ± 0.89	40.86 ± 6.72	32.87 ± 4.00	36.30 ± 5.24
Octyl acetate	n.d.	n.d.	n.d.	n.d.	62.88 ± 14.32	80.79 ± 4.07	71.90 ± 11.38	77.42 ± 10.88
4-ethylphenol	n.d.	n.d.	n.d.	n.d.	113.16 ± 13.89	148.24 ± 6.28	235.35 ± 1.70	198.55 ± 1.00
Guaiacol	303.34 ± 8.36	2545.30 ± 147.73	5166.65 ± 1877.41	383.59 ± 42.30	108.34 ± 24.61	127.40 ± 7.94	141.82 ± 3.15	132.49 ± 0.73
Homoguaiacol	2942.72 ± 70.19	2301.42 ± 130.73	n.d.	2160.31 ± 310.94	1687.59 ± 144.46	1768.13 ± 29.52	1616.80 ± 40.84	1625.07 ± 82.97
Phenol	147.20 ± 9.79	617.12 ± 58.46	741.90 ± 78.05	310.49 ± 41.03	58.87 ± 13.05	104.07 ± 3.93	61.19 ± 0.97	64.04 ± 13.61
Squalene	983.78 ± 26.99	1751.57 ± 197.02	9067.37 ± 823.75	2709.52 ± 453.40	n.d.	n.d.	n.d.	n.d.
Styrene	n.d.	n.d.	165.34 ± 17.93	92.32 ± 14.35	n.d.	n.d.	n.d.	n.d.
α-cubebene	157.24 ± 23.93	127.64 ± 13.68	51.35 ± 7.69	107.26 ± 17.07	37.76 ± 3.80	55.05 ± 4.85	85.28 ± 2.34	49.56 ± 9.76
4-methyldihydro-2-(3H)-furanone	n.d.	n.d.	n.d.	n.d.	82.23 ± 15.51	108.06 ± 4.44	83.61 ± 2.32	93.86 ± 6.33
α -terpineol	n.d.	n.d.	n.d.	n.d.	22.98 ± 4.26	40.06 ± 1.26	23.49 ± 0.41	24.29 ± 2.63

Results indicate mean values ± standard deviation of three replicate.

Table 6
Sensory scores of olives collected at the end (day 200) of manufacturing process during both the 2012 and 2013 years.

Sensory attributes	2012 year				2013 year			
	Trial A	Trial B	Trial C	Trial D	Trial A	Trial B	Trial C	Trial D
Odour:								
green olive aroma	7.45 ± 0.18^{a}	7.82 ± 0.14^{b}	6.87 ± 0.09^{a}	7.21 ± 0.03^{a}	6.83 ± 0.10^{b}	7.45 ± 0.18^{c}	6.11 ± 0.21^{a}	6.85 ± 0.10^{b}
off-odours	1.09 ± 0.02^{a}	1.33 ± 0.06^{a}	2.71 ± 0.18^{b}	1.02 ± 0.07^{a}	1.08 ± 0.03^{a}	1.35 ± 0.13^{a}	2.26 ± 0.13^{b}	0.90 ± 0.03^{a}
Taste:								
crunchiness	5.14 ± 0.04^{a}	5.30 ± 0.07^{a}	4.98 ± 0.08^{a}	5.17 ± 0.10^{a}	4.84 ± 0.08^{a}	5.08 ± 0.07^{a}	5.15 ± 0.07^{a}	5.21 ± 0.11^{a}
sweet	2.22 ± 0.02^{a}	2.20 ± 0.08^{a}	2.61 ± 0.07^{a}	2.30 ± 0.06^{a}	2.56 ± 0.11^{a}	2.91 ± 0.07^{a}	2.70 ± 0.05^{a}	2.59 ± 0.06^{a}
acid	3.73 ± 0.10^{a}	4.13 ± 0.07^{a}	4.01 ± 0.10^{a}	4.22 ± 0.03^{a}	3.68 ± 0.03^{a}	3.81 ± 0.11^{a}	4.03 ± 0.07^{a}	3.88 ± 0.03^{a}
bitter	4.59 ± 0.03^{a}	4.13 ± 0.03^{a}	5.36 ± 0.09^{b}	4.80 ± 0.03^{a}	4.22 ± 0.14^{a}	4.06 ± 0.06^{a}	5.02 ± 0.08^{b}	4.34 ± 0.05^{a}
salty	3.59 ± 0.22^{a}	3.82 ± 0.09^{a}	3.58 ± 0.14^{a}	3.44 ± 0.06^{a}	3.36 ± 0.03^{a}	3.42 ± 0.18^{a}	3.55 ± 0.13^{a}	3.45 ± 0.07^{a}
complexity	4.81 ± 0.10^{b}	4.97 ± 0.23^{b}	4.00 ± 0.03^{a}	4.03 ± 0.10^{a}	5.24 ± 0.07^{b}	4.82 ± 0.25^{ab}	4.10 ± 0.11^{a}	4.48 ± 0.04^{a}
off-flavours	1.09 ± 0.03^{a}	0.93 ± 0.04^{a}	1.99 ± 0.07^{b}	1.15 ± 0.08^{a}	1.32 ± 0.04^{ab}	1.63 ± 0.12^{b}	2.55 ± 0.13^{c}	0.98 ± 0.01^{a}

a–c: different letters indicate significant differences between experimental trials for the same sample for $P \leq 0.05$.

presence of those compounds suggests their enzymatic origin by lipoxygenase activity on polyunsaturated fatty acids of drupes. A similar pathway has been identified in the biosynthesis of volatile compounds of olive oil (Kiritsakis, 1998; Angerosa et al., 2000; Salas et al., 2000; Williams and Harwood, 2000; Ridolfi et al., 2002; Salas, 2004;) as well as in the brine of fermented table olives by LAB and yeasts (Sabatini et al., 2008). 2-phenylethanol could be produced in yeasts by L-phenylalanine catabolism (Sabatini et al., 2009).

Esters were mainly found during the 2013 year and the highest concentrations were estimated for the olives of trials A and B. Hexadecanoic acid, nonanoic acid, 1,4-butanediol, benzaldehyde-2,5-dimethyl, benzaldehyde-3-ethyl, 2-nonanone, squalene and

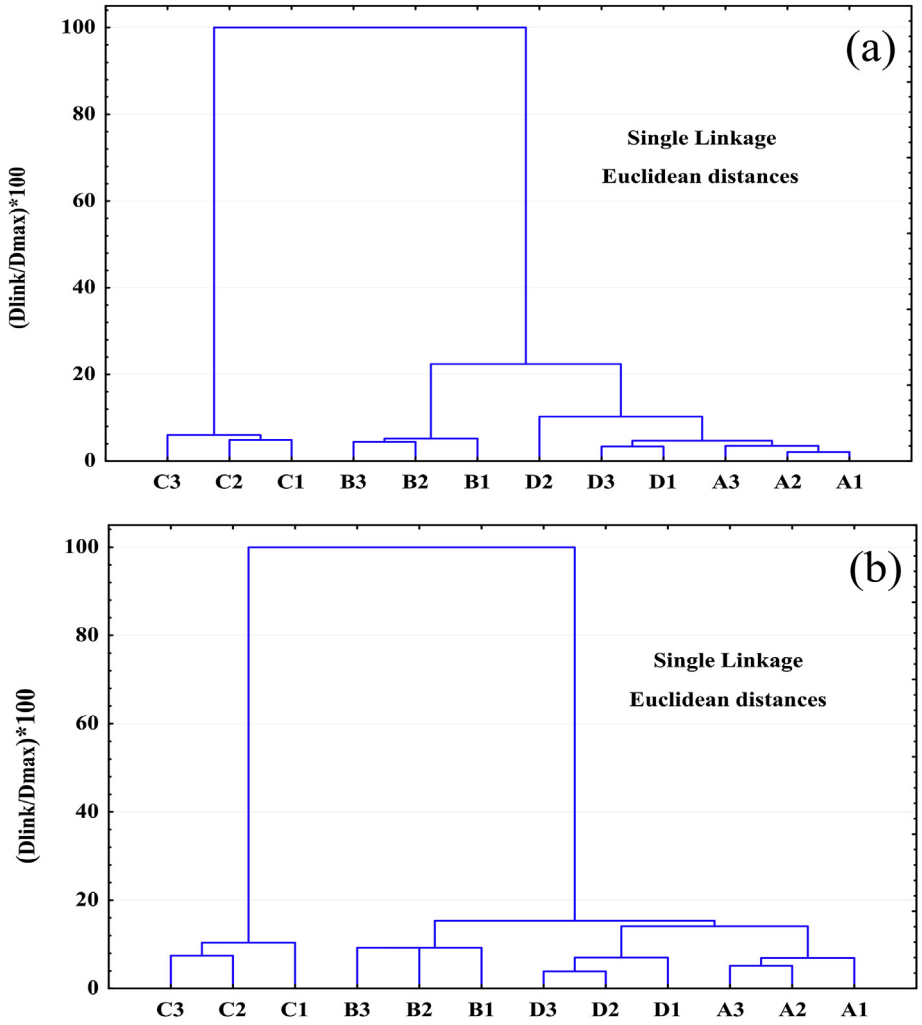

Fig. 2. Dendrogram of brine samples resulting from HCA based on values of microbial and pH changes during the 2012 (Fig. a) and 2013 (Fig. b) campaigns. The dissimilarity among samples was measured by Euclidean distance, whereas cluster aggregation was achieved by single linkage. Letters (A, B, C and D) correspond to experimental trials. The numbers associated to each letters correspond to replicates per each experimental trials.

styrene were revealed only in 2012 campaign. While 2-ethylhexanoic and heptanoic acid, butyrolactone, benzyl acetate, cis-3-hexenylacetate, ethyl dihydrocinnamate, ethyl lactate, methyl hexadecanoate, methyl hydrocinnamate, methyl salicylate were revealed only in 2013 campaign and reached the highest values in the trials A and B.

Only hydrocarbons, squalene and styrene, and phenols, guaiacol and phenol, were estimated at the highest concentrations for trial C especially during 2012. In particular styrene could be formed by decarboxylation of trans-cinammic acid (Shimada et al., 1992) which is produced by L-phenylalanine deamination.

The results of the sensory analysis are reported in Table 6. Samples performed with PdCs differed significantly ($p < 0.05$) from trials C and D used as controls. The main differences were estimated in terms of green olive aroma, bitter, complexity and off-flavours (taste). In details, the trial B showed the highest values of green olive aroma, and at the same time the lowest in terms of bitter taste. Very low values both of off-odours and off-flavours were found in samples of trials A and D. On the other hand, trial C showed values of off-odours and bitter significantly higher than other trials.

3.6. Multivariate statistical analysis

Multivariate data analysis has been widely applied in food processes (Berrueta et al., 2007) and it has found recently an extensive application in table olive research with promising results (Bautista-Gallego et al., 2011; Rodríguez-Gómez et al., 2013, 2012a, 2012b). The Barlett's sphericity test was applied to all data matrix inputs and differences statistically ($p < 0.0001$) significant were found among trials.

3.6.1. pH and microbiological data

HCA visibly discriminated samples of trials A and D from the others (Fig. 2). It is an unsupervised method that recognizes and distributes data grouping, according to their affinity, in clusters of progressive dissimilarity, as described in a dendrogram. On the basis of this approach, the areas and values both of pH and microbial groups of microorganisms included in the study, have been proven to be a useful variables to discriminate samples. The dendrograms visibly showed that the C cluster is recognized as different from the macro-cluster containing A, B and D samples. The cluster analysis provides further insight by classifying B cluster as different from that represented by A and D samples. In fact, the latter two trials were showed as closely grouped because of their short distance among samples.

The correlation analysis among variables showed that there were many significant relationships among them. Thus, data were appropriate to be subjected to PCA in order to condense the information into a reduced number of Factors. The results of the PCA (Fig. 3) showed that only two and three eigen-values higher than 1, which accounted for 74.97, 15.14% and 67.54, 11.75 and 9.05% of variability, were found for the years 2012 and 2013, respectively. This indicated that the initial 18 variables might be expressed as linear combination only of two and three Factors explaining 90.11%

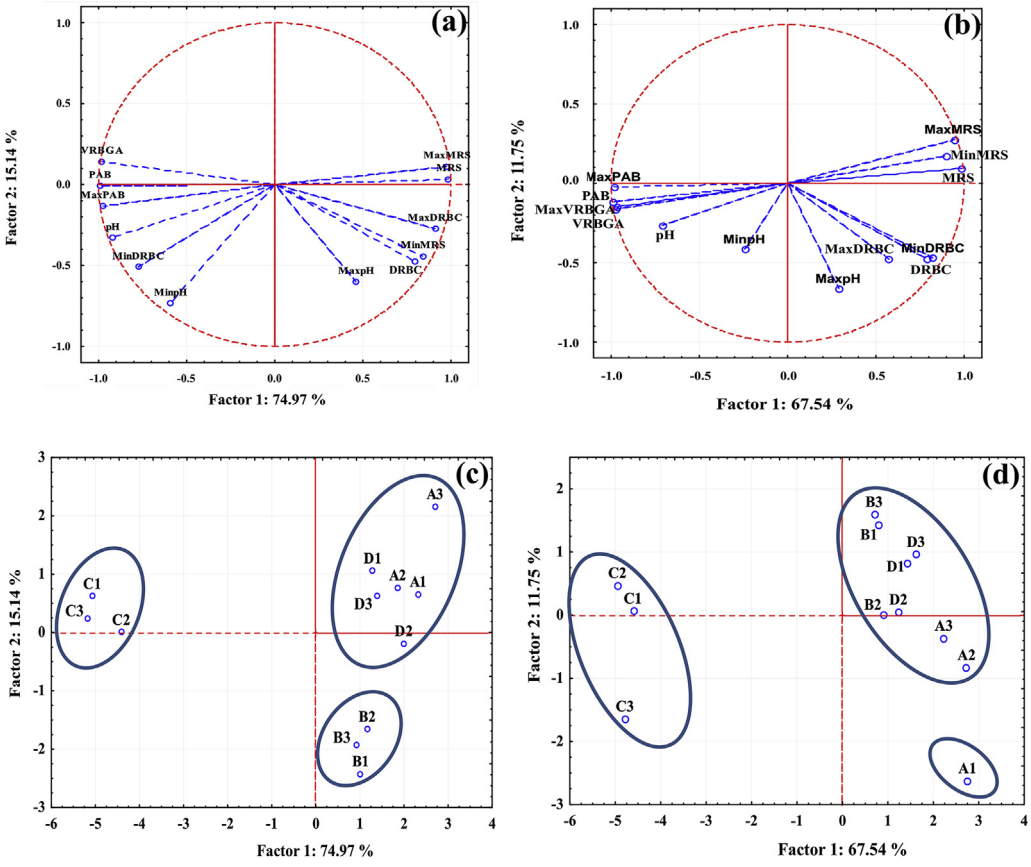

Fig. 3. PCA analysis based on the values of microbial and pH changes estimated during the 2012 [Fig. a (loading plot); Fig. b (score plot)] and 2013 [Fig. c (loading plot); Fig. d (score plot)] campaigns. Abbreviations: MRS, Man-Rogosa-Sharpe agar for mesophilic rod LAB; DRBC, dichloran rose bengal chloramphenicol agar for total yeasts and filamentous fungi; VRBGA, violet red bile glucose agar for *Enterobacteriaceae*; PAB, *Pseudomonas* agar base for pseudomonads. Max and Min corresponds to the maximum and minimum values of pH and microbial populations detected on the corresponding medium used for counts. Letters (A, B, C and D) correspond to the experimental trials. The numbers associated to each letter correspond to replicates per each trial. Circles have been reported only to display the distribution of trials onto graphic representation of PCA.

(2012 year) and 88.34% (2013 year) of the total variance. The components of the PCA were correlated to variables as shown in the Fig. 3a (2012) and Fig. 3b (2013). The discrimination of trials can be visualized in the plot of scores (Fig. 3c and d). In details, the Fig. 3c shows the projection of the cases (experimental trials) onto the planes as a function of Factors 1 and 2 (90.11% of the total variance explained) for the 2012. All replicates of the experimental trials were grouped into three main groups of which the trial A resulted closely related to the trial B and mainly along the Factor 1. On the other hand, the variables associated to the Factor 2 significantly contributed to discriminate trial C from the others thesis. Results similar to that reported above were found during the 2013. Excepted the replicate A1, all replicates of A, B and D were clearly separated from that of trial C. In both years, the experimental trials A, B and D were mainly influenced by values of MRS, MaxMRS, DRBC, MaxDRBC (positively) and pH (negatively) variables. On the contrary, the C was mainly related to the negative values of VRBGA, MaxVRBGA, MaxPAB, PAB and pH variables. Thus, both the multivariate statistical approaches (HCA and PCA) showed that the olives produced according to trials A and B might be closely related to that of control D (inoculated with starter), and, at the same time, to gain the advantages of PdC methods that means the reduction of starter amount to be inoculated, as well as the high strain diversity of LAB.

3.6.2. VOCs and sensory scores

The explorative multivariate analysis based on PCA was performed also on data set obtained from VOC (Fig. 4) and sensory analysis (Fig. 5). Globally, results found by PCA were in agreement with those obtained by clustering analysis (for pH and microbiological data), because the use of PdC could mainly influence the composition of final product.

For the PCA of VOCs, a total of four (2012 year) factors (accounting for 63.45, 18.69, 10.10 and 3.42% of total variance) and six (2013 years) factors (explaining 51.06, 20.87, 8.44, 6.27, 4.83 and 3.40% of total variance), that showed eigen-value higher than 1.00, were found. The F1 and F2 components, selected from the PCA, explain 63.45 and 18.69% (2012) and 51.06 and 20.87% (2013) of total variance, respectively (Fig. 4).

In both campaigns, the experimental samples of trials A and B, analyzed at 200 days of olive process, were located in the quadrant characterized by positive values of F1 component that explained more than 60% of total variance. At the same time, the trials A and B were clearly separated from each other, as well as from both the control thesis C and D. In fact, the lowest loading values of F1 component were estimated for the control trial C, in both the campaigns.

The effect of year on strain composition of experimental trials might explain the differences estimated in terms of VOC and

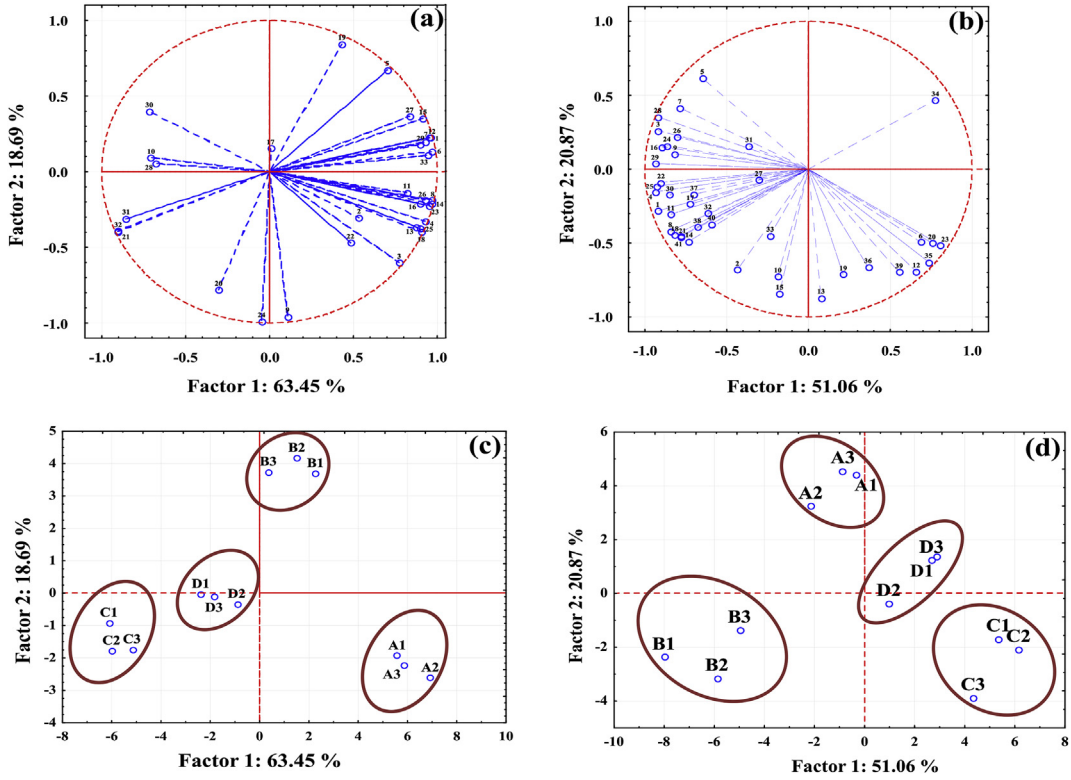

Fig. 4. PCA analysis based on the values of VOCs of samples collected at the end (day 200) of olive productions in 2012 [Fig. a (loading plot); Fig. b (score plot)] and 2013 [Fig. c (loading plot); Fig. d (score plot)] campaigns. Numbers in the loading plots: 1, acetic acid; 2, butanoic acid; 3, hexadecanoic acid; 4, hexanoic acid; 5, nonanoic acid; 6, octanoic acid; 7, pentanoic acid; 8, propionic acid; 9, 1.4-butanediol; 10, 1-butanol-3-methyl; 11, 1-hexanol; 12, 1-octanol; 13, 2-nonen-1-ol; 14, benzyl alcohol; 15, cis-hexen-1-ol; 16, phenylethyl alcohol; 17, 2-butenal-2-methyl; 18, 2-decenal (E); 19, benzaldehyde; 20, benzaldehyde-2.5-dimethyl; 21, benzaldehyde-3-ethyl; 22, nonanal; 23, octanal; 24, phenylacetaldehyde; 25, 2-nonanone; 26, 3-hydroxybutanone; 27, cyclopentanone; 28, guaiacol; 29, homoguaiacol; 30, phenol; 31, squalene; 32, styrene; 33, α-cubebene for the 2011 campaign (Fig. 2a). 1, 2-ethylhexanoic acid; 2, acetic acid; 3, butanoic acid; 4, heptanoic acid; 5, hexanoic acid; 6, octanoic acid; 7, pentanoic acid; 8, propionic acid; 9, 1-butanol-3-methyl; 10, 1-hexanol; 11, 1-octanol; 12, 2-nonen-1-ol; 13, benzyl alcohol; 14, cis-hexen-1-ol; 15, phenylethyl alcohol; 16, 2-butenal-2-methyl; 17, 2-decenal (E); 18, benzaldehyde; 19, nonanal; 20, octanal; 21, phenylacetaldehyde; 22, 3-hydroxybutanone; 23, 4-hylacetophenone; 24, butyrolactone; 25, cyclopentanone; 26, benzyl acetate; 27, cis-3-hexenylacetate; 28, ethyl dihydrocinnamate; 29, ethyl lactate; 30, methyl hexadecanoate; 31, methyl hydrocinnamate; 32, methyl salicylate; 33, octyl acetate; 34, vanillin; 35, 4-ethylphenol; 36, guaiacol; 37, homoguaiacol; 38, phenol; 39, α-cubebene; 40, 4-methyldihydro-2-(3H)-furanone; 41, α-terpineol for the 2011 campaign (Fig. 2b). Letters (A, B, C and D) correspond to the experimental trials. The numbers associated to each letter correspond to replicates per each trials. Circles have been reported only to display the distribution of trials onto graphic representation of PCA.

sensory results that were represented by score-plot (Fig. 4) and bi-plot (Fig. 5) graphics.

With regards to PCA of sensory scores, a positive correlation of both trials A and B to green olive aroma and complexity (taste) was found in both years. On the other hand, control thesis C, carried out by spontaneous fermentation, was clearly separated from the other trials, in detail along the Factor 1. In both years, all replicates C were characterized by the high loading values associated to bitter, off-odours and off-flavours variables. A clear separation of replicates D from the others was also estimated, in particular along the Factor 2. In details, after the PCA, three significant (higher than 1.00) eigen-values were estimated in both campaigns. For the 2012 year,

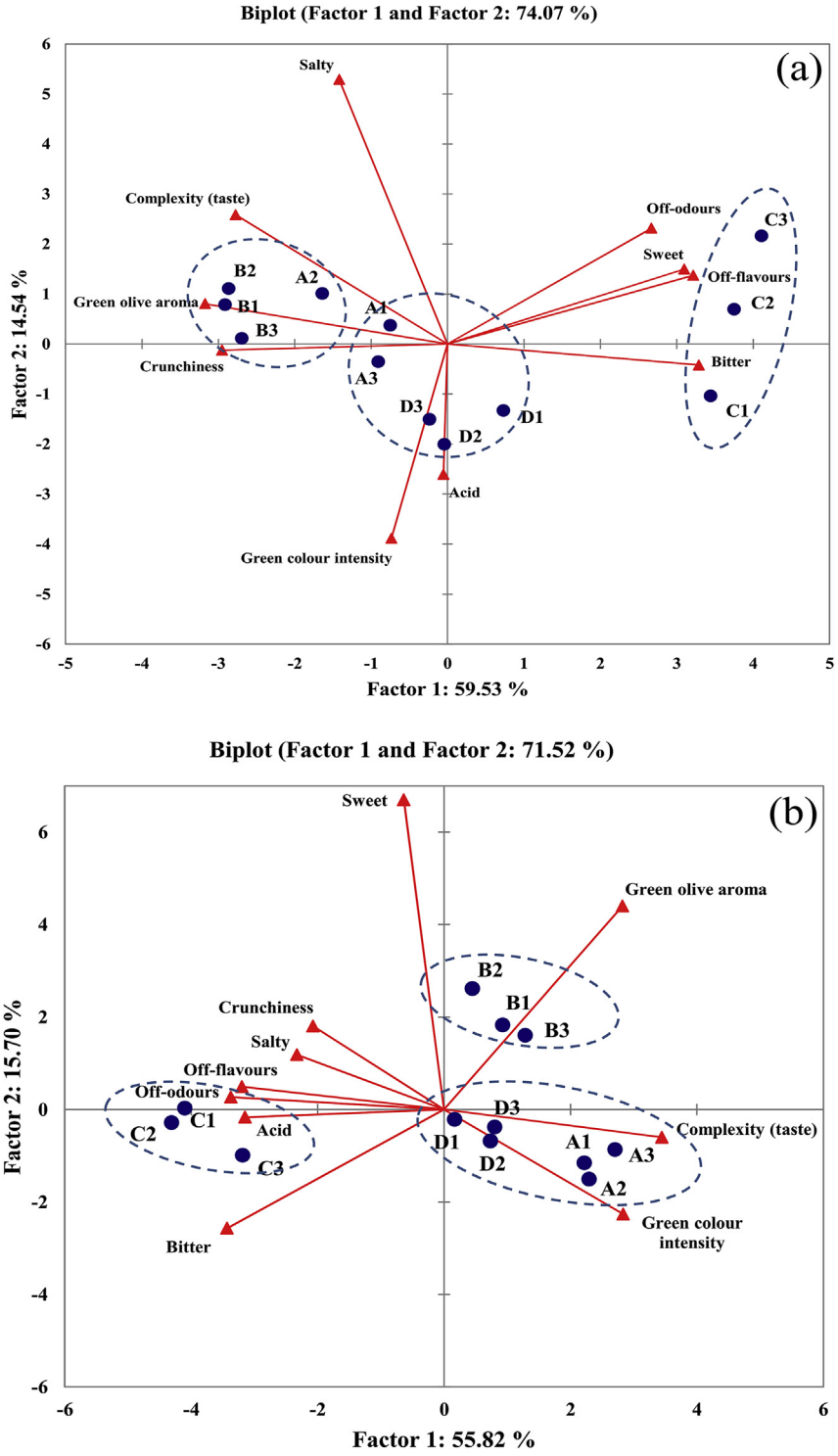

Fig. 5. PCA for sensory data of olives at the end of process (200 day) during the 2012 (Fig. a) and 2013 (Fig. b) campaigns. Biplot graphs show relationships among factors, variables and treatments. Letters (A, B, C and D) correspond to the experimental trials. The numbers associated to each letters correspond to replicates per each trial. Circles have been reported only to display the distribution of trials onto graphic representation of PCA.

the Factor 1 explained 59.53 and Factor 2 14.64% of total variability; for the 2013, Factor 1 and Factor 2 accounted for 55.82 and 15.70% of total variance, respectively.

4. Conclusions

This study provided an overview on the microbial ecology of fermented table olives produced by using the PdC method. The addition of partially fermented brines used as PdC into new brine, before the beginning of olive fermentation, favours the dominance of several *L. pentosus* strains. At the same time, the high strain diversity of *L. pentosus* population, as well as its annual variability, could positively affect the quality of final products. These results suggested PdC as a valuable method to favour the growth of autochthonous *L. pentosus* strains with technological aptitude that are able to drive the entire fermentation process.

The microbial diversity at species level both of LAB and yeast populations was in agreement with those commonly reported for table olive production. The concentration of spoilage microbial groups was estimated at very low levels in all experimental thesis and in both years. Furthermore, data obtained by VOCs and sensory analysis showed that olives produced with use of spontaneously fermented PdC were characterized by the highest scores of sensory complexity and none undesired off-odours and off-flavours were detected.

Although this research was performed in triplicates and in two consecutive olive campaigns, further investigations in different companies and with different olive varieties are being prepared to deepen the knowledge on the effects of PdC on quality of fermented table olives.

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