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Effect of the mechanical harvest of drupes on the quality characteristics of green fermented table olives

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Abstract

BACKGROUND: Because of damage caused by mechanical harvesting, the drupes for table olive production are traditionally hand harvested. Until now, no data have been available on the microbiological and chemical features of mechanically harvested drupes during fermentation.

RESULTS: Drupes mechanically harvested and inoculated with *Lactobacillus pentosus* OM13 were characterized by the lowest concentrations of potential spoilage microorganisms. On the other hand, drupes mechanically harvested and subjected to spontaneous fermentation showed the highest concentration of *Enterobacteriaceae* and pseudomonads during transformation. The lowest decrease of pH (4.20) was registered for the trials inoculated with the starter culture. Differences in terms of volatile organic compounds were estimated among trials. Multivariate analysis showed that the olives processed from the drupes mechanically harvested and inoculated with starter were closely related to control production (drupes manually harvested) in terms of microbiological and pH values. Sensory analysis evidenced negative evaluations only for the uninoculated trials.

CONCLUSION: Drupes mechanically harvested and subjected to a driven fermentation with *Lactobacillus pentosus* OM13 determined the production of table olives with appreciable organoleptic features. Thus mechanical harvesting performed using a trunk shaker equipped with an inverse umbrella and the addition of starter lactic acid bacteria represents a valuable alternative to manual harvesting for table olive production at the industrial level.

Supporting information may be found in the online version of this article.

Keywords: lactic acid bacteria; yeasts; *Lactobacillus pentosus* OM13; Nocellara del Belice table olive fermentation; mechanical harvest; manual harvest

INTRODUCTION

Drupes to be transformed into table olives have to be intact. Skin and flesh of drupes are extremely sensitive to scratches and wounds that may occur during harvesting. For this reason, only hand harvesting carried out with accuracy can avoid fruit damage. Mechanical harvesting is not generally applied to drupes to be transformed into table olives; this kind of harvesting might damage (scratches, wounds on skins) drupes, which can not be further processed.[1,2] However, susceptibility to damage depends on the variety, texture, maturity stage, water content, firmness, temperature, size and shape, as well as internal fruit factors such as cell wall, strength and elasticity, cell shape and structure.[3–5] Furthermore, as stated by Jiménez-Jiménez *et al.*,[6] the mechanical damage from impact is strictly related to the impact energy level and the time after impact.

The mechanical harvesting of drupes for olive oil production has been deeply studied[7,8] and this practice is widespread. Conversely, knowledge of the effect of this harvest on table olives is quite limited.[9–11] Mechanical harvesting, mostly applied to drupes for table olives, is performed using large trunk-shaking or vibrating machines that operate at a well-defined vibration frequency and shaking time.[12–14] Recently, Gambella *et al.*[15] tested three coating materials (silicone, vulcanized rubber and natural rubber) with different thicknesses and rotational speeds to reduce the percentage of damaged fruits.

Olive drupes cannot be eaten unprocessed because of the presence of oleuropein, which is a bitter glucoside consisting of glucose, elenolic acid and *o*-diphenol hydroxytyrosol compounds.[16] A variety of technological methods are commonly applied to produce table olives.[17,18] The 'Greek' processing style, also known as

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the 'natural method', is a common method employed in Mediterranean countries,[19] even though productions carried out at an industrial level rely on starter lactic acid bacteria (LAB), often *Lactobacillus plantarum* and/or *Lactobacillus pentosus*.[16,17,20,21] Generally, the direct inoculation of commercial starter cultures into olive brine ensures a rapid increase of LAB populations, with a sudden decrease in pH. This procedure limits the risk of off-flavour generation due to growth of spoilage microrganisms.[16,22–24] For this reason, the use of selected strains is becoming a common practice for table olive fermentation.[24–27] The International Olive Council (IOC) estimated a total world production of 2.5 million tons of olives for the 2012/2013 campaign, with 76 000 tons located in Italy.[28]

Sicily (southern Italy) is the most important Italian region for table olive production.[29] A consistent part of this production is based on Nocellara del Belice cultivar and the resulting product enjoys the status of Protected Denomination of Origin (PDO) obtained through Regulation EC No. 134/1998. Traditionally, the harvesting of Nocellara del Belice drupes is time consuming because it is performed manually. The present research was aimed at studying the applicability of a trunk shaker machine equipped with an inverted umbrella catching the detached, falling fruits of Nocellara del Belice to reduce the number of damaged drupes, as well as to limit the extent of damage and to evaluate the influence of this practice on the final product. To this purpose, the microbiological, chemical and sensory parameters were monitored during table olive processing.

EXPERIMENTAL

Experimental table olive production and sample collection

Table olive transformation was carried out on drupes of the cultivar Nocellara del Belice produced by olive groves (located in Castelvetrano, Trapani province, Sicily, Italy; 37° 36′ 46″ N/12° 50′ 52″ E), the main area for Nocellara del Belice PDO production. Olive drupes were mechanically harvested using a trunk shaker equipped with an inverse umbrella (model 'SICMA F3 umbrella olive harvester'; SICMA Srl, Acconia di Curinga, Catanzaro, Italy). The machine was equipped with a high-frequency and self-breaking vibrating head mounted over a telescopic arm; the reverse umbrella (7 m in diameter), case (containing up to 500 kg of drupes) and unload unit were hydraulically controlled. Samples of drupes were collected in 2012 and 2013 from the mass of fruits obtained shaking 50 trees per year.

The samples of olive drupes were transferred to two vats (180 L volume). Each vat contained 150 kg olives and 30 L brine composed of NaCl 9% (w/v). One vat was inoculated with 0.15 g kg^{-1} of the freeze-dried autochthonous strain *L. pentosus* OM13, already tested for Nocellara del Belice table olives,[22] and represented the trial mechanical A (MCA). The other vat was uninoculated and represented the trial mechanical B (MCB), which was spontaneously fermented. Two additional trials performed with drupes manually harvested were included in the experimental plan: manual A (MNA) inoculated as reported above for the trial MCA and manual B (MNB) subjected to spontaneous fermentation. The fermentation of all trials was carried out at room temperature for 210 days and periodically monitored. Samples of brine (about 50 mL) were collected before starter culture inoculation, immediately after its addition and at 3, 6, 9, 15, 30, 50, 70, 90, 130, 170 and 210 days of fermentation. The experiment was performed in triplicate (three vessels per trial) in two consecutive years (2012 and 2013).

Physicochemical and microbiological analyses

The pH values of brine samples were determined by pH meter (BASIC 20+; Crison Instruments SA, Barcelona, Spain). Salt concentration was routinely analysed as reported by Garrido Fernández *et al.*[30]

Decimal dilutions of brines were prepared in Ringer's solution (Sigma-Aldrich, Milan, Italy) and different microbial groups, such as mesophilic rod LAB, yeasts, *Enterobacteriaceae*, pseudomonads, staphylococci and coagulase-positive staphylococci (CPS), enumerated as reported by Martorana *et al.*[31] Analyses were performed in triplicate. All media and supplements used were supplied from Oxoid (Thermofisher, Basingstoke, UK).

Isolation and phenotypic grouping of LAB

Presumptive LAB (at least four colonies with the same colour, morphology, margin, surface and elevation) were collected from the highest plated dilutions following their growth on MRS agar. The isolates were purified by successive subculturing and the purity of the isolates was checked microscopically. Gram-positive (Gregersen KOH method) and catalase-negative isolates (determined in presence of H_2O_2 5%, v/v) were stored in broth containing 20% (v/v) glycerol at −80 °C pending further experimentation.

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 medium used for isolation, without citrate, from which certain LAB can result in gas formation. Obligate homofermentative metabolism was determined by the absence of growth in the presence of a mixture of pentose carbohydrates (xylose, arabinose and ribose; 8 g L^{-1} each) in place of glucose.

Genotypic investigation of LAB at strain and species level

DNA from LAB isolates was extracted using the InstaGene Matrix kit (Bio-Rad Laboratories, Hercules, CA, USA) 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.*[32] The identification at species level was performed by multiplex PCR analysis based on the *rec*A gene with species-specific primers for *Lactobacillus pentosus*, *L. plantarum* and *L. paraplantarum*, as described by Torriani *et al.*[33]

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.*[34] DNA sequencing reactions were performed at Primm-Biotech Srl (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 of the sole type strains within the database EZTaxon, located at http://www.ezbiocloud.net/eztaxon.

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 subculturing steps onto DRBC medium and subjected to genetic characterization.

DNA extraction was performed as reported above. All selected isolates were preliminarily 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.*[35] 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. The D1/D2 region was amplified and polymerase chain reaction (PCR) products were visualized as described by Settanni *et al.*[36] The reaction of DNA sequencing and the identities of sequences were determined as reported above.

Volatile organic compounds (VOCs)

Volatile compounds were determined at 210 days of fermentation by solid phase micro-extraction technique in head space followed by gas chromatography–mass spectrometry (HS-SPMEGC-MS).[25,37] Drupes were homogenized and transferred (0.50 g) into 2 mL vials with pierceable silicone rubber septa coated with polytetrafluoroethylene (PTFE) film. Fifty microliters of 2-pentanol-4-methyl methanol solution (0.981 μg mL^{-1}) were used as internal standard. A Supelco SPME (Bellefonte, PA) holder and fibre was coated with divinylbenzene–carboxen–polydimethylsiloxane. The vials were heated at a controlled temperature (40 ± 0.5 °C) in order to reach equilibrium and 30 min exposure time. The GC-MS conditions were used as described by Corona.[38] 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, TG House, Cambridge, UK). Semi-quantitative determination was carried out by the internal standard method. The calibration curve was constructed with readings on five 2-pentanol-4-methyl methanol solutions with concentrations ranging from 1.5 to 8 μg mL^{-1} ($R^2 = 0.994$). All analyses were performed in triplicate.

Sensory evaluation

Evaluation of the sensory profiles of the experimental olives was performed using a descriptive method (UNI 10957, 2003)[39] as reported by Aponte *et al.*[22] The analysis was applied to olives at the end of fermentation (day 210).

Twelve judges (six females and six males, 22–35 years old) were trained in preliminary sessions using different samples of commercial table olives of the cultivar Nocellara del Belice, in order to develop a common vocabulary for the description of the sensory attributes of the experimental samples and to familiarize 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 panellists were selected for sample evaluation. Thus 15 descriptors were included in the analysis for the external aspect (green colour intensity), odour (green olive aroma, complexity and off-odours), taste (crispness, easy stone detachment from the flesh, juicy, sweet, sour, bitter, salt, astringent and complexity) and off-flavours. 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.

Statistical and explorative multivariate analysis

Data of pH, microbiological investigation and sensory evaluation were analysed using a generalized linear model (GLM) that included the effects of samples; Student's *t*-test was used for mean comparison. Post hoc Tukey's 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 analysis of variance (ANOVA) test was applied to identify significant differences among olive attributes. Combined ANOVA of pH values and microbiological counts was performed on data collected over 2 years of experimentation. All parameters were analysed by two-way ANOVA within each year. Simple effects tests were used to examine significant two-way interactions and the least significant difference (LSD) procedure was used for pairwise comparison.

In addition, explorative multivariate analysis was employed to investigate the relationship among data obtained from the different experimentations. A hierarchical cluster analysis (HCA) (joining, tree clustering) was carried out for grouping the trials according to their similarity, measured by Euclidean distances, whereas cluster aggregation was based on the single linkage method.[40] HCA is a graphical representation of a matrix of distances such as the dendrogram where the objects (strains) are joined together in a hierarchical ascendant analysis from the closest one, i.e. the most similar, to the furthest apart, which is the most different.

Furthermore, the principal component analysis (PCA) was employed to investigate the 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, staphylococci, CPS and pH values.[41,42] Areas were calculated by integration using OriginPro 7.5 software (OriginLab Corporation, Northampton, MA, 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.[41]

PCA was also employed to investigate the relationship among olive samples based on VOC profile, as well as sensory analysis.[43,44]

The number of principal factors was selected according to the Kaiser criterion[45] and only factors with eigenvalues higher than 1.00 were retained. All data were preliminarily evaluated using Barlett's sphericity test,[46,47] in order to check the statistically significant difference among samples within each dataset.

Statistical data processing and graphical construction were achieved using the SPSS software package (SPSS Inc., Chicago, IL, USA), STATISTICA software version 10 (StatSoft Inc., Tulsa, OK, USA) and XLStat software version 7.5.2 (Addinsoft, New York, NY, USA) for Excel.

RESULTS

pH values and microbiological loads

The results of pH measurements and microbial analyses of the brines collected during the entire process of table olive production are reported in Table 1.

The pH value was 7.3 at the beginning of the transformation (day 0) and 4.2 at the end of the process (day 210) for all trials in both years of research. Trial MCA showed the highest decrease of pH at day 6 of fermentation and reached pH values significantly lower than MCB at day 30. From day 50 onwards, the pH decreased approximately to 4.2 for all treatments. However, the most rapid decrease of pH was registered for MCA and MNA.

The microbial groups mostly represented on untreated olives mechanically harvested were yeasts (almost 5.0 log CFU g^{-1} in both years) and LAB (around 2.0 log CFU g^{-1} in both years). The concentrations of *Enterobacteriaceae* and pseudomonads were comparable to that of LAB. Yeasts and LAB were more represented than other microbial groups even when manually harvested olives

Table 1. Values of pH and microbial concentrations of samples collected during 2012 and 2013 table olive production

	Days of fermentation											
	0	3	6	9	15	30	50	70	90	130	170	210
pH 2012												
Trial MCA	7.2 ± 0.3a	5.2 ± 0.1a	4.0 ± 0.2a	4.0 ± 0.2a	3.8 ± 0.1a	3.7 ± 0.0a	3.8 ± 0.1a	3.7 ± 0.2a	4.0 ± 0.1a	4.4 ± 0.2a	4.3 ± 0.1a	4.2 ± 0.2a
Trial MCB	7.3 ± 0.1a	5.7 ± 0.1b	5.4 ± 0.2b	5.3 ± 0.1b	4.2 ± 0.0a	4.2 ± 0.1a	4.3 ± 0.1b	4.3 ± 0.2a	4.1 ± 0.3a	4.4 ± 0.1a	4.5 ± 0.3a	4.3 ± 0.2a
Trial MNA	7.3 ± 0.2a	5.2 ± 0.2a	4.3 ± 0.3a	4.1 ± 0.2a	3.7 ± 0.2a	3.9 ± 0.2a	3.7 ± 0.2a	3.9 ± 0.2a	4.1 ± 0.2a	4.3 ± 0.1a	4.2 ± 0.1a	4.0 ± 0.1a
Trial MNB	7.2 ± 0.2a	6.6 ± 0.1c	6.3 ± 0.2c	5.7 ± 0.2b	4.9 ± 0.2b	4.3 ± 0.2a	4.4 ± 0.2b	4.1 ± 0.3a	4.1 ± 0.2a	4.3 ± 0.2a	4.7 ± 0.1a	4.4 ± 0.1a
pH 2013												
Trial MCA	7.3 ± 0.1a	5.0 ± 0.2a	4.9 ± 0.3a	4.4 ± 0.2a	4.2 ± 0.1a	3.9 ± 0.1a	4.1 ± 0.1a	4.3 ± 0.1a	4.1 ± 0.2a	4.0 ± 0.2a	4.1 ± 0.2a	4.1 ± 0.1a
Trial MCB	7.3 ± 0.1a	6.1 ± 0.3b	6.0 ± 0.2b	5.3 ± 0.3b	4.9 ± 0.1b	4.6 ± 0.1a	4.2 ± 0.2a	4.3 ± 0.2a	4.4 ± 0.3a	4.3 ± 0.1a	4.4 ± 0.2a	4.3 ± 0.2a
Trial MNA	7.4 ± 0.2a	5.2 ± 0.2a	4.9 ± 0.3a	4.4 ± 0.1a	4.1 ± 0.2a	4.1 ± 0.2a	4.2 ± 0.3a	4.0 ± 0.1a	4.2 ± 0.5a	4.0 ± 0.4a	4.2 ± 0.2a	4.0 ± 0.3a
Trial MNB	7.1 ± 0.1a	5.3 ± 0.2a	5.6 ± 0.1b	5.3 ± 0.2b	4.7 ± 0.4b	4.5 ± 0.3a	4.4 ± 0.3a	4.4 ± 0.2a	4.0 ± 0.2a	4.1 ± 0.3a	4.1 ± 0.1a	4.1 ± 0.1a
MRS 2012												
Trial MCA	7.0 ± 0.2b	6.2 ± 0.1c	7.3 ± 0.0b	6.9 ± 0.3b	7.0 ± 0.1b	7.3 ± 0.1b	6.9 ± 0.3b	6.8 ± 0.1c	5.9 ± 0.3b	5.7 ± 0.2a	5.8 ± 0.1b	5.2 ± 0.1a
Trial MCB	1.0 ± 0.2a	4.7 ± 0.1b	4.5 ± 0.2a	4.4 ± 0.1a	5.1 ± 0.2a	5.6 ± 0.3a	5.1 ± 0.1a	5.2 ± 0.2a	4.8 ± 0.2a	5.1 ± 0.3a	4.7 ± 0.1a	5.0 ± 0.2a
Trial MNA	7.0 ± 0.4b	6.7 ± 0.2d	6.9 ± 0.4b	7.2 ± 0.1b	7.1 ± 0.4b	7.0 ± 0.3b	7.4 ± 0.1c	6.3 ± 0.3b	6.0 ± 0.1b	5.3 ± 0.2a	5.9 ± 0.1b	5.7 ± 0.2b
Trial MNB	1.1 ± 0.1a	4.0 ± 0.1a	4.2 ± 0.1a	4.6 ± 0.0a	5.3 ± 0.1a	5.5 ± 0.2a	5.8 ± 0.3b	5.3 ± 0.3a	4.5 ± 0.2a	5.3 ± 0.3a	5.0 ± 0.2a	4.8 ± 0.3a
MRS 2013												
Trial MCA	7.2 ± 0.1b	7.2 ± 0.2d	7.1 ± 0.0c	7.5 ± 0.1b	7.5 ± 0.2c	6.8 ± 0.1b	6.9 ± 0.1c	7.1 ± 0.3c	6.2 ± 0.3c	6.0 ± 0.3b	6.1 ± 0.1b	6.0 ± 0.2b
Trial MCB	1.1 ± 0.2a	4.7 ± 0.3b	4.5 ± 0.1a	4.4 ± 0.2a	4.7 ± 0.3a	5.6 ± 0.1a	5.2 ± 0.2a	5.2 ± 0.3a	5.3 ± 0.2b	5.2 ± 0.3a	4.8 ± 0.4a	4.7 ± 0.3a
Trial MNA	7.3 ± 0.3b	6.7 ± 0.3c	7.2 ± 0.2c	7.2 ± 0.2b	7.0 ± 0.2b	7.2 ± 0.3b	7.3 ± 0.2c	7.1 ± 0.1c	5.9 ± 0.2c	6.2 ± 0.3b	6.0 ± 0.3b	5.9 ± 0.5b
Trial MNB	0.8 ± 0.4a	1.3 ± 0.5a	3.2 ± 0.2a	4.2 ± 0.3a	4.5 ± 0.4a	5.8 ± 0.3a	6.0 ± 0.3b	5.8 ± 0.3b	4.7 ± 0.1a	4.8 ± 0.4a	5.8 ± 0.2b	5.9 ± 0.3b
DRBC 2012												
Trial MCA	4.6 ± 0.3a	4.1 ± 0.2a	4.7 ± 0.2a	6.7 ± 0.2c	6.1 ± 0.2a	6.7 ± 0.1b	6.4 ± 0.1b	5.6 ± 0.2a	5.5 ± 0.2b	5.8 ± 0.3b	4.5 ± 0.3a	4.6 ± 0.3a
Trial MCB	4.3 ± 0.1a	3.9 ± 0.1a	5.8 ± 0.1b	5.2 ± 0.1a	5.9 ± 0.3a	5.3 ± 0.3a	5.6 ± 0.2a	5.5 ± 0.1a	5.0 ± 0.3a	5.2 ± 0.2a	4.8 ± 0.1a	5.9 ± 0.1b
Trial MNA	4.0 ± 0.3a	3.7 ± 0.3a	5.0 ± 0.1a	6.0 ± 0.2b	6.0 ± 0.1a	6.5 ± 0.0b	5.9 ± 0.2a	6.4 ± 0.2b	5.4 ± 0.2b	6.0 ± 0.3b	5.1 ± 0.2a	4.1 ± 0.2a
Trial MNB	4.0 ± 0.2a	3.5 ± 0.1a	5.6 ± 0.2b	5.3 ± 0.3a	5.7 ± 0.2a	5.0 ± 0.3a	5.8 ± 0.4a	5.6 ± 0.3a	4.8 ± 0.2a	5.4 ± 0.2a	5.1 ± 0.2a	5.7 ± 0.3b
DRBC 2013												
Trial MCA	4.9 ± 0.1b	4.3 ± 0.3a	4.1 ± 0.3a	4.7 ± 0.2b	5.7 ± 0.2a	5.7 ± 0.3a	4.3 ± 0.1a	4.2 ± 0.3a	4.9 ± 0.3b	5.4 ± 0.1b	5.6 ± 0.3a	4.2 ± 0.1a
Trial MCB	4.9 ± 0.2b	3.9 ± 0.1a	5.8 ± 0.3c	5.2 ± 0.2b	5.9 ± 0.3a	5.3 ± 0.1a	5.0 ± 0.2a	5.5 ± 0.2c	6.2 ± 0.2c	4.3 ± 0.1a	5.0 ± 0.2a	6.0 ± 0.1c
Trial MNA	4.2 ± 0.1a	4.4 ± 0.1a	4.5 ± 0.2a	4.1 ± 0.2a	5.8 ± 0.1a	5.5 ± 0.1a	4.6 ± 0.3a	5.0 ± 0.3b	5.0 ± 0.3b	5.0 ± 0.2b	5.2 ± 0.4a	4.1 ± 0.3a
Trial MNB	4.0 ± 0.2a	4.2 ± 0.2a	5.1 ± 0.1b	5.0 ± 0.3b	5.9 ± 0.3a	5.5 ± 0.2a	5.3 ± 0.2a	5.6 ± 0.3c	4.2 ± 0.3a	4.4 ± 0.3a	4.8 ± 0.3a	5.3 ± 0.2b
VRBGA 2012												
Trial MCA	n.d.	1.2 ± 0.0b	0.9 ± 0.4a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Trial MCB	n.d.	2.0 ± 0.1c	2.1 ± 0.3b	1.0 ± 0.1a	0.6 ± 0.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Trial MNA	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 MNB	n.d.	0.9 ± 0.3a	1.2 ± 0.2a	1.9 ± 0.1b	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
VRBGA 2013												
Trial MCA	n.d.	1.1 ± 0.1a	1.1 ± 0.3a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Trial MCB	n.d.	2.1 ± 0.2b	2.2 ± 0.4b	3.0 ± 0.1a	1.8 ± 0.2	0.6 ± 0.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Trial MNA	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 MNB	n.d.	1.2 ± 0.2a	1.0 ± 0.3a	2.8 ± 0.2a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PAB 2012												
Trial MCA	n.d.	2.1 ± 0.2a	2.0 ± 0.1a	0.9 ± 0.1a	0.3 ± 0.1a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Trial MCB	n.d.	2.4 ± 0.2a	3.3 ± 0.3b	4.1 ± 0.3b	4.4 ± 0.1b	5.5 ± 0.3a	5.5 ± 0.2b	5.1 ± 0.1b	n.d.	n.d.	n.d.	n.d.
Trial MNA	n.d.	1.8 ± 0.3a	1.7 ± 0.3a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Trial MNB	n.d.	2.1 ± 0.2a	3.1 ± 0.4b	3.9 ± 0.4b	4.2 ± 0.2b	5.2 ± 0.3a	5.0 ± 0.3a	4.6 ± 0.3a	n.d.	n.d.	n.d.	n.d.
PAB 2013												
Trial MCA	n.d.	2.2 ± 0.2b	2.6 ± 0.1b	3.9 ± 0.0b	4.9 ± 0.2b	3.3 ± 0.3b	3.0 ± 0.3a	2.2 ± 0.4a	n.d.	n.d.	n.d.	n.d.
Trial MCB	n.d.	3.5 ± 0.2c	4.3 ± 0.3c	5.6 ± 0.1d	5.9 ± 0.1c	6.0 ± 0.2c	6.0 ± 0.3b	4.2 ± 0.3b	n.d.	n.d.	n.d.	n.d.
Trial MNA	n.d.	1.6 ± 0.2a	1.1 ± 0.1a	2.0 ± 0.2a	4.0 ± 0.2a	1.9 ± 0.2a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Trial MNB	n.d.	3.7 ± 0.1c	2.3 ± 0.3b	4.7 ± 0.1c	5.5 ± 0.2c	5.7 ± 0.3c	5.6 ± 0.3b	3.8 ± 0.2b	n.d.	n.d.	n.d.	n.d.

Results of microbial loads are expressed as log CFU mL^{-1} and indicate the mean values ± standard deviation of three plate counts. Abbreviations: MCA, olive drupes inoculated with *L. pentosus* OM13 and mechanically harvested; MCB, olive drupes uninoculated and mechanically harvested; MNA, olive drupes inoculated with *L. pentosus* OM13 and manually harvested; MNB, olive drupes uninoculated and manually harvested; 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; n.d., not detected (value < detection limit of method); different letters (a–c) indicate significant differences among experimental trials for the same sample and the same medium ($P < 0.05$). Loads on BP agar have not been reported in the table since all samples showed concentration of staphylococci less than the detection limit of the method.

Table 2. Molecular identification of LAB species during the table olive production

Lactobacillus species	Strain	Isolation source (day of sampling)	Size of multiplex PCR[a] amplicon	% similarity[b] (accession no. of closest relative) by: BLAST	% similarity[b] (accession no. of closest relative) by: EzTaxon	Sequence length (bp)	Acc. no.
L. pentosus	2OCRBL80	MCA (70d)	218	99 (KF923751.1)	99.60 (D79211)	1512	KP256078
	2OCRBL81	MNB (90d)	218	99 (AB362677.1)	99.60 (D79211)	1512	KP256077
	1OCRBL388	MNA (130d)	218	99 (KF923751.1)	99.46 (D79211)	1505	KP256089
	1OCRBL492	MNA (210d)	218	99 (KF923751.1)	99.13 (D79211)	1504	KP256090
	1OCRBL133	MCB (9d)	218	98 (KF923751.1)	98.33 (D79211)	1512	KP256081
L. plantarum	BL277-1-OC	MNB (30d)	318	99 (KJ802480.1)	99.20 (ACGZ01000098)	1513	KT268295

Abbreviations: MCA, olive drupes inoculated with *L. pentosus* OM13 and mechanically harvested; MCB, olive drupes uninoculated and mechanically harvested; MNA olive drupes inoculated with *L. pentosus* OM13 and manually harvested; MNB, olive drupes uninoculated and manually harvested.
[a] Results obtained by multiplex PCR analysis of the *rec*A gene with species-specific primers for *L. pentosus*, *L. plantarum* and *L. paraplantarum*.[33]
[b] Results obtained by the 16S rRNA sequence search.

were analysed. For these olives, pseudomonads showed lower levels than those estimated for the olives subjected to mechanical harvesting, while *Enterobacteriaceae* could not be detected. The brines analysed before addition did not host any of these microbial groups. None of the microbial groups previously reported were at detectable levels in the brines added for fermentation.

Owing to the use of the starter culture, trials MCA and MNA showed higher concentrations of LAB than MCB and MNB, whereas an opposite trend was registered for the growth of potential spoilage microorganisms, which was estimated at very low levels for the inoculated trials. Immediately after the inoculation of the starter culture, LAB were about 7.0 log CFU mL^{-1} in both trials MCA and MNA. LAB were significantly higher than yeasts. Up to the 130 day, trials MCA and MNA showed a significant increase of both groups. Conversely, trials MCB and MNB showed the lowest LAB and yeast concentrations during the entire period of observations in both years. From day 130 onwards, LAB and yeasts showed almost constant concentration values until the end of the observations. Trial MCB showed the highest concentration of *Enterobacteriaceae* (around day 9) and pseudomonads (around day 50) in both years. Staphylococci and CPS were not detected in any sample.

Isolation, genotypic characterization and distribution of LAB

A total of 1742 colonies were collected from the highest plated dilutions of cell suspensions, and 1481 rods were considered presumptive LAB cultures, as being Gram positive and catalase negative. All cultures were able to grow at 15 °C, unable to develop at 45 °C and were facultatively homofermentative (grew in the presence of pentose carbohydrates, but CO_2 was not produced from glucose). Owing to the high number of isolates, about 40% of cultures (selected on the basis of the isolation source, colony morphology, experimental trial and year of production) were subjected to RAPD analysis, which allowed the identification of 23 different strains. Results from multiplex PCR analysis and 16S rRNA gene sequencing revealed the presence of a major group of *L. pentosus* (Table 2) composed of 20 strains (Fig. 1). The other three strains were identified as *L. plantarum*.

Lactobacillus pentosus dominated the LAB population in all trials and in both years of observation. *Lactobacillus plantarum* was mainly isolated between 50 and 70 days in both spontaneously fermented trials.

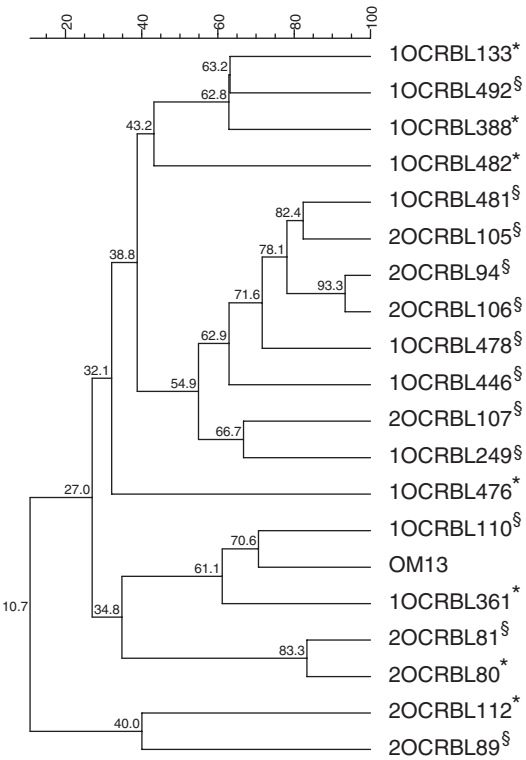

Figure 1. Dendrogram of *Lactobacillus pentosus* strains isolated during table olive preparation in both years. Symbols: *strains isolated during year 2012; §strains isolated during year 2013; OM13, commercial starter culture.

In terms of biodiversity within the *L. pentosus* species, trials MCB and MNB showed the highest number of strains. As expected, the commercial starter OM13 inoculated in MCA and in MNA was the strain most frequently isolated during fermentation in both trials and in both years. No *L. pentosus* strain of year 2012 was then found during year 2013.

Isolation, identification and distribution of yeasts

A total of 3108 yeast colonies were collected from DRBC agar. Based on colony and cell morphology, 593 isolates were subjected to molecular identification. After restriction analysis of 5.8S-ITS region, the isolates were clustered into 11 groups (Table 3).

Table 3. Molecular identification of yeasts isolated during table olive production

Species	Strain	Isolation source (day of sampling)	RP	5.8S-ITS PCR	Size of restriction fragments *Cfo*I	*Hae*III	*Hin*fI	% similarity[a] (accession no. of closest relative) by:	Acc. no.
Candida boidinii	1OCRY307	MCB (30d)	I	700	329 + 299	700	390	99 (KC442246.1)	KP256103
	1OCRY366	MCA (90d)	II	740	346 + 312 + 82	733	378 + 182 + 147	99 (KC442246.1)	KP256104
	1OCRY264	MNA (30d)	III	780	335 + 302	740	377	99 (KC442246.1)	KP256105
	1OCRY446	MCB (170d)	IV	750	343 + 309 + 81	750	393 + 195 + 158	99 (EU293427.1)	KP256106
	1OCRY300	MCB (30d)	V	740	329 + 299	740	390	99 (GU373760.1)	KP256107
Candida diddensiae	2OCRY20	MCA (6d)	VI	695	300 + 184 + 142 + 68	450 + 143 + 91	341	99 (U45750.1)	KP256101
Candida membranifaciens	1OCRY126	MNB (6d)	VII	650	314 + 297	420 + 150 + 80	334	99 (EF362752.1)	KP256097
Wickerhamomyces anomalus	1OCRY107	MCB (3d)	VIII	600	600	600	305	99 (KM246030.1)	KP256095
	1OCRY127	MCA (170d)	IX	620	598	619	329	100 (HM107788.1)	KP256098
	1OCRY180	MCA (30d)	X	650	598	600	331	99 (KC510047.1)	KP256099
	1OCRY267	MNB (50d)	XI	670	583	625	325	99 (JX049437.1)	KP256100

Abbreviations: MCA, olive drupes inoculated with *L. pentosus* OM13 and mechanically harvested; MCB, olive drupes uninoculated and mechanically harvested; MNA, olive drupes inoculated with *L. pentosus* OM13 and manually harvested; MNB, olive drupes uninoculated; RP, restriction profile and manually harvested. All values for the 5.8S-ITS PCR and 26S PCR and restriction fragments are given in bp.
[a] According to BlastN search of D1/D2 26S rRNA gene sequences in NCBI database.

Sequencing of the D1/D2 domain of the 26S rRNA gene identified seven species: *Candida boidinii* (groups I–V), *Candida diddensiae* (group VI), *Candida membranifaciens* (group VII) and *Wickerhamomyces anomalus* (groups VIII–XI). *C boidinii* and *W. anomalus* mainly dominated the yeast population in both years. In detail, *C. boindii* was frequently isolated during the fermentations of MCA and MNA. Blastomycetes isolated from MCB and MNB were mainly represented by *W. anomalus* and C. *diddensiae*; *C. membranifaciens* was isolated only at day 6 in trial MNB (year 2012).

Analysis of VOCs and sensory evaluation

The results of VOC analyses carried out on olive samples at day 210 are reported in Table 4 for both years. Acids, alcohols and aldehydes were detected at the highest concentrations in both campaigns. Hydrocarbons reached high concentrations in 2012. Among acids, acetic, hexadecanoic, nonanoic, butanoic and octanoic acids showed the highest values in MNA and MNB; the main compounds within the class of alcohols were phenylethyl alcohol, 1-butanol-3-methyl and benzyl alcohol. *cis*-Hexen-1-ol was estimated at high concentration in MCA and MCB in 2013. Aldehydes were mainly represented by 2-decenal (E), octanal and benzaldehyde; on the other hand, hydrocarbons and phenols were represented by squalene and homoguaiocol, respectively.

Results of the sensory analysis are reported in Table 5. Significant ($P < 0.05$) differences were mainly found among the trials. The main differences were estimated in terms of green olive aroma, complexity (odour), bitterness, complexity and off-flavours (taste). In detail, the MCA and MNA trials showed the highest scores for green colour intensity, green olive aroma and taste complexity, and the lowest scores for bitter and astringent taste. On the other hand, MCB showed values of off-odours and off-flavours significantly higher than the other trials.

Statistical and explorative multivariate analysis

The combined ANOVA over years 2012 and 2013 showed no significant ($P < 0.05$) interactions between years and the dependent variables corresponding to pH values and microbial loads detected on MRS, DRBC and BP media.

HCA classified the trials in accordance with their mutual dissimilarity and relationship using the 18 variables selected on the basis of results from pH determination and microbial analysis (Fig. 2). In both years, replicates of all trials were clearly separated into two mega-clusters (MCA, MNA and MCB, MNB) in accordance with method of fermentation and regardless of harvesting technique. Furthermore, within each mega-cluster, two groups at a low level of dissimilarity were found on the basis of the fermentation method. The lowest level of dissimilarity was estimated among trials MNA and MCA in 2012.

The results obtained by monitoring microbial counts and pH values were also subjected to PCA (Fig. 3). The components of the PCA were correlated with variables as shown in Fig. 3(a) (2012) and Fig. 3(c) (2013), and the corresponding values of factor loadings are reported in Table S1 (supporting information). The discrimination of trials can be visualized in the plot of the scores (Fig. 3b, d). In detail, Fig. 3(b) shows the projection of the cases (representing the three replicates per each trials) on to the planes as a function of factors 1 and 2 (66.96% of the total variance explained), in 2012. The trials were significantly separated along factor 1 on the basis of the fermentation method, regardless of the harvest technique. These results confirmed those obtained by HCA. Furthermore, in 2013, the trials MCA and MNA were closely related to one another also along factor 2 (Fig. 3d).

With regard to VOCs, PCA results are shown in Fig. 4. In order to construct a graphical representation of loading (Fig. 4a, c) and score (Fig. 4b, d) plots, the first two components F1 and F2 were selected. The score plots (Fig. 4b, d) clearly represented the four trials in separated areas of PCA quadrants. The harvest techniques mainly affected the composition of VOCs for the samples in 2013,

Table 4. Concentration of volatile organic compounds (μg kg^{-1}) at the end (day 210) of olive production during both 2012 and 2013 campaigns

	2012 campaign				2013 campaign			
Compound	Trial MCA	Trial MCB	Trial MNA	Trial MNB	Trial MCA	Trial MCB	Trial MNA	Trial MNB
Acetic acid	5448.1 ± 206.0	6145.0 ± 218.9	9530.5 ± 308.9	6747.9 ± 213.4	6374.2 ± 192.1	7742.8 ± 305.8	10907.8 ± 218.3	5305.0 ± 177.3
Butanoic acid	219.8 ± 21.9	239.2 ± 28.0	300.2 ± 22.3	257.1 ± 15.2	258.8 ± 24.0	301.4 ± 35.3	137.9 ± 19.2	20.2 ± 2.6
Heptanoic acid	n.d.	n.d.	n.d.	n.d.	22.6 ± 1.1	19.3 ± 2.9	45.3 ± 6.3	17.3 ± 1.0
Hexadecanoic acid	758.5 ± 75.4	1312.6 ± 153.6	1797.4 ± 133.7	1896.6 ± 112.2	1031.1 ± 110.3	1653.9 ± 193.5	498.1 ± 69.3	176.5 ± 22.5
Hexanoic acid	577.2 ± 129.1	249.6 ± 29.2	268.3 ± 25.5	212.5 ± 13.0	121.5 ± 26.4	100.4 ± 11.7	57.6 ± 8.0	79.7 ± 3.2
2-Ethylhexanoic acid	n.d.	n.d.	n.d.	n.d.	7.9 ± 0.4	10.5 ± 2.3	26.6 ± 3.7	13.2 ± 0.4
Nonanoic acid	1170.1 ± 230.1	651.3 ± 76.2	483.1 ± 46.0	275.0 ± 48.5	160.6 ± 30.5	81.7 ± 9.6	256.3 ± 35.7	94.5 ± 12.1
Octanoic acid	1051.5 ± 206.8	437.8 ± 51.2	408.3 ± 38.9	273.1 ± 21.9	162.9 ± 29.2	54.3 ± 6.4	57.6 ± 8.0	107.5 ± 13.7
Pentanoic acid	475.9 ± 90.5	306.7 ± 35.9	308.3 ± 29.3	197.0 ± 15.8	73.8 ± 13.2	38.7 ± 4.5	59.7 ± 8.3	11.6 ± 1.5
Propionic acid	213.6 ± 11.1	76.3 ± 8.9	180.6 ± 23.5	51.1 ± 4.1	242.8 ± 50.6	96.2 ± 11.2	151.6 ± 21.1	94.8 ± 12.1
1,4-Butanediol	38.6 ± 7.2	72.1 ± 10.0	55.6 ± 5.9	53.5 ± 10.3	61.6 ± 4.8	90.9 ± 12.5	n.d.	12.0 ± 1.0
1-Butanol-3-methyl	295.7 ± 49.8	477.4 ± 65.9	686.2 ± 72.3	1506.2 ± 458.7	399.0 ± 47.5	601.5 ± 83.1	358.3 ± 25.0	286.3 ± 23.9
1-Hexanol	108.3 ± 18.2	132.2 ± 16.0	131.3 ± 13.8	132.8 ± 23.4	172.0 ± 47.6	166.6 ± 20.2	145.7 ± 10.2	203.6 ± 13.4
1-Octanol	851.8 ± 143.4	201.6 ± 27.8	289.9 ± 16.2	168.0 ± 26.9	1149.3 ± 136.9	254.0 ± 35.1	776.7 ± 54.2	143.0 ± 7.5
2-Nonen-1-ol	762.1 ± 31.5	374.3 ± 51.7	423.5 ± 23.7	350.8 ± 56.2	195.3 ± 14.0	471.7 ± 65.2	25.7 ± 1.8	40.9 ± 1.7
Benzyl alcohol	602.2 ± 24.9	493.4 ± 68.2	672.6 ± 32.3	150.7 ± 24.1	643.3 ± 46.3	621.7 ± 85.9	707.9 ± 49.4	648.4 ± 38.4
cis-Hexen-1-ol	728.2 ± 14.5	575.6 ± 79.5	326.8 ± 15.7	32.9 ± 2.2	759.8 ± 54.7	725.2 ± 21.2	493.8 ± 38.7	345.2 ± 20.4
Phenylethyl alcohol	1001.4 ± 252.0	1374.6 ± 189.9	1998.7 ± 96.1	1824.0 ± 321.6	1783.5 ± 432.3	1732.0 ± 239.3	1411.6 ± 164.46	945.3 ± 57.0
2-Butenal-2-methyl	39.1 ± 1.5	45.0 ± 2.0	49.7 ± 2.4	58.1 ± 1.3	38.2 ± 1.7	42.3 ± 1.8	29.8 ± 1.6	17.1 ± 1.1
2-Decenal (E)	1672.8 ± 66.1	351.3 ± 15.3	832.8 ± 115.9	387.2 ± 21.8	1634.7 ± 24.4	330.2 ± 14.4	1620.9 ± 148.4	125.3 ± 8.2
Benzaldehyde	85.4 ± 3.4	579.5 ± 25.3	345.7 ± 19.3	304.6 ± 51.4	83.4 ± 10.4	544.7 ± 23.8	116.5 ± 19.3	372.0 ± 59.6
Benzaldehyde-2,5-dimethyl	85.4 ± 3.4	579.5 ± 25.3	345.7 ± 19.3	304.6 ± 61.3	19.9 ± 2.3	54.6 ± 2.4	14.5 ± 2.4	34.7 ± 1.0
Benzaldehyde-3-ethyl	n.d.	43.1 ± 1.9	21.8 ± 1.4	46.2 ± 2.6	20.7 ± 2.7	40.6 ± 1.8	20.8 ± 3.4	23.2 ± 0.5
Nonanal	201.1 ± 7.9	207.0 ± 9.0	272.2 ± 18.1	389.0 ± 21.9	196.6 ± 2.7	194.6 ± 4.8	181.1 ± 13.0	196.3 ± 3.8
Octanal	701.8 ± 12.3	169.1 ± 7.4	229.3 ± 33.9	100.7 ± 2.3	708.9 ± 14.1	158.9 ± 6.9	285.4 ± 5.9	1044.3 ± 20.5
Phenylacetaldehyde	29.6 ± 0.5	71.5 ± 3.1	72.4 ± 4.0	89.1 ± 2.0	41.1 ± 1.1	19.2 ± 1.0	39.7 ± 1.9	60.9 ± 1.2
Vanillin	146.8 ± 21.0	115.4 ± 5.0	n.d.	n.d.	208.6 ± 5.3	108.4 ± 4.7	136.6 ± 2.3	76.3 ± 1.5
2-Nonanone	267.1 ± 4.7	167.9 ± 5.9	105.7 ± 5.5	76.5 ± 1.5	269.8 ± 7.4	157.8 ± 5.6	46.3 ± 0.8	n.d.
3-Hydroxybutanone	n.d.	n.d.	151.4 ± 7.8	123.6 ± 2.4	18.5 ± 2.1	21.8 ± 0.8	33.8 ± 0.6	29.7 ± 1.6
4-Ethylacetophenone	n.d.	n.d.	n.d.	n.d.	7.9 ± 0.9	n.d.	11.2 ± 0.9	54.9 ± 2.9
4-Methyldihydro-2-(3*H*)-furanone	n.d.	n.d.	n.d.	n.d.	n.d.	5.7 ± 0.8	107.7 ± 8.4	84.8 ± 4.6
Butyrolactone	345.6 ± 20.6	415.7 ± 57.4	n.d.	n.d.	97.3 ± 3.0	90.4 ± 2.5	43.1 ± 3.4	51.7 ± 2.8
Cyclopentanone	n.d.	n.d.	345.1 ± 17.9	233.4 ± 4.6	75.6 ± 2.1	22.1 ± 1.1	99.5 ± 1.7	94.9 ± 5.0
Benzyl acetate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	64.5 ± 7.4	68.2 ± 1.6
cis-3-Hexenylacetate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	35.3 ± 1.8	35.0 ± 1.9
Ethyl dihydrocinnamate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	30.9 ± 0.9	n.d.
Ethyl lactate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	37.7 ± 1.7	16.3 ± 2.7
Methyl hexadecanoate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	266.7 ± 47.1	133.2 ± 22.4
Methyl hydrocinnamate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	15.5 ± 2.7	91.3 ± 15.4
Methyl salicylate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	44.9 ± 7.9	31.5 ± 1.3
Octyl acetate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	95.4 ± 16.8	87.0 ± 3.9
4-Ethylphenol	n.d.	n.d.	n.d.	n.d.	87.7 ± 1.7	n.d.	212.9 ± 16.6	224.5 ± 35.8
Guaiacol	242.2 ± 2.7	3901.8 ± 539.0	458.4 ± 23.7	7161.8 ± 296.4	242.2 ± 2.4	491.5 ± 7.9	141.9 ± 11.1	149.8 ± 5.1
Homoguaiacol	1850.7 ± 219.0	874.8 ± 120.8	2601.0 ± 145.3	n.d.	2257.9 ± 267.2	1102.3 ± 152.3	1775.3 ± 138.6	1773.8 ± 223.2
Phenol	642.2 ± 37.1	758.8 ± 14.8	374.5 ± 18.0	811.7 ± 33.6	115.3 ± 10.7	956.2 ± 12.1	51.5 ± 4.0	57.3 ± 3.1
α-Terpineol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	28.9 ± 5.1	26.2 ± 1.3
Squalene	1185.0 ± 110.1	11820.4 ± 1032.9	3382.5 ± 162.7	9779.8 ± 404.7	1386.4 ± 128.8	2018.6 ± 178.8	n.d.	n.d.
Styrene	n.d.	94.2 ± 13.0	114.0 ± 5.5	181.5 ± 7.5	n.d.	118.7 ± 16.4	n.d.	n.d.
α-Cubebene	107.6 ± 6.0	148.0 ± 8.0	132.9 ± 6.4	56.3 ± 4.8	122.2 ± 6.6	162.7 ± 8.8	39.5 ± 4.5	78.8 ± 2.5

Abbreviations: MCA, olive drupes inoculated with *L. pentosus* OM13 and mechanically harvested; MCB, olive drupes uninoculated and mechanically harvested; MNA, olive drupes inoculated with *L. pentosus* OM13 and manually harvested; MNB, olive drupes uninoculated and manually harvested. Results indicate mean values ± standard deviation of three replicate; n.d., not detected (value less than detection limit of method).

Table 5. Sensory scores of olives collected at the end (day 210) of the manufacturing process during both the 2012 and 2013 campaigns

	2012 campaign				2013 campaign			
Descriptors	Trial MCA	Trial MCB	Trial MNA	Trial MNB	Trial MCA	Trial MCB	Trial MNA	Trial MNB
Aspect								
Green colour intensity	6.2 ± 0.2b	5.3 ± 0.4a	6.4 ± 0.1b	5.9 ± 0.6b	6.3 ± 0.2b	5.2 ± 0.2a	6.6 ± 0.3b	5.8 ± 0.3a
Brightness	5.7 ± 0.2a	5.5 ± 0.4a	5.8 ± 0.2a	5.5 ± 0.1a	5.6 ± 0.4a	5.1 ± 0.1a	5.8 ± 0.2a	5.5 ± 0.3a
Odour								
Green olive aroma	7.3 ± 0.3c	5.5 ± 0.3a	7.4 ± 0.4c	6.4 ± 0.3b	7.5 ± 0.4b	5.5 ± 0.4a	7.4 ± 0.4b	6.6 ± 0.2b
Complexity	6.3 ± 0.4c	4.4 ± 0.3a	6.5 ± 0.3c	5.5 ± 0.4b	5.7 ± 1.7b	4.7 ± 0.3a	7.0 ± 0.2c	5.3 ± 0.3b
Off-odours	1.1 ± 0.2a	3.4 ± 0.4c	1.1 ± 0.1a	2.2 ± 0.1b	1.1 ± 0.2a	3.3 ± 0.3c	1.0 ± 0.2a	2.2 ± 0.2b
Taste								
Crispness	5.2 ± 0.1a	5.2 ± 0.1a	4.8 ± 0.1a	4.9 ± 0.1a	5.2 ± 0.1a	5.2 ± 0.2a	4.9 ± 0.2a	4.8 ± 0.1a
Easy stone	3.8 ± 0.1a	4.7 ± 0.1a	3.9 ± 0.1a	4.3 ± 0.3a	3.9 ± 0.1b	2.4 ± 0.2a	4.2 ± 0.1b	3.5 ± 0.1b
Juicy	5.5 ± 0.4b	3.5 ± 0.4a	5.3 ± 0.1b	4.6 ± 0.4a	5.5 ± 0.4c	3.5 ± 0.3a	5.4 ± 0.2c	4.6 ± 0.4b
Sweet	2.6 ± 0.4a	2.1 ± 0.3a	2.9 ± 0.2a	2.5 ± 0.2a	2.7 ± 0.4b	2.1 ± 0.3a	2.9 ± 0.2b	2.5 ± 0.2b
Sour	3.6 ± 0.4a	4.4 ± 0.3b	3.8 ± 0.2a	4.3 ± 0.4b	3.5 ± 0.3a	4.2 ± 0.1b	4.0 ± 0.1a	4.3 ± 0.4b
Bitter	2.8 ± 0.4b	5.0 ± 0.2c	2.2 ± 0.1a	2.9 ± 0.2b	3.4 ± 0.2a	5.4 ± 0.3b	3.2 ± 0.1a	4.2 ± 0.0b
Salt	3.4 ± 0.3b	3.9 ± 0.2b	2.5 ± 0.2a	3.7 ± 0.0b	3.2 ± 0.1a	4.2 ± 0.1b	3.4 ± 0.1a	4.3 ± 0.0b
Complexity	5.8 ± 0.3b	4.0 ± 0.2a	6.6 ± 0.2c	5.7 ± 0.2b	5.0 ± 0.0c	3.2 ± 0.1a	5.2 ± 0.0c	4.4 ± 0.1b
Astringent	2.2 ± 0.2a	3.8 ± 0.1b	2.4 ± 0.1a	3.4 ± 0.1b	3.1 ± 0.0a	4.1 ± 0.0b	3.1 ± 0.0a	3.6 ± 0.2a
Off-flavours	1.2 ± 0.1a	3.9 ± 0.1c	1.3 ± 0.0a	3.3 ± 0.2b	1.1 ± 0.1b	2.7 ± 0.2d	0.9 ± 0.1a	2.1 ± 0.1c

Abbreviations: MCA, olive drupes inoculated with *L. pentosus* OM13 and mechanically harvested; MCB, olive drupes uninoculated and mechanically harvested; MNA, olive drupes inoculated with *L. pentosus* OM13 and manually harvested; MNB, olive drupes uninoculated and manually harvested. Different letters (a–d) indicate significant differences between experimental trials for the same sample for $P \leq 0.05$.

when trials were significantly separated along the F1 component. The correlation of VOC variables with the main factors of PCA are reported in Tables S2 and S3 for 2012 and 2013, respectively.

The multivariate statistical analysis was concluded by using data of sensory analysis of olives. Biplot graphical representations were constructed as illustrated in Fig. 5(a, b). The four trials resulting mainly separated along the F1 component. Trials MCB and MNB were closely associated with bitter, astringent, salt and off-odour descriptors, in both years. Conversely, MCA and MNA showed the closest correlation with green olive aroma and complexity (odour and taste).

Barlett's sphericity test was applied to all data matrix inputs, and statistically significant differences ($P < 0.0001$) were found among trials.

DISCUSSION

The aim of the present study was to evaluate the effect of different harvesting methodologies and processing conditions on the quality of fermented table olives of the cultivar Nocellara del Belice. To our knowledge, no study has so far been carried out to evaluate the effects of the mechanical harvesting of drupes on the final characteristics of table olives. Limited information has been published on the rheological characteristics of drupes just after mechanical harvesting,[48–50] but no data are available on the microbial and chemical features of the product during fermentation.

The results showed that the mechanical harvesting of drupes subjected to the driven fermentation by *L. pentosus* OM13 as starter culture did not affect negatively the microbial characteristics of the transformation process. However, besides LAB and yeasts, *Enterobacteriaceae* were also found. These microorganisms, at high concentrations, might spoil the organoleptic quality and safety of table olives acting as alterative and/or pathogenic microorganisms.[51,52] Many species belonging to the pseudomonad group are commonly identified as food spoilage bacteria, but also as human pathogens. *Pseudomonas* spp. can be characterized by high levels of proteolitic, lipolitic and pectinolitic activities[53,54] damaging the final product.

The inoculation of LAB starter into the brines of MCA and MNA significantly reduced the growth of potential spoilage and/or pathogenic microorganisms. The use of selected LAB strains as starters determines a rapid acidification of the product and inhibits the growth of potential spoilage populations.[55] In this work, both trials inoculated with the starter culture showed a significant decrease of pH within the sixth day of fermentation, a trend also registered by other authors.[56] From day 70 onward, and in particular for year 2012, pH values significantly increased. Although LAB clearly dominated the microbial population of the inoculated trials and the production of lactic acid reduced the pH, yeast concentration was estimated at detectable levels until the end of the process. Yeasts might oxidize lactic acid and the increase of pH might be significant.[57] Interestingly, the combined analysis of variance of data showed no significant interactions between years and dependent variables, especially for pH values and LAB concentrations. These two parameters are defining for the chemical and sensory quality of the final product because they might inhibit significantly the growth of spoilage/pathogenic microorganisms.

Several LAB species might have different influence on the quality of table olives, but the species commonly associated with this production process are *L. pentosus* and *L. plantarum*.[16,22,58] In our study, *L. pentosus* was the main LAB species found in all trials during both years. This species is commonly recognized as one of the most technologically relevant LAB owing to its high aptitude to ferment olives. For this reason, its dominance during table olive production might represent a guarantee of quality of the final in terms of microbiological and chemical characteristics.

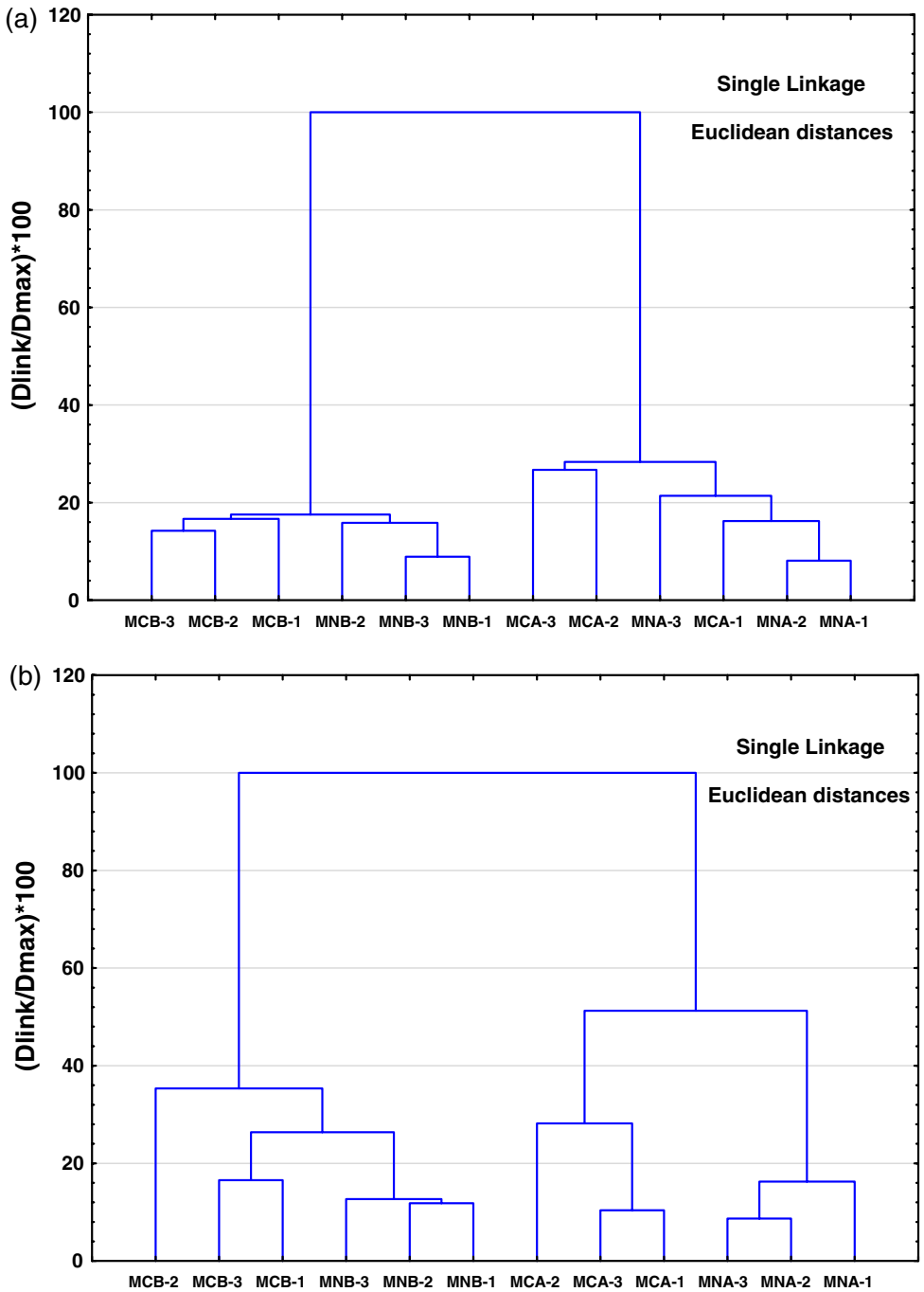

Figure 2. Dendrogram of brine samples resulting from HCA analysis based on values of microbial and pH changes during the 2012 (a) and 2013 (b) campaigns. The dissimilarity among samples was measured by Euclidean distance, whereas cluster aggregation was achieved by single linkage. Abbreviations: MCA, MCB, MNA and MNB correspond to codes used for the experimental trials. The numbers associated with codes correspond to replicates per each experimental trials.

Several autochthonous strains belonging to *L. pentosus* were found during fermentation even in presence of the commercial starter in both years. The presence of several *L. pentosus* strains during olive transformation is reported to improve the complexity of the sensory profile of the final product.[22,42]

The yeast species isolated in this work are commonly associated with the table olive environment, mostly with the fermentation of green table olives.[59,60] In particular, the species *W. anomalus* mainly dominated the yeast population during the entire production of MNA and MCB olives. Some previous studies[25,61] reported that table olive fermentation might be carried out by yeasts. Yeasts are relevant in directly brined green and black natural olive fermentations, where fruits are not treated with sodium hydroxide and LAB are partially inhibited due to the presence of phenolic compounds.[30] However, an overgrowth of fermentative yeasts could induce a high production of CO_2 that could damage drupe texture.[62]

Thus LAB and yeasts in table olives have an essential role in olive preservation. It has been clearly shown in other works on table olives[55] and/or in other food production[63,64] that, although

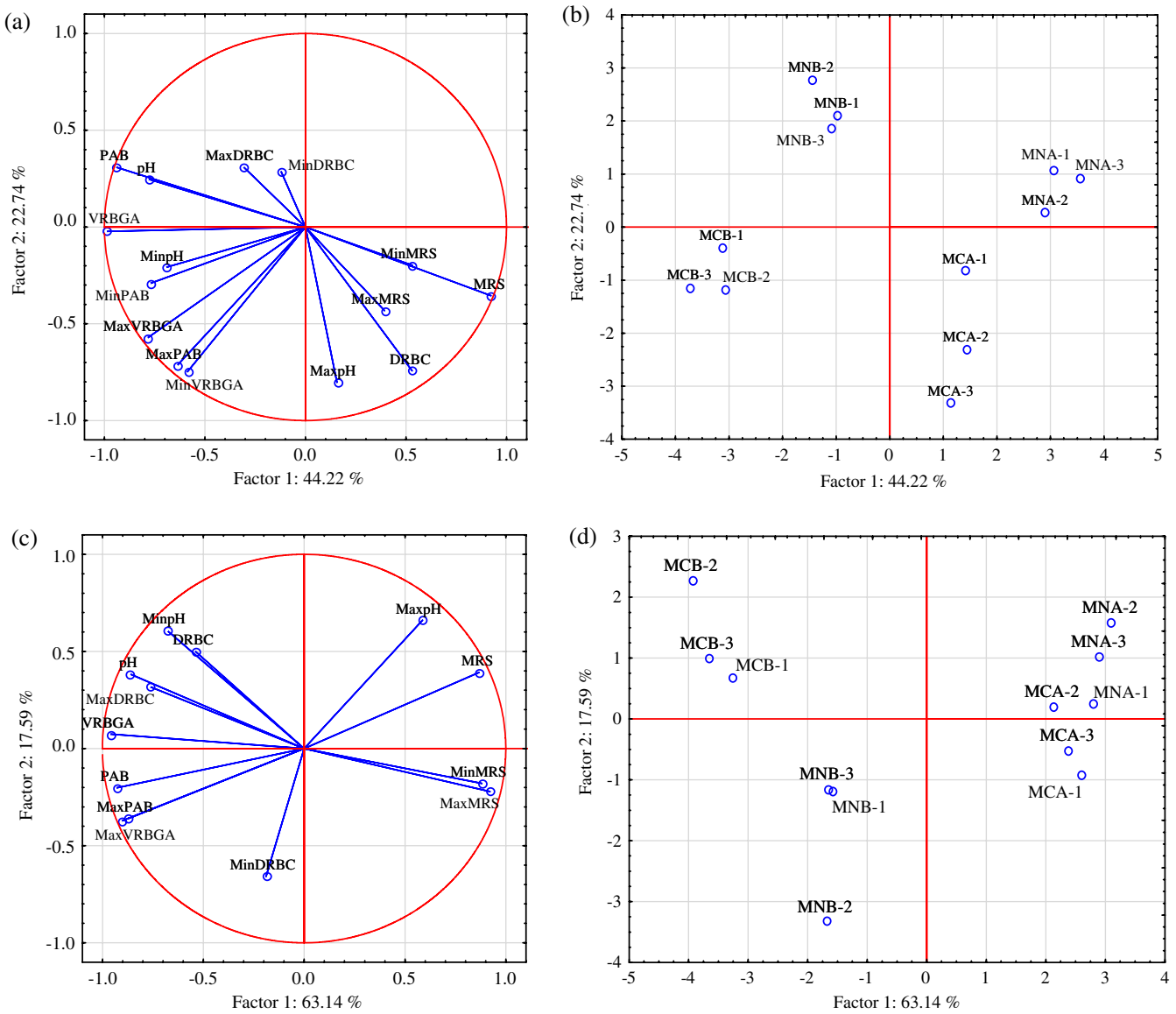

Figure 3. PCA analysis based on the values of microbial and pH changes estimated during the 2012 (a: loading plot; b: score plot) and 2013 (c: loading plot; d: score plot) campaigns. Abbreviations: MRS, Man–Rogosa–Sharpe agar for mesophilic rod LAB; DRBC, dichloran rose bengal chloramphenicol agar for total yeasts and filamentous fungi; VRBGA, violet red bile glucose agar for *Enterobacteriaceae*; PAB, *Pseudomonas* agar base for pseudomonads. 'Max' and 'Min' correspond to the maximum and minimum values of pH and microbial populations detected on the corresponding medium used for counts. The codes (MCA, MCB, MNA and MNA) correspond to the experimental trials and the numbers associated with codes correspond to replicates per trial.

at very low concentrations, both yeasts and LAB could affect the chemical composition, such as pH and concentration of VOCs, of many foods.

In this regard, high concentrations of acids, such as acetic and nonanoic acids as well as 1-octanol and 2-decenal (E) were found, in this work, in olives inoculated with starter. High concentrations of these compounds indicate alcoholic and heterolactic fermentation.[65,66] Experimental olives also showed many alcohols at high concentrations; the presence of *cis*-hexen-1-ol and 1-hexanol in table olives is commonly associated with herbaceous flavours – a pleasant sensory descriptor of many fruit and vegetable fermented foods.

Multivariate data analysis has been widely applied in food processes,[67] including table olive production.[41,43,68,69] HCA visibly discriminated trials on the basis of type of fermentation. However, as shown in the dendrograms, low dissimilarity was found among samples collected from the mass of drupes harvested manually or mechanically. The areas and values of both pH and microbial groups of microorganisms included in the study were proven to be useful variables to discriminate samples. HCA is an unsupervised method that recognizes and distributes data, according to their affinity, in clusters of progressive dissimilarity. In detail, the HCA is a graphical representation of a matrix of distances such as the dendrogram where the objects (trials) are joined together in a hierarchical ascendant analysis from the closest one – i.e. the most similar – to the furthest apart – i.e. the most different.

Since the correlation analysis among variables showed that there were many significant relationships among them, data were subjected to PCA in order to condense the information into a reduced number of factors. Both multivariate statistical approaches (HCA and PCA) showed that the olives produced according to the same type of fermentation were closely related regardless of the type

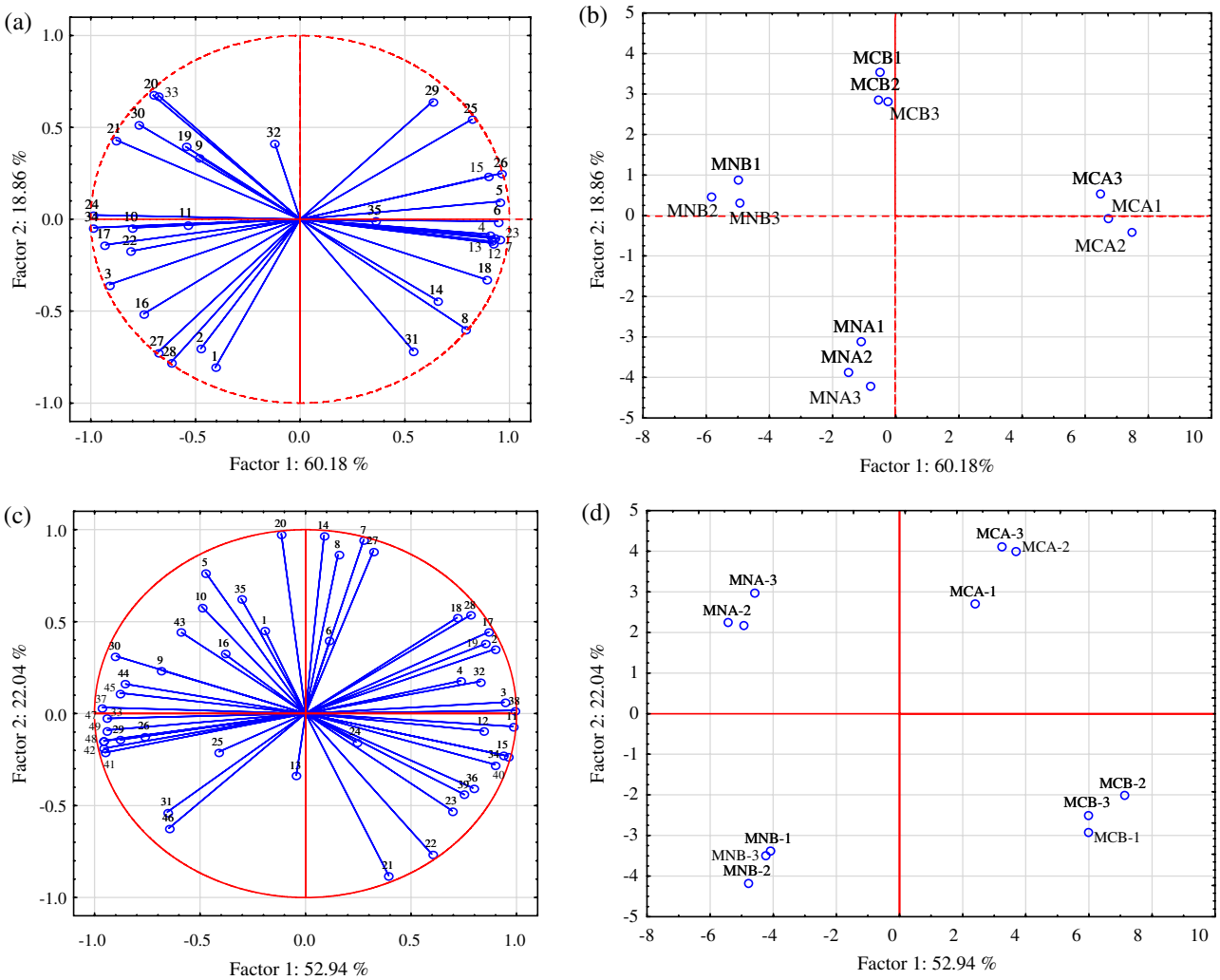

Figure 4. PCA analysis based on the values of VOCs of samples collected at the end (day 210) of olive production in 2012 (a: loading plot; b: score plot) and 2013 (c: loading plot; d: score plot) campaigns. Numbers in the loading plots: 1, acetic acid; 2, butanoic acid; 3, hexadecanoic acid; 4, hexanoic 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, vanillin; 26, 2-nonanone; 27, 3-hydroxybutanone; 28, cyclopentanone; 29, butyrolactone; 30, guaiacol; 31, homoguaiacol; 32, phenol; 33, squalene; 34, styrene; 35, α-cubebene for Fig. 2(a); 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, 2-ethylhexanoic acid; 10, heptanoic acid; 11, 1,4-butanediol; 12, 1-butanol-3-methyl; 13, 1-hexanol; 14, 1-octanol; 15, 2-nonen-1-ol; 16, benzyl alcohol; 17, *cis*-hexen-1-ol; 18, phenylethyl alcohol; 19, 2-butenal-2-methyl; 20, 2-decenal (E); 21, benzaldehyde; 22, benzaldehyde-2.5-dimethyl; 23, benzaldehyde-3-ethyl; 24, nonanal; 25, octanal; 26, phenylacetaldehyde; 27, vanillin; 28, 2-nonanone; 29, 3-hydroxybutanone; 30, cyclopentanone; 31, 4-ethylacetophenone; 32, butyrolactone; 33, 4-methyldihydro-2-(3*H*)-furanone; 34, guaiacol; 35, homoguaiacol; 36, phenol; 37, 4-ethylphenol; 38, squalene; 39, styrene; 40, α-cubebene; 41, benzyl acetate; 42, *cis*-3-hexenylacetate; 43, ethyl dihydrocinnamate; 44, ethyl lactate; 45, methyl hexadecanoate; 46, methyl hydrocinnamate; 47, methyl salicylate; 48, octyl acetate; 49, α – terpineol for the Fig. 2(c). Abbreviations: the codes (MCA, MCB, MNA and MNA) correspond to the experimental trials and the numbers associated with codes correspond to replicates per each trial.

of harvesting. Differences in terms of VOC composition estimated among trials might be explained on the basis of variability at strain level found within the *L. pentosus* populations. On the whole, results found by PCA were in agreement with those obtained by clustering analysis based on pH and microbiological data.

CONCLUSIONS

Our study provides an overview of the microbial ecology and chemical parameters of fermented table olives produced with drupes subjected to mechanical harvesting performed using a trunk shaker equipped with an inverse umbrella. Interestingly, statistical analysis of the data showed that, independent of the year of harvest, the addition of starter ensured a rapid decrease in pH and high concentrations of LAB. In addition, the experimental protocol reduced the growth of spoilage microorganisms during the entire process. The scores associated with undesired off-odours and off-flavours were superimposable on that estimated for the controls performed with manual harvesting. Thus mechanical harvesting performed using the trunk shaker equipped with an inverse umbrella and the addition of starter cultures represents a valuable alternative to manual harvesting for table olive production.

Further investigations will be carried out on other olive varieties for a more thorough validation of this harvest method for table olive production.

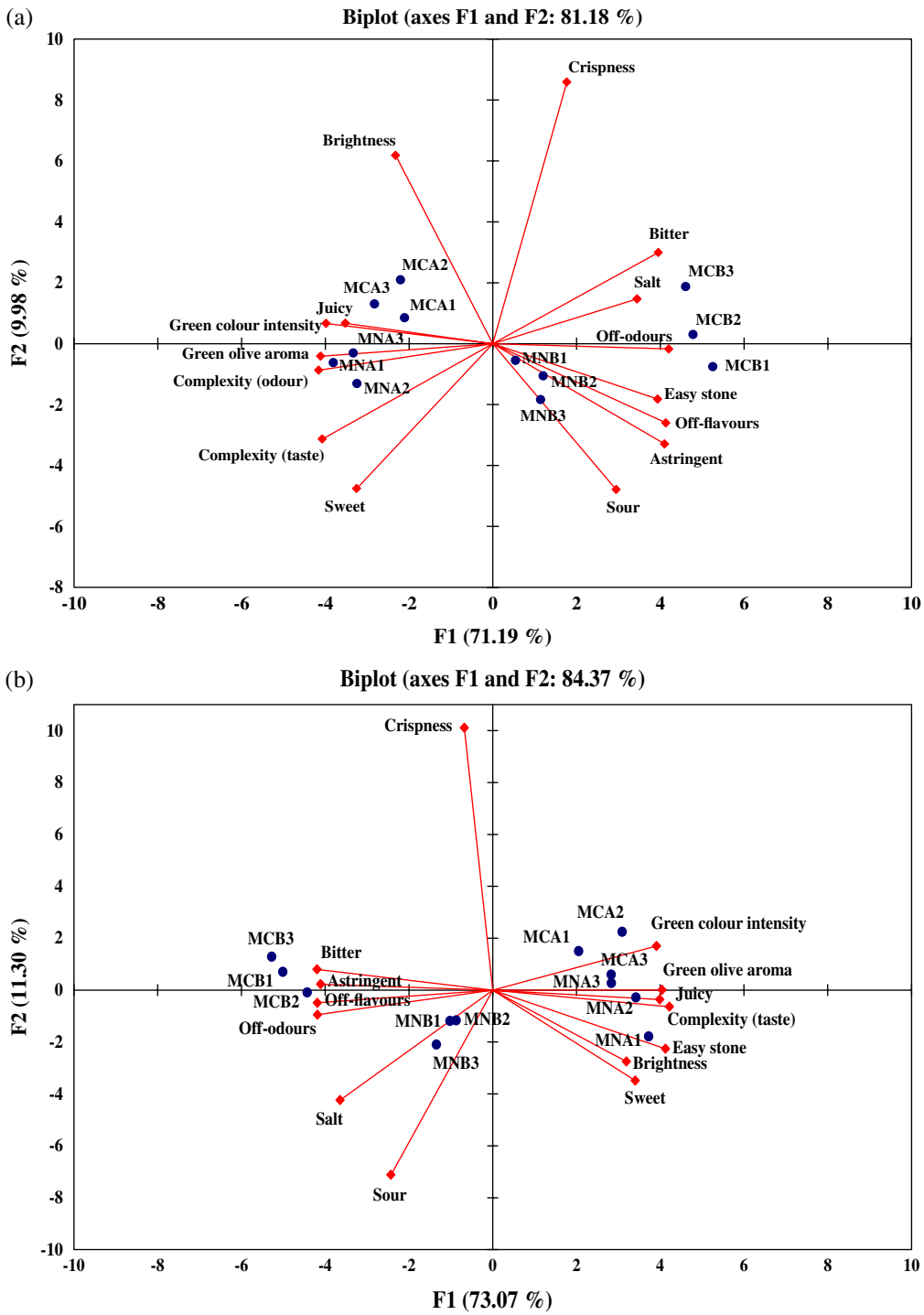

Figure 5. PCA for sensory data of olives at the end of the process (day 210) during the 2012 (a) and 2013 (b) campaigns. Biplot graphs show relationships among factors, variables and treatments. Abbreviations: the codes (MCA, MCB, MNA and MNA) correspond to the experimental trials and the numbers associated with codes correspond to replicates per each trial.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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