



## Study of green Sicilian table olive fermentations through microbiological, chemical and sensory analyses

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### ABSTRACT

The production of five different green table olive cultivars was studied by a combined strategy consisting of chemical, microbiological and sensory analyses. Cultivable microflora of samples collected during processing was monitored by plate counts on seven synthetic culture media. In all samples Enterobacteriaceae, Pseudomonaceae, staphylococci, lactic acid bacteria and spore-forming bacteria were undetectable. Yeasts and moulds were countable from the day 42 (2 log CFU/ml) till the end of fermentation (6 log CFU/ml). The use of three different approaches for microorganism detection, including a culture-independent methodology, revealed the presence of barely three yeast species during the entire fermentation period: *Candida parapsilosis*, *Pichia guilliermondii* and *Pichia kluyveri*. Biochemical features of technological interest were evaluated for 94 strains in order to investigate their potential role in fermentation of green Sicilian table olives.

Olive drupes sampled at picking and periodically during fermentation were also carpologically analyzed, revealing that all the cultivars were suitable for table olive fermentation process. After 120 days of fermentation all products met acceptable commercial standards, although GC–MS analysis evidenced several differences among varieties in terms of aroma components. Results from sensory evaluation led to the conclusion that a revision of technological procedures may improve the final quality of product.

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### 1. Introduction

Fermentation techniques associated to animal products have become increasingly sophisticated, whereas fermentation of vegetables, particularly olives, remains craft-based and empirical. This is surprising as the economic importance of olives worldwide is indisputable: almost two million tons every year, 38% of which is produced in Spain, Italy and Greece (IOOC, 2008).

The unified qualitative standard applying to table olives in international trade defines table olives as “the sound fruit of specific varieties of cultivated olive tree (*Olea europaea sativa*, Hoffm., Link), harvested at the proper stage of ripeness and [...] processed as specified in this standard. Such processing may include the addition of various products or spices of good quality” (IOOC, 2004). The main purpose of processing is, at least, the

removal of fruit bitterness by hydrolysis of the phenolic compounds, especially oleuropein. Fernandez-Diez (1983) reported that the table olive preparations of the greatest importance in the world are: (i) Spanish- (or Sevillian-) style green olives in brine; (ii) Californian-style black ripe olives and (iii) Greek-style, natural black olives in brine. In the first process, bitterness is removed by adding lye; in the Greek and Californian style processes, fruits are placed directly in brine and an acidified solution, respectively, and oleuropein removal is slow and only partial (Garrido-Fernández, 1997). The fermentation of Spanish-style treated olives is due to lactic acid bacteria (LAB), while in Greek and Californian processed black olives the organisms responsible for fermentation are yeasts, and LAB represent a small proportion of the total microflora (Garrido-Fernández, 1997). However, there are many other traditional table olive elaboration recipes that are less known in the international market (Panagou et al., 2003) and constitute niche products. In Sicily (Southern Italy), table olives are often processed without LAB starter cultures and by the addition of lactic acid in order to minimize the Gram negative bacterial population and to selectively

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promote the growth of certain wild LAB. After harvesting, green olives are transported to the factory, sorted to separate damaged fruits, washed to remove superficial dirt and finally brined in a 6–10% salt solution. Unlike 'Spanish-style' green olives, they do not undergo preliminary treatment in sodium hydroxide (lye) before fermentation. Thus, the debittering mechanism applied in this process consists in dilution and subsequent hydrolysis of oleuropein. Washing waters remove a significant proportion of olive polyphenols, mainly hydroxytyrosol glucoside and oleuropein (Arroyo-López et al., 2007). In these conditions relatively high temperatures must be considered essential for the success of fermentation. Tassou et al. (2002) proved that fermentation conducted at less than 18 °C in Greek-style black olives led to products with a detectable bitter taste making the product unfit for consumption.

The traditional production areas of table olives in Sicily generally enjoy good climatic conditions during fermentation. However, in most processing plants, fermenters are exposed to the open air and, hence, the fermentation temperature follows environmental fluctuations. This is particularly important in the winter where low temperatures may prevail determining delay of microbial activity. Despite the apparent beneficial effects of temperature on table olive fermentation, temperature control is a complex and expensive procedure not generally applicable within current industrial technology of Sicily, since a low production cost is essential for a successful market competition. Consequently, sodium chloride concentration and pH of the brine are the main control parameters acting during fermentation (Garrido-Fernández et al., 1997).

In light of the above, the aim of the present work was to evaluate the physico-chemical and microbiological changes during a particular type of green seasoned table olives fermentation in Sicily: i) without previous lye treatment of olives; ii) brine supplemented at the onset of fermentation with lactic acid; iii) fermentation carried out at room temperature; iv) without starter adding. Since table olive quality is closely related to cultivar characteristics, (Montaño et al., 2003; Poiana and Romeo, 2007) the fermentations of four different Sicilian cultivars and a Spanish green olive, were monitored from a chemical, microbiological and sensorial point of view.

## 2. Methods

### 2.1. Plant material

In October 2006, olive from four green Sicilian table olive cultivars (Brandofino, Castriciana, Nocellara del Belice, and Passalunara) and the Spanish Manzanilla were collected at a maturity stage suitable for processing. Fruits were harvested at green stage from 10-year-old trees trained to vase spaced 5 × 5 m, immediately transported to the manufacturer, and selected for brining.

### 2.2. Olive processing

After washing with tap water, 16 kg of drupes of each cultivar were put into 40-litre glass containers filled with freshly prepared 8% NaCl brine and acidified with 1 ml/l lactic acid (95%) (Sigma, Milan, Italy). The fermentation of each cultivar was performed in triplicate: three lots of olives coming from different plots of land. Fermentations took place on the same days in the same conditions. Room temperature was daily registered.

### 2.3. Carpological and chemical analysis

Drupe quality evolution was monitored by carpological (weight, pulp-pit ratio, diameter, height) and chemical (soluble solid content, pH and titratable acidity) analysis. The olives

(30 fruits randomly sampled from the entire amount) were analyzed in three different stages: at harvest and after 60 and 120 days in brine. Soluble solid content was measured by using an Atago Palette PR-32 digital refractometer (Atago Co., Ltd., Tokyo, Japan); titratable acidity (expressed as g/l of oleic acid) was determined with a Crison S compact titrator, (Crison Instruments, SA, Alella, Barcelona, Spain).

### 2.4. Microbiological analysis

Microbiological analyses were performed on olives and on olives and brines at 2 days and then weekly up to 84 days of fermentation. For each sample aliquots of 10 g of olives and 25 ml of their brines were diluted with 0.9% NaCl solution (315 ml), homogenized with a Stomacher Lab-Blender 400 (Seward Medical, London, United Kingdom) for 2 min, serially diluted and plated in triplicate for both microbial enumeration and isolation. The media and the conditions were as follows: Plate Count Agar (PCA) incubated at 30 °C for 48 h for mesophilic bacteria as well as for spore-forming bacteria after treatment of cell suspensions at 80 °C for 10 min; MRS agar with 0.17 g/l of cycloheximide (Sigma), incubated at 30 °C for 4 days in anaerobiosis (Anaerogen kit, Oxoid, Basingstoke, United Kingdom) for LAB; Violet Red Bile Glucose Agar (VRBGA), incubated at 37 °C for 18–24 h for Enterobacteriaceae; Baird-Parker agar base, with Egg Yolk Tellurite Emulsion, incubated at 37 °C for 48 h for staphylococci; Dichloran Rose-Bengal Chloramphenicol agar base (DRBC) with the addition of Chloramphenicol Selective Supplement incubated at 25 °C for 3–4 days for yeasts and moulds; *Pseudomonas* agar base, with CFC Supplement, incubated at 30 °C for 48 h for *Pseudomonas* spp. All the media and the supplements used were provided by Oxoid.

### 2.5. Isolation and identification of microorganisms

A total of 94 colonies were randomly picked from DRBC agar plates seeded with the highest sample dilutions to analyze yeast populations. Each colony was purified by repeated streaking on Malt Extract agar (Oxoid), and incubation at 25 °C for 48 h. All isolates were stored at 4 °C on Malt Extract agar (Oxoid) slants.

DNAs from pure cultures were extracted according to a standard procedure. Briefly, cultures in stationary phase were centrifuged at 29 000 × g for 5 min. Cellular pellets were washed twice in STE (NaCl, Tris-HCl, EDTA pH 7.5), resuspended in ET (EDTA, Tris-HCl), added of 0.5 U/μl of Lyticase (Sigma) and incubated for 16 h at 37 °C. After incubation, 40 μl of Triton X-100 (15% water solution) and 1 vol. of ammonium acetate 5 mol/l were added to the sample that was then centrifuged at 17 000 × g for 5 min at 4 °C. The supernatant was precipitated with 1 vol. of isopropanol and centrifuged at 29 000 × g for 5 min. Finally, pellets were dried and resuspended in 100 μl of DNA Rehydration Solution (Promega, Madison, WI, USA) by incubation at 55 °C for 60 min.

The isolated yeasts were characterized by the PCR-RFLP (Restriction Fragment Length Polymorphism) method developed by Esteve-Zarzoso et al. (1999). PCR was performed according to the authors by using primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). PCR conditions were as follows: initial denaturation at 95 °C for 5 min and 40 cycles of denaturation at 95 °C for 1 min, primer annealing at 58 °C for 2 min and DNA extension at 72 °C for 3 min. A final extension was completed at 72 °C for 10 min. Amplicons were run on a 1% agarose gel (wt/vol) (Invitrogen, Cergy-Pontoise, France) to evaluate their quality and size. For RFLP analysis, 20 μl aliquots of PCR products were digested for 3h at 37 °C by the restriction endonucleases *Cfo* I, *Hinf* I and *Hae* III (Promega). The restriction fragments were separated on 2% agarose gels and the fragment sizes were evaluated for

comparison with the 1 Kb Ladder plus (Invitrogen) by using Quantity One (Gel Doc EQ, Bio-Rad, Marnes-la-Coquette, France).

Representative strains of each PCR-RFLP profile were identified at the species level by sequencing of the D1/D2 region of the 26S rRNA gene. PCR mix was prepared as reported above except for the primers used that were NL-1 (5'-GCATATCAATAAGCGGAGGAAAAG-3') and NL-4 (5'-GGTCCGTGTTCAAGACGG-3') (Kurtzman and Robnett, 1997). PCR conditions were as follows: initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 1 min, primer annealing at 52 °C for 45 s and DNA extension at 72 °C for 1 min. A final extension was completed at 72 °C for 7 min.

The amplified fragments were purified from agarose gel 1.5% (wt/vol) by Qiaquick Gel Extraction Kit (Qiagen, Milan, Italy) according to the supplier's instructions. The DNA sequences were determined by the dideoxy chain termination method (Sanger et al., 1977) by using the forward primers (NL-4) described by Kurtzman and Robnett (1997). Research for DNA similarity was performed with the National Centre of Biotechnology Information GenBank (Altschul et al., 1997).

## 2.6. PCR amplification of the yeast community ITS rDNA

DNA was directly extracted from brine samples as well as from the bulk of cells obtained by suspending all the colonies developed on the DRBC agar plate surfaces in a suitable volume of quarter strength Ringer's solution (Oxoid). In both cases DNA was extracted by using the Wizard DNA purification kit (Promega) through a procedure modified according to Ercolini et al. (2001). ITS amplification was performed as previously described. Species identification was obtained by comparison of size amplicons with known species.

## 2.7. Useful technological properties

Proteolytic activity was evaluated by method described by Fadda et al. (2004); while for lipolytic activity two different protocols were used (Fadda et al., 2004; Hernández et al., 2007). The assimilation of lactic acid and citric acid was evaluated according to Disegna et al. (1997).  $\beta$ -glucosidase activity was assayed by using 4-MUG (Fluka, Milan, Italy), according to the method proposed by Fia et al. (2005). Hydrogen sulphide production was evaluated on the basis of the colour of the colonies (cream or brown) on Biggy agar (Difco, Detroit, MI). Two enzyme activities, pectolytic and xylanolytic, were studied in order to determine polysaccharolytic activity. Both analyses were performed according to the method described by Hernández et al. (2007).

## 2.8. Olive aroma compounds analysis

Volatile compounds at 60 and 120 days of fermentation were identified by Solid Phase Micro-Extraction technique in Head Space followed by Gas Chromatography/Mass Spectrometry (HS-SPME-GC/MS) (Pawliszyn, 1999). 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. 5  $\mu$ l of 2-Pentanol-4-methyl methanol solution (0.822  $\mu$ g/ml) were used as an internal standard. Three different fibers, Polydimethylsiloxane (PDMS) 100  $\mu$ m, Divinyl-Benzene(DVB)/Carboxen/PDMS, and Carboxen/DVB 50/30  $\mu$ m, were tested in order to select the one with the best extraction efficiency. Selection was based on the  $F_{ij}$  criterion function introduced by Zuba et al. (2002) and modified by Hamm et al. (2003).  $F_{ij}$  values were respectively 0.478 for PDMS fiber, 0.889 for the CAR/DVB and 1.437 for PDMS/CAR/DVB. The vials were heated at controlled temperature (40  $\pm$  0.5 °C) in order to reach equilibrium. On the basis of preliminary tests, 30 min exposure time proved suitable for fiber

saturation and for reproducibility of the extraction procedure. The equilibrium condition was reached after 3 h of sample conditioning.

Collected data were processed with the instrument data system. Chromatographic and spectrometric results showed excellent reproducibility ( $SD \leq 4\%$ ). Olive volatile compounds were identified first by a critical and reasoned comparison with mass spectral data within NIST 2002 library. Subsequently, some of the identified compounds were verified on the LRI list. In addition, other compounds were compared to their related standards. 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  $\mu$ g/ml to 8  $\mu$ g/ml ( $R^2 = 0.995$ ).

## 2.9. Sensory evaluation

A descriptive method (UNI 10957, 2003) was used to define the sensory profile. A descriptive panel of ten judges was employed. The judges were trained in some preliminary sessions, using different samples of canned olives, in order to develop a common vocabulary for the description of the sensory attributes of green table olive samples and to familiarize themselves with scales and procedures. Each attribute term was extensively described and explained to avoid any doubt about the relevant meaning. On the basis of the frequency of citation (>60%) fifteen descriptors were selected to be inserted in the card: intensity of the green colour, brightness (aspect), odour green olives, off-odour (odour), crispness, easy peeling and juiciness (rheological), sweet, acid, bitter, salt (taste), astringent (tactile in mouth), green olive flavour, off-flavour (flavour), overall. Random samples were evaluated by assigning a score between 1 (absence of the sensation) and 9 (extremely intense) in individual booths under incandescent white lighting at the sensory laboratory of the DOFATA Department (University of Catania, Sicily). Water at room temperature was used to rinse sample before tasting. A computerized data collection program was used (FIZZ, Software Solutions for Sensory Analysis and Consumer Tests, Biosystemes, Couternon, France). Three evaluations were monthly performed starting from January 2007 (90, 120 and 150 days of fermentation).

## 2.10. Data analysis

The sensory data for each attribute were submitted to one-way Analysis of Variance (ANOVA), considering as an independent variable the days of maintenance and as a dependent variable the sensory attribute. Significance was tested with the F test. The mean values were submitted to the multiple comparison test using the LSD (Least Significant Difference) procedure that allows the attributes which differentiate the samples to be determined.

# 3. Results

## 3.1. Physico-chemical evolution of olives

According to carpological data, drupes of the five cultivars showed a similar trend in quality characteristics. Fruit weight, as well as the longitudinal and transversal diameter of the drupes, significantly decreased from picking onward (Table 1). Soluble solid content and pH were used to monitor the fermentation process. The pH presented a slow decrease within the first 60 days of fermentation in all cases. The drop in values ranged from 0.22 for the cultivar Nocellara del Belice to a maximum of 0.38 for the cultivar Brandofino. By day 60, pH had declined more quickly, reaching the minimum value (4.14) for the cultivar Passalunara (Table 1). Soluble solid content decreased during monitoring due to the olive

**Table 1**  
Evolution of physico-chemical parameters during ripening for the five cultivars considered.

Cultivar	Fruit weight (g)			Fruit diameter (mm)			Fruit length (mm)			pH			Titratable acidity (g/l oleic acid)		
	0 <sup>a</sup>	60	120	0	60	120	0	60	120	0	60	120	0	60	120
Manzanilla	4.14 <sup>a</sup> ± 0.70 <sup>b</sup>	3.90 <sup>b</sup> ± 0.72	3.79 <sup>c</sup> ± 0.73	17.59 <sup>a</sup> ± 1.02	16.60 <sup>b</sup> ± 1.87	15.85 <sup>c</sup> ± 1.81	20.78 <sup>a</sup> ± 1.33	19.70 <sup>b</sup> ± 2.90	19.35 <sup>c</sup> ± 3.36	0.88 <sup>ns</sup> ± 0.22	0.88 <sup>ns</sup> ± 0.22	0.90 <sup>ns</sup> ± 0.18	0.85 <sup>a</sup> ± 0.06	1.02 <sup>b</sup> ± 0.05	1.21 <sup>c</sup> ± 0.07
Passalunara	5.89 <sup>a</sup> ± 1.00	5.38 <sup>b</sup> ± 1.05	4.66 <sup>c</sup> ± 0.51	22.65 <sup>a</sup> ± 1.13	20.92 <sup>b</sup> ± 2.38	18.89 <sup>c</sup> ± 1.17	25.93 <sup>a</sup> ± 2.02	24.11 <sup>b</sup> ± 2.29	22.65 <sup>c</sup> ± 1.14	1.08 <sup>ns</sup> ± 0.14	1.08 <sup>ns</sup> ± 0.14	1.05 <sup>ns</sup> ± 0.18	0.86 <sup>a</sup> ± 0.07	1.20 <sup>b</sup> ± 0.06	1.68 <sup>c</sup> ± 0.07
Castriciana	5.22 <sup>a</sup> ± 0.63	5.08 <sup>b</sup> ± 0.60	4.00 <sup>c</sup> ± 0.69	18.29 <sup>a</sup> ± 0.87	18.44 <sup>b</sup> ± 1.40	15.70 <sup>c</sup> ± 1.26	24.44 <sup>a</sup> ± 1.34	19.43 <sup>b</sup> ± 2.91	21.60 <sup>c</sup> ± 1.54	0.85 <sup>ns</sup> ± 0.24	0.85 <sup>ns</sup> ± 0.24	0.88 <sup>ns</sup> ± 0.15	0.74 <sup>a</sup> ± 0.04	0.95 <sup>b</sup> ± 0.03	1.24 <sup>c</sup> ± 0.04
Nocellara	6.40 <sup>a</sup> ± 0.106	5.51 <sup>b</sup> ± 1.48	5.07 <sup>c</sup> ± 1.40	21.69 <sup>a</sup> ± 1.90	19.77 <sup>b</sup> ± 3.40	18.60 <sup>c</sup> ± 1.91	23.19 <sup>a</sup> ± 1.78	27.79 <sup>b</sup> ± 1.91	21.30 <sup>c</sup> ± 1.98	0.97 <sup>ns</sup> ± 0.15	0.97 <sup>ns</sup> ± 0.15	0.95 <sup>ns</sup> ± 0.22	0.99 <sup>a</sup> ± 0.03	1.04 <sup>b</sup> ± 0.03	1.11 <sup>c</sup> ± 0.05
Brandofino	8.38 <sup>a</sup> ± 1.30	7.44 <sup>b</sup> ± 1.80	6.38 <sup>c</sup> ± 1.19	21.84 <sup>a</sup> ± 1.35	20.00 <sup>b</sup> ± 2.55	18.20 <sup>c</sup> ± 1.89	30.52 <sup>a</sup> ± 2.14	28.32 <sup>b</sup> ± 3.27	26.00 <sup>c</sup> ± 2.10	1.15 <sup>ns</sup> ± 0.20	1.15 <sup>ns</sup> ± 0.20	1.17 <sup>ns</sup> ± 0.21	1.06 <sup>a</sup> ± 0.04	1.00 <sup>b</sup> ± 0.05	1.09 <sup>c</sup> ± 0.07
Cultivar	Fruit soluble solid content (brix <sup>c</sup> )			pH			Titratable acidity (g/l oleic acid)								
	0	60	120	0	60	120	0	60	120	0	60	120	0	60	120
Manzanilla	14.79 <sup>a</sup> ± 1.41	12.70 <sup>b</sup> ± 0.71	9.72 <sup>c</sup> ± 1.45	5.38 <sup>a</sup> ± 0.06	5.02 <sup>b</sup> ± 0.05	4.23 <sup>c</sup> ± 0.10	4.23 <sup>c</sup> ± 0.10	4.23 <sup>c</sup> ± 0.10	4.23 <sup>c</sup> ± 0.10	0.85 <sup>a</sup> ± 0.06	0.85 <sup>a</sup> ± 0.06	0.85 <sup>a</sup> ± 0.06	0.85 <sup>a</sup> ± 0.06	1.02 <sup>b</sup> ± 0.05	1.21 <sup>c</sup> ± 0.07
Passalunara	13.29 <sup>a</sup> ± 1.36	12.75 <sup>b</sup> ± 0.85	9.67 <sup>c</sup> ± 0.96	5.22 <sup>a</sup> ± 0.13	4.92 <sup>b</sup> ± 0.05	4.14 <sup>c</sup> ± 0.08	4.14 <sup>c</sup> ± 0.08	4.14 <sup>c</sup> ± 0.08	4.14 <sup>c</sup> ± 0.08	0.86 <sup>a</sup> ± 0.07	0.86 <sup>a</sup> ± 0.07	0.86 <sup>a</sup> ± 0.07	0.86 <sup>a</sup> ± 0.07	1.20 <sup>b</sup> ± 0.06	1.68 <sup>c</sup> ± 0.07
Castriciana	13.92 <sup>a</sup> ± 1.91	13.89 <sup>b</sup> ± 1.97	11.20 <sup>c</sup> ± 1.82	5.52 <sup>a</sup> ± 0.07	5.27 <sup>b</sup> ± 0.12	4.18 <sup>c</sup> ± 0.69	4.18 <sup>c</sup> ± 0.69	4.18 <sup>c</sup> ± 0.69	4.18 <sup>c</sup> ± 0.69	0.74 <sup>a</sup> ± 0.04	0.74 <sup>a</sup> ± 0.04	0.74 <sup>a</sup> ± 0.04	0.74 <sup>a</sup> ± 0.04	0.95 <sup>b</sup> ± 0.03	1.24 <sup>c</sup> ± 0.04
Nocellara	14.01 <sup>a</sup> ± 2.77	14.02 <sup>b</sup> ± 2.54	11.60 <sup>c</sup> ± 1.97	5.25 <sup>a</sup> ± 0.18	5.03 <sup>b</sup> ± 0.11	4.26 <sup>c</sup> ± 0.10	4.26 <sup>c</sup> ± 0.10	4.26 <sup>c</sup> ± 0.10	4.26 <sup>c</sup> ± 0.10	0.99 <sup>a</sup> ± 0.03	0.99 <sup>a</sup> ± 0.03	0.99 <sup>a</sup> ± 0.03	0.99 <sup>a</sup> ± 0.03	1.04 <sup>b</sup> ± 0.03	1.11 <sup>c</sup> ± 0.05
Brandofino	15.10 <sup>a</sup> ± 3.34	13.75 <sup>b</sup> ± 1.46	9.40 <sup>c</sup> ± 1.50	5.49 <sup>a</sup> ± 0.09	5.11 <sup>b</sup> ± 0.07	4.35 <sup>c</sup> ± 0.07	4.35 <sup>c</sup> ± 0.07	4.35 <sup>c</sup> ± 0.07	4.35 <sup>c</sup> ± 0.07	1.06 <sup>a</sup> ± 0.04	1.06 <sup>a</sup> ± 0.04	1.06 <sup>a</sup> ± 0.04	1.06 <sup>a</sup> ± 0.04	1.00 <sup>b</sup> ± 0.05	1.09 <sup>c</sup> ± 0.07

<sup>a</sup> Days of fermentation.

<sup>b</sup> Results expressed as mean ± standard deviation. Means along a row with a different letter were significantly different ( $p < 0.05$ ).

fermentation process (Garrido-Fernández et al., 1997; Durán Quintana et al., 1999): Cultivars Brandofino, Manzanilla and Passalunara experienced a constant decrease, while in cultivars Nocellara del Belice and Castriciana soluble solid content started to decrease by day 60 (Table 1). Actually, for all the cultivars, soluble solid content declined faster from 60 to 120 days, with drops ranging from 2.42 for the cultivar Nocellara del Belice to 4.35 for Brandofino. Nocellara fruits showed the highest soluble solid content and Passalunara the lowest at both 60 and 120 days of ripening (Table 1).

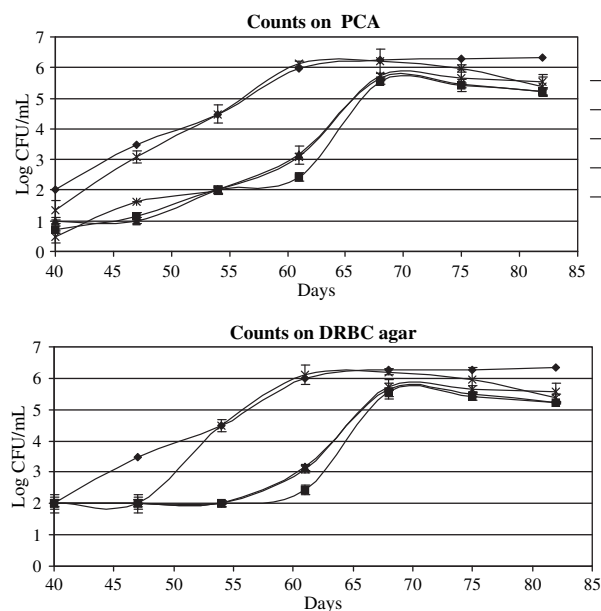
Total titratable acidity expressed as oleic acid, mainly represented by organic acids, is important due to the buffering capacity during fermentation (Brighigna, 1984). A titratable acidity increment was observed for Manzanilla, Castriciana and Passalunara during the whole period of immersion in brine. By contrast, in Brandofino and Nocellara no significant titratable acidity variation was recorded. The highest value (1.68) was registered for Passalunara at 120 days of fermentation (Table 1).

### 3.2. Microbiological changes

Samples of olives for microbiological analysis were weekly collected till the 84th day of fermentation. Within the first 42 days, counts on the seven media included in this study remained at undetectable levels ( $< 2 \log \text{CFU/ml}$ ). After day 42, the microbial population of each cultivar mainly consisted of yeasts (about  $2 \log \text{CFU/ml}$ ); LAB were only detected in Brandofino cultivar (about  $2 \log \text{CFU/ml}$ ), while Pseudomonaceae, Staphylococcaceae, Enterobacteriaceae and spore-forming bacteria remained undetectable up to the end of fermentation. Yeast microflora increased from  $2 \log \text{CFU/ml}$  to  $6 \log \text{CFU/ml}$  at the 63rd day for Brandofino and Nocellara del Belice and at the 70th day for the other three cultivars (Fig. 1). Moreover, as shown by Fig. 1, counts on PCA at  $30^\circ \text{C}$  perfectly matched those on DRBC agar, suggesting that yeasts are the sole microorganisms to be detected.

### 3.3. Yeast strains identification

A total of 94 yeasts was isolated during the manufacturing of table olives from five different cultivars and at different ripening



**Fig. 1.** Microbial evolution during table olive fermentation of total microflora and yeasts. Symbols mean:  $\blacklozenge$ , Brandofino;  $\blacksquare$ , Castriciana;  $\blacktriangle$ , Manzanilla;  $\times$ , Nocellara del Belice;  $*$ , Passalunara.

times (Table 2). Yeasts were grouped according to the PCR-RFLP method developed by Esteve-Zarzoso et al. (1999). PCR amplification of the ITS region allowed strains to be referred to three classes according to amplicon size: four strains exhibited amplicons of approximately 550 bp, six of about 500 bp, while the remaining 84 strains presented ITS amplicons of 450 bp. Digestion by using three restriction endonucleases (*Cfo* I, *Hinf* I and *Hae* III) presumptively confirmed the existence of three species (Fig. 2). Representative strains of each PCR-RFLP profile were identified at the species level by sequencing the D1/D2 region of the 26S rRNA gene (Table 2). According to this evidence six strains were referred to the species *Candida* (*C.*) *parapsilosis*, four to the species *Pichia* (*P.*) *guilliermondii* and 84 to the species *Pichia kluyveri*, confirming previous identifications obtained by restriction analysis. *Pichia guilliermondii* and *Candida parapsilosis* representatives were recovered only from cultivar Brandofino during the sampling at 42 days of processing (Table 2).

#### 3.4. Technological properties of yeast strains

One of the objectives of this study was to investigate the ability of the isolated yeasts to exert properties of technological interest and to contribute to the desirable organoleptic characteristics of the fermented product. Five *C. parapsilosis* strains and one *P. guilliermondii* were able to degrade Tween 80 and tributyrin, thus showing an esterase activity. No yeast strain was able to degrade sodium lactate, whereas strains of *P. guilliermondii* and *C. parapsilosis* were able to metabolize sodium citrate (data not shown). No yeast strain exhibited proteolytic activity on skim milk PCA. At different extent, all strains were H<sub>2</sub>S producers (data not shown). β-glucosidase activity was recorded for all the strains belonging to the species *P. guilliermondii* and for one strain of *C. parapsilosis* (data not shown). All strains belonging to the species *P. guilliermondii* were proved to possess a faint pectolytic and xylanolytic activity, strains of *C. parapsilosis* only exhibited a negligible

pectolytic activity, while no strains of *P. kluyveri* were able to express these traits (data not shown).

#### 3.5. Monitoring of cultural yeast population dynamics during seasoned table olive ripening

Dynamics of the dominating cultivable yeasts during seasoned green olive ripening were also investigated using ITS analysis of the microbial biomass grown on DRBC countable agar plates. This approach confirmed the results obtained by conventional isolation methods: the same three yeast species were detected, but the distribution in samples and over time appeared completely different (Table 2). *P. kluyveri* still was the main species detected, since amplicons of 450 bp appeared in most of the samples. By contrast, *Pichia guilliermondii* and *C. parapsilosis* did not emerge from samples of the cultivar Brandofino but were recovered in samples of the cultivars Castriciana, Manzanilla and Nocellara del Belice (Table 2).

As a third approach, DNA was directly extracted from the same samples considered above. In ITS patterns one band of 450 bp appeared throughout the olive-making process, proving that *P. kluyveri* is the only dominating species up to the end of ripening (Table 2). Amplicons referable to the species *P. guilliermondii* and *C. parapsilosis* were retrieved in all the cultivars, except Castriciana, but only within the first days of monitoring (Table 2).

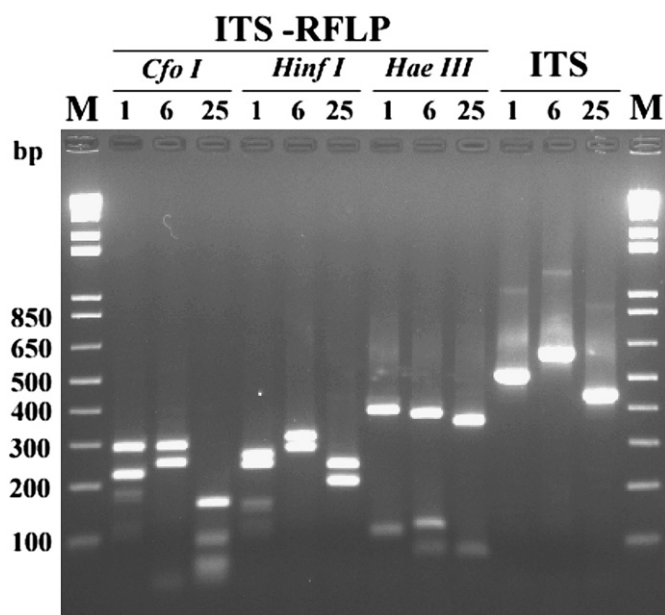
#### 3.6. Olive aroma compounds

The aroma component is a very important factor to monitor changes during olive storage. A total of 52 compounds were characterized by GC–MS analysis in samples after 60 days of ripening (Table 3). Several classes of chemical compounds were identified: alcohols, esters, aldehydes, acids, terpene derivatives and hydrocarbons. Hexanol, heptanol, octanol and 2-nonen-1-ol as well as heptanal, octanal and other aldehydes were found in a similar

**Table 2**  
Yeast species occurring during manufacturing as revealed by culture-dependent (isolation on DRBC) and culture-independent (DNA extracted from bulk cells harvested from DRBC viable counts and for DNA directly extracted from samples) approaches.

	Cultivar	Days of ripening						
		40	47	54	61	68	75	82
Yeast species isolated	Brandofino	<i>C. parapsilosis</i> (6) <sup>a</sup> <i>P. guilliermondii</i> (4)	<i>P. kluyveri</i> (3)	<i>P. kluyveri</i> (2)	<i>P. kluyveri</i> (4)	<i>P. kluyveri</i> (4)	<i>P. kluyveri</i> (4)	<i>P. kluyveri</i> (4)
	Castriciana				<i>P. kluyveri</i> (4)	<i>P. kluyveri</i> (4)	<i>P. kluyveri</i> (4)	<i>P. kluyveri</i> (4)
	Manzanilla				<i>P. kluyveri</i> (4)	<i>P. kluyveri</i> (4)	<i>P. kluyveri</i> (3)	<i>P. kluyveri</i> (3)
	Nocellara del Belice			<i>P. kluyveri</i> (3)	<i>P. kluyveri</i> (3)	<i>P. kluyveri</i> (4)	<i>P. kluyveri</i> (4)	<i>P. kluyveri</i> (4)
	Passanulara				<i>P. kluyveri</i> (4)	<i>P. kluyveri</i> (4)	<i>P. kluyveri</i> (3)	<i>P. kluyveri</i> (4)
DNA extracted from bulk cells	Brandofino		<i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>
	Castriciana	<i>P. kluyveri</i>		<i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i> <i>P. guilliermondii</i>
	Manzanilla	<i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>
	Nocellara del Belice	<i>C. parapsilosis</i> <i>P. kluyveri</i>	<i>C. parapsilosis</i> <i>P. kluyveri</i>	<i>C. parapsilosis</i> <i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>
	Passanulara					<i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>
DNA extracted from samples	Brandofino	<i>C. parapsilosis</i> <i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>
	Castriciana	<i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>
	Manzanilla	<i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>
	Nocellara del Belice	<i>C. parapsilosis</i> <i>P. kluyveri</i>	<i>C. parapsilosis</i> <i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>
	Passanulara	<i>P. guilliermondii</i> <i>C. parapsilosis</i> <i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. guilliermondii</i> <i>C. parapsilosis</i> <i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i>

<sup>a</sup> Number of isolates in parenthesis (n° = 94).



**Fig. 2.** Restriction pattern analysis of ITS amplicons of three strains isolated from seasoned green table olives. Lanes 2–10 report ITS amplicons of strains D1, D6 and D 25 digested with endonucleases *Cfo* I (lanes 2–4), *Hinf* I (lanes 5–7) and *Hae* I (lanes 8–10). Lanes 11–13 show ITS amplicons of the three strains; M, 1-Kb DNA ladder plus (Invitrogen srl) was used as molecular weight marker.

concentration range for the five cultivars. Cultivar Manzanilla exhibited the highest concentration of acetylic esters, methyl and trimethyl-substitutive hydrocarbons and methyl esters compared to the other cultivars, whereas in Passalunara the largest amount of terpenes, such as copaene and alpha muurolene, was recovered (Table 3). Thirty-one compounds were identified in olives after 120 days of ripening (Table 3). Besides the characteristic alcohols and aldehydes, methyl esters and acetate compounds had notably decreased, while some fatty acids, missing in the first round, were detected. In fact, octanoic and nonanoic acid were found at high concentrations in all cultivars analyzed (Table 3).

According to data obtained, during storage olives showed considerable changes in their quality profile, with a decrease in the number of aromatic compounds identified and, at the same time, the appearance of new compounds, determining a modification of the final flavour.

### 3.7. Sensory analysis

Sensory descriptors were generally affected by olive variety. In the cultivar Castriciana results did not show significant differences between samples for all attributes except that for green olives flavour ( $p \leq 0.05$ ). In detail, olives at 120 days exhibited for this descriptor the highest intensity (data not shown) and, even if not significantly, at the same time olives resulted to be characterized by a greater brightness, intensity of green olives odour, crispness, sourness, bitterness and astringency (data not shown). Results of Manzanilla did not reveal not significant differences between samples for all descriptors: olives at 150 days still showed a high intensity of green olives odour, sour and bitter (data not shown). In Brandofino, only juiciness was significantly different ( $p \leq 0.05$ ) showing the lowest intensity for samples at 120 days. The descriptor green olives flavour discriminated fruits of Nocellara del Belice ( $p \leq 0.01$ ), being more intense at 150 days of ripening. The Passalunara olives were characterized by descriptors sweet and green olives flavour for  $p \leq 0.05$  (data not shown).

Results of ANOVA at 90 days did not show significant differences among samples for all attributes except for crisp ( $p \leq 0.001$ ) and juicy ( $p \leq 0.01$ ) (Fig. 3a). At 120 days, samples still did not show significant differences among them (Fig. 3b). At 150 days samples were distinguishable only by crisp attribute for  $p \leq 0.05$  (Fig. 3c). Sensory descriptors remained unchanged over time only for cultivar Manzanilla. As a general consideration, the “overall” assessment was below average for the five cultivars. The best score was reached in February (120 days) by the cultivar Passalunara (5.2). Ultimately olives of all cultivars obtained the worst results at the third evaluation (Fig. 3c).

## 4. Discussion

The natural fermentation of olives relies upon indigenous microflora from raw material or containers used for storage. This practice may lead to fluctuations in the final characteristics of the product, such as quality and flavour, and may determine its spoilage (Lanciotti et al., 1999; Panagou et al., 2003). As widely reported in the literature, the predominant microorganisms in Spanish-style treated olives are LAB; yeasts, instead, are the organisms responsible for the fermentation of olives in natural processing (Panagou et al., 2003). In the proper development of fermentation of the Sicilian technology monitored, we would expect an equilibrium between yeast and lactic acid bacteria populations. As a matter of fact, in the present study, the application of three different approaches, including a culture-independent one, revealed the presence of just three yeast species during the entire fermentation period.

The main factors that could have limited the adaptation of LAB to the brine environment are the ambient temperature, the initial salt concentration, the nutrient availability and the presence of natural inhibitory compounds, since the fruits were not subjected to lye treatment (Fleming et al., 1973; Ruiz-Barba et al., 1991, 1993). Salt concentration should not be inhibitory when applied at the onset of fermentation, since a balance between brine and flesh is quickly attained (Garrido-Fernández et al., 1997; Marsilio and Lanza, 1998), reaching a final salt concentration which can be easily tolerated by most. However, in the case of fermentation without prior lye treatment, the diffusion of salt is slower owing to the epidermis of the fruits, which acts as a barrier. Similarly, the diffusion into the brine of reducing sugars as well as other nutrients, including vitamins and amino acids, mainly present in olive flesh, is protracted owing to the epidermis of the fruits. The growth of LAB is known to be retarded in brines with 8% salt concentration (Özay and Borcakli, 1996; Tassou et al., 2002) regardless of fermentation temperature. Thus, in this study, LAB growth may have been overcome by salt-tolerant yeasts which provided the products with less free acidity and relatively higher pH values. Moreover, room temperature during fermentation was never higher than 22 °C with an average value of 18 °C  $\pm$  3 throughout the monitoring period. As pointed out by Tassou et al. (2002), the use of a relatively high temperature (>18 °C) must be considered essential for the success of olive fermentation. In the conditions of salt concentration and temperature of the present work, it comes as no surprise that yeasts thrived throughout fermentation. Yeast populations ranging from 3 to 5 Log CFU/ml have been determined in brine during fermentation of different kinds of olives (Marquina et al., 1992; de Castro et al., 2002; Tassou et al., 2002).

*P. kluyveri* appears the most representative species within yeast population. This microorganism, widely investigated for its killer phenotype (Sangorrín et al., 2007), has already been recovered from brines with the same NaCl content by Oliveira et al. (2004), but it has been recently indicated as part of the dominant yeast microflora in olive fermentation (Hurtado et al., 2008). *P. guilliermondii* is

**Table 3**  
Aromatic compounds identified (x) in drupes of the five olive cultivars analyzed at 60 and 120 day of fermentation.

RT <sup>a</sup>	Compound at 60 days	Cultivar					RT	Compound at 120 days	Cultivar				
		B <sup>b</sup>	M	N	P	C			B	M	N	P	C
1,78	Ethyl Acetate	x	x	x	x	x	1,90	Acetic acid	x			x	x
2,24	Butanal, 3-methyl			x	x	x	3,51	1-Pentanol	x		x		
2,36	Butanoic acid, 3-hydroxy			x	x		3,56	1-Pentanol, 4-methyl-	x				x
3,30	1-Pentanol	x	x	x	x	x	4,80	Hexanal	x	x			
4,16	Acetic acid, butyl ester	x		x	x		5,42	Heptane, 2,3-dimethyl	x				
4,81	Hexanal		x	x	x	x	5,48	Hexane, 2,3,4-trimethyl		x	x	x	
5,48	Hexane, 2,3,4-trimethyl		x				8,78	1-Butanol, 3-methyl- acetate			x		x
5,52	1-Pentanol, 2-ethyl	x		x	x	x	12,04	Acetic acid, pentyl ester					x
7,65	3-Hexen-1-ol (Z)	x		x			10,13	Heptanal	x	x			
7,95	o-Xylene	x				x	16,45	1-Heptanol	x			x	
8,78	1-Butanol, 3-methyl-, acetate	x	x	x			17,48	1,7-Heptanediol				x	x
9,11	Hexane, 2,3-dimethyl			x	x		17,63	Octanal	x	x	x		
12,04	Acetic acid, pentyl ester			x	x	x	22,66	1-Octanol	x		x		x
12,45	2-Buten-1-ol, 3-methyl			x	x		23,93	2-Nonen-1-ol	x	x	x	x	x
12,59	Hexanoic acid, methyl ester	x	x			x	28,72	Octanoic Acid			x		
15,27	Benzene, 1,2,3-trimethyl	x	x				30,32	3-Octen-2-one, 7-methyl-		x			
16,45	1-Heptanol		x				31,42	Acetic acid, 2-phenylethyl	x		x	x	x
17,63	Octanal		x				31,90	2-Nonenal, (E)				x	
17,88	Pentane, 2,2,3,4-tetramethyl			x	x	x	32,09	2-Decenal, (E)	x	x	x		x
18,02	Heptane, 2,2,4-trimethyl		x				33,36	Nonanoic acid	x	x	x	x	x
18,28	4-Hexen-1-ol, acetate	x	x	x	x	x	34,39	2,4-Decadienal, (E,E)		x			
18,77	Acetic acid, hexyl ester	x		x	x	x	36,75	2-Dodecenal, (E)	x				
19,02	Benzyl Alcohol	x					36,95	2-Tridecenal, (E)		x	x		x
20,31	Pentane, 3-ethyl-2,2- dimethyl			x		x	37,08	2-Propenoic acid, 3-phenyl		x			x
21,80	Pentane, 2,2,3,4-tetramethyl			x	x	x	37,15	Vanillin				x	
22,66	1-Octanol		x				37,85	Alpha-Cubebene	x				
23,93	2-Nonen-1-ol, (E)		x				43,16	Butylated Hydroxytoluene		x			x
23,56	Phenylethyl Alcohol	x		x	x		43,73	1-Decanol, 2-ethyl	x				
24,75	Isooctanol	x	x										
25,17	1,7-Octadien-3-one, 2-methyl		x	x	x								
25,32	Octanoic acid, methyl ester		x										
28,03	1-Nonanol	x											
29,49	2-Decen-1-ol (E)		x										
29,33	2-Dodecenol	x			x	x							
30,47	3-Tetradecene (Z)	x											
30,56	1-Octene, 6-methyl-6-methylene			x									
30,74	Decanoic acid, 2-methyl		x										
31,42	Acetic acid, 2-phenylethyl	x	x	x	x	x							
32,09	2-Decenal (E)		x										
34,23	1-Undecene, 4-methyl	x											
36,35	1-Heptanol, 2-propyl	x											
36,56	Hexane, 2,3,4-trimethyl			x	x								
36,95	2-Octenal (E)		x										
37,90	Copaene	x			x	x							
38,59	1-Octanol, 2-butyl				x								
39,13	7-Octen-1-ol, 3,7-dimethyl		x										
43,05	alpha-Muurolene	x		x									
43,20	Butylated Hydroxytoluene	x		x	x								
51,92	Methyl tetradecanoate	x	x										

<sup>a</sup> RT: Retention time.

<sup>b</sup> B: Brandofino; M: Manzanilla; N: Nocellara del Belice; P: Passalunara; C: Castriciana.

a species related to high volatile phenol production (Martorell et al., 2006; Barata et al., 2006), but has rarely been associated to olive environments (Arroyo-López et al., 2007). Indeed, within the genus *Pichia* the species commonly isolated from olive fermentation in Spain, Portugal and Italy are *Pichia anomala* and *Pichia membranifaciens* (Fernández González et al., 1993; Marquina et al., 1992; Lanciotti et al., 1999). *C. parapsilosis* has been isolated at low rates from olive brine during fermentation (Marquina et al., 1992; Fernández González et al., 1993; Ruiz-Barba and Jiménez-Díaz, 1995; Hernández et al., 2007; Hurtado et al., 2008). Strongly fermentative yeasts such as *Saccharomyces cerevisiae*, *Candida diddensiae*, and *C. parapsilosis* appear very early on olive brines and seem to be well adapted to the first stage of fermentation (Ruiz-Barba and Jiménez-Díaz, 1995; Hurtado et al., 2008).

Results showed that the activity of the yeast population, with high counts throughout the fermentation stage, could play a key role in the final sensory characteristics of green table olives.

Although some polysaccharolytic strains of yeast have been associated with softening and gas-pocket formation in olives (Vaughn et al., 1972; Durán Quintana et al., 1986), subsequent investigations attributed to the yeast population the role of contributing to the organoleptic characteristics of table olives (Garrido-Fernández et al., 1995; Sánchez et al., 2000; Arroyo-López et al., 2008). However, very few studies deal with the characterization of this yeast population and its possible beneficial role during fermentation. In the present survey, no strain belonging to the species *P. kluyveri* was able to degrade sodium lactate or sodium citrate making the proliferation of *P. kluyveri* during green table olive fermentation a welcome occurrence. Oliveira et al. (2004) recorded lactose assimilation capability in all the strains of *P. kluyveri* analyzed; and, similarly, most of the strains studied by Hernández et al. (2007) were able to reduce lactate from 10% to 55%. By contrast none of our *P. kluyveri* strains showed  $\beta$ -glucosidase activity, and, since the technological production process does not

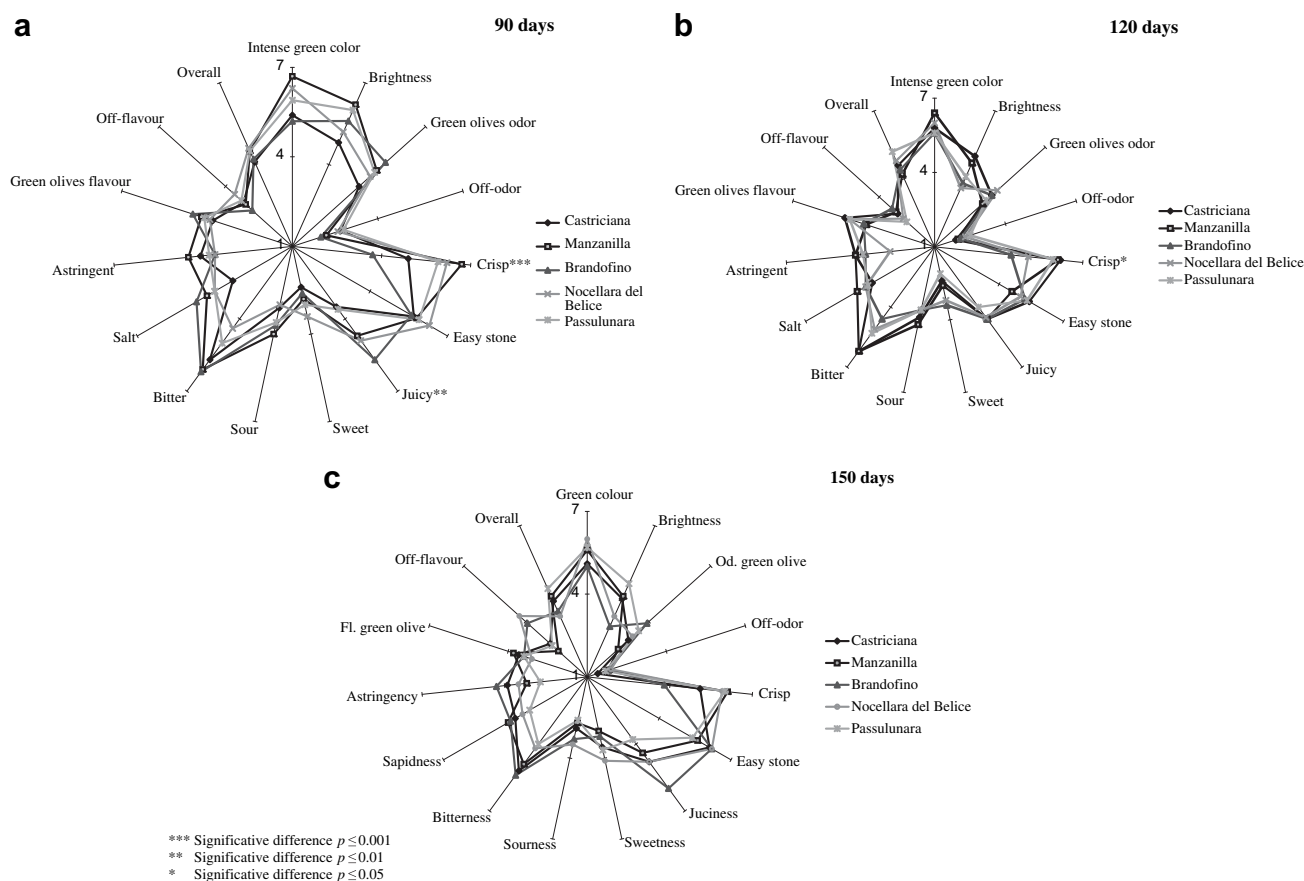


Fig. 3. Evaluation of sensory attributes of table olives after 4, 5 and 6 months of fermentation.

include NaOH treatment, the contribute of microorganisms to oleuropein hydrolysis by the  $\beta$ -glucosidase enzyme appears of indisputable importance. Five strains of the species *P. guilliermondii* and one of *C. parapsilosis* isolated from brines, exhibited the  $\beta$ -glucosidase activity, but they, albeit to a poor extent, also showed pectolytic and/or xylanolytic capabilities (agents of softening and gas-pocket formation). Indeed, among the 94 yeast strains investigated in this survey, no strain could be strictly considered a suitable candidate for a starter culture.

The modest results obtained from sensorial evaluation suggest that technological procedures should be revised and new stages introduced so as to improve final product quality. In this regard, it must be highlighted that no substantial differences emerged in considering different table olive cultivars, and as a consequence, it cannot be indicated a cultivar best adapted to the technological conditions that characterize table olive production in this area of Sicily. As a matter of fact, carpological data on olives at picking evidenced that all the five cultivars fitted for processing as table olives, even though different quality characteristics emerged. Based on IOOC (1999), all the cultivars could be classified as high-weight fruits. According to Brighigna (1984) classification, based on pulp/pit ratio, the Brandofino cultivar with a pulp percentage higher than 86% (pulp/pit > 6), and cultivars Castriciana, Nocellara del Belice and Manzanilla with pulp percentages of 84–85% (flesh/pit > 5) can be considered “very good” for table olive processing, whereas cultivar Passalunara (flesh/pit ratio of 4.91) can be just defined “good”. Moreover, at the end of fermentation, even though fruits of the cultivars, as an effect of the salt concentration in brine, became wrinkled and decreased in size, they were still compatible with commercial standards.

Regarding aroma profiles, numerous studies have been carried out on olive oil, while limited is the knowledge on the qualitative-quantitative composition of volatile compounds of table olives (Sabatini and Marsilio, 2008; Bianchi, 2003; Garrido-Fernández et al., 1997). In the present study, headspace solid-phase micro-extraction (HS-SPME) was employed to define the volatile profiles of table olives. The analysis evidenced compounds deriving from the degradation of polyunsaturated fatty acids through a chain of enzymatic reactions known as the lipoxygenase pathway (Morales et al., 1995; Angerosa et al., 2000). Others volatile compounds found in table olives were hydrocarbons, such as octane and octene and aldehydes such as heptanal, octanal, 2 nonenal (E) which are due to autoxidation reactions (Morales et al., 1997). During the six months of brining there have been changes in the dynamics of proliferative microorganisms and consequently in the volatile compound metabolic pathways.

In conclusion, the selection of well-adapted yeast strains with desirable biochemical properties would appear the most reasonable approach for producing high-quality green table olives. In order to improve sensory characteristics and to ensure final product homogeneity, further studies should be performed on the microbial evolution in environmental conditions different from those characterizing the Sicilian olive production kept at minimal level for economical competitiveness.

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