Evaluation of a single strain starter culture, a selected inoculum enrichment, and natural microflora in the processing of Tonda di Cagliari natural table olives: Impact on chemical, microbiological, sensory and texture quality

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Abstract

The processing of natural table olives is still empirical and far to be controlled. Taking natural fermentation (NF) as control, the performances of a single strain LAB starter culture (SSL) and a selected inoculum enrichment (SIE) were compared. The results showed that the SIE was more efficient in controlling spoiling microflora (Enterobacteriaceae spp.) compared to SSL and NF. Indeed, Enterobacteriaceae in SIE samples were not detectable starting from 10 days, while in SSL and NF samples starting from 30 days. Both starters rapidly acidified the brine, lowering the pH to safety levels (<4.0) after 12 days, while NF samples reached pH 4.3 after 45 days, and these values were kept constant up to 150 days. Moreover, both starters showed debittering activity, as stated through sensory analyses and HPLC analysis of phenols. Compared to NF samples, SIE and SSL extracts retained more hydroxytyrosol at the end of the process, and this could partially account for their higher scavenging activity. Texture profile analysis (TPA) showed that olives processed with SIE inoculum were firmer and more elastic, compared to SSL inoculated olives, thus resulting more similar to NF samples.

1. Introduction

Natural table olives are obtained by fruits of Olea europea L. put directly in brine, in which they undergo a complete or partial fermentation (IOC, 2004), mainly due to microbial metabolism (Romero et al., 2004). The product can be preserved or not by the addition of acidifying agents. The main variables affecting the fermentation process are both intrinsic, such as the olive cultivar itself (Medina et al., 2010), the indigenous microbiota present over the fruit surface (Nychas, Panagou, Parker, Waldron, & Tassou, 2002), and technological, mainly the salt concentration of brines, the processing temperature and hygienic practices (Tassou, Panagou, & Katsaboxakis, 2002). The processing is aimed at the debittering of the fruit via: 1) diffusion from fruit to brine of the bitter compound oleuropein, present in the fruit in its glycosylate form, 2) oleuropein enzymatic hydrolysis, carried out by microbiota with b-glycosidase and esterase activity (Garrido-Fernandez, Fernandez Díaz, & Adams, 1997; Tassou et al., 2002). Moreover, flavour and aroma characteristics are greatly improved by the fermentation process. The preservation of the product throughout the processing is due to acidity development and

consequent pH decline, and to the bacteriostatic activity of sodium chloride (i.e. decrease of a_w, increase of osmotic pressure).

At the beginning of the fermentation, spoilage or even pathogenic species could develop, but usually rapidly succumb to yeasts and LAB (Lactic Acid Bacteria), being more sensitive to salt concentration and acidification of brines. The growth of LAB in naturally fermented olives depends largely on the processing conditions (Abriouel, Benomar, Lucas, & Galvez, 2010). Yeasts can exert both positive or negative role, depending on the species involved (Arroyo-Lopez et al., 2012).

One of the major drawbacks of spontaneous fermentation is the slow processing time, linked to the debittering process that, as usually observed in Tonda di Cagliari traditional productions, can last up to 12e14 months.

At present, there is a growing interest in the development of starter cultures with desirable features. This topic has been recently reviewed by Corsetti, Perpetuini, Schirone, Tofalo, and Suzzi (2012).

In the cited studies, single or dual strain starter cultures have been used, while examples of the use of more complex mixes of LAB cultures, together in the same starter, are not reported. Nevertheless, the experience has shown that back-slopping (i.e. inoculation of the raw material with a small quantity of a previously successfully fermented batch) accelerates the initial phase of fermentation, resulting in the promotion of desirable changes during the whole process, an overall more predictable process, and a product with improved characteristics (Corsetti et al., 2012).

This strategy could be successfully enhanced and implemented, selecting autochthonous mesophilic lactobacilli present during the fermentation process, and adding them to the indigenous population. In this way, the initial number of desirable microorganisms can be increased, ensuring a more reliable and faster process than spontaneous fermentation (Aponte et al., 2012). Selected inoculum enrichment (SIE) represents a new concept in the use of LAB starters in table olive processing at an industrial scale.

A complex of autochthonous isolates, coming from successful spontaneous fermentations have undergone a natural selection. Therefore, they result to be more adapted to the specific brine conditions during fermentation (pH), a_w, concentration of nutrients and antimicrobial compounds, temperature, competitive microflora (Aponte et al., 2012). The aim of this work was to evaluate the technological performances, during the processing of table olives, of two starters: a Single Strain LAB (SSL) starter culture and a Selected Inoculum Enrichment (SIE), made up of an undefined number of strains, taking natural fermentation (NF) as control. Fermentation profile has been traced monitoring microbial counts, pH, titratable acidity and volatile acidity development. Phenolic profile, antioxidant capacity, along with instrumental texture analyses and sensory evaluations have been carried out to define the impact of the technologies employed on the quality features during the processing and in the final products.

2. Materials and methods

2.1. Samples

Olives from the variety Tonda di Cagliari coming from an irrigated olive orchard, located in the south of Sardinia (Italy), were harvested mechanically, in the last decade of October 2013, at the green-yellow ripe stage. Fruits were selected discarding the defective ones, calibrated

(fruit diameter 17e20 mm), carefully washed in tap water, under continuous stirring, allowed to drip the excess of water, and then transferred to the laboratory. Olives were placed in sanitized plastic vats that had a capacity of 60 kg of olives and 40 L of brine (7% NaCl, kept constant throughout the process). An experimental design with 3 replicates and 3 repetitions was used. Three different fermentation conditions were evaluated, namely: 1) 3 vats inoculated with a single strain of Lactobacillus plantarum (SSL); 2) 3 vats inoculated with an undefined mixed culture of Lactobacillus pentosus strains (SIE), isolated from previous successful fermentations; 3) 3 vats under natural fermentation (NF), as control.

Then, experimental vats were transferred to an acclimatized room. The temperature was set at 27 C, until a steady-state pH was reached, for all the batches. Then, temperature was set to 24 C for the rest of the experiment.

2.2. Starter cultures origin and inocula preparation

SSL starter culture, made of a single strain belonging to the species Lb. plantarum (strain S1T10A, isolated from Sicilian “Nocellara Etnea” olives by Cocolin et al., 2013), was supplied by Turin University.

SIE starter culture, an undefined mix of strains belonging to the species Lb. pentosus, was obtained inoculating in FH broth medium (Isolini, Grand, & Glattli, 1990€) all the colonies grown on FH agar plates, seeded for mesophilic lactobacilli counts in previous successfully naturally fermented olives. The broth cultures obtained were concentrated and kept frozen at e 80 C until use. Both SSL and SIE frozen cultures were reactivated in MRS broth. An aliquot of 0.5 ml of each broth culture, grown overnight, at 37 C, was spread onto 120 mm MRS agar plates, anaerobically incubated, at 37 C, for 24 h.

A total of 45 petri dishes per starter culture were prepared according to Cocolin et al. indications (personal communication, October 2013). The colonies grown were collected washing the plates surface with 4 ml of PBS (Phosphate Buffered Solution). The total volume collected was split in 3 aliquots of 60 ml to be used for inoculating each of the 3 plastic vats.

2.3. Physico-chemical analyses

The analyses of olive brines for pH and titratable acidity (expressed as grams of lactic acid per 100 ml brine), were carried out using standard laboratory methods. Volatile acidity (expressed in grams of lactic acid per 100 ml brine) was carried out as follows: 10 ml of brine were put in a flask with the addition of 1 g of tartaric acid. Then, volatile acids were distilled under steam current using a distillation apparatus, using decarbonized distilled water as steam feeding. The distillate was collected (250 ml) and titrated with NaOH 0.1 N, using phenolphthalein as indicator.

Sodium Chloride in brines was determined according to the Mohr method. Briefly, 1 ml of brine was diluted with 50 ml of distilled water, adding K_2CrO_4 as indicator, titrating until turning of colour with $AgNO_3$ 0.1 N.

2.4. Total phenols determination

Phenolic content was determined with the Folin Ciocalteau method. Briefly, 10 g of homogenized olives were put in a test tube with screw cap, with 20 ml of a 80/20 v/v methanol/water solution. After 30 min of stirring, the solution was centrifuged at 4000 rpm for 10 min. The methanol-water phase (phenolic extract) was collected, and diluted (1/2). 100 mL of extract or gallic acid standard were left to react with 500 mL of Folin-Ciocalteau reagent (Sigma Aldrich), for 5 min at room temperature, then 3 ml of 20% Na_2CO_3 solution and ultra

pure water were added to a 10 ml final volume. After 80 min at room temperature, in the darkness, the reaction mix was analysed with a Varian Cary 50 UV-VIS spectrophotometer (Varian Inc. The Netherlands), at l¼ 725 nm, optical path 10 mm. For quantification, a calibration curve was obtained using gallic acid as external standard (200e2000 mg/kg). Results were expressed as mg/kg of gallic acid.

2.5. HPLC determination of phenolic compounds

2.5.1. Extraction of phenolic fraction

Phenolic compounds were extracted from olives according to the IOC method for determination of biophenols in olive oils by HPLC (IOC, 2009), with some minor changes. Briefly, three g of homogenized olives were extract twice with 15 ml of a methanol/ water (80/20, v/v) solution, and 10 ml of hexane. The tubes were agitated for 20 min in rotatory shaker, the organic layer was separated. The two extracts were combined, filtered through a 0.45 mm PTFE syringe filter (Whatman Inc., Clinton, NJ, USA), and dried in rotavapor (t ¼ 30 C). The residue was dissolved in 15 ml of ethyl acetate plus 2 g of anhydrous $MgSO_4$ to remove the remaining water fraction. One mL of the ethyl acetate solution was dried under a gentle N_2 stream, recollected with 1 ml of methanol and injected in HPLC for the analysis.

2.5.2. HPLC analysis of phenols

A HPLC 1100 (Agilent Technologies, Milan, Italy) coupled with DAD detector UV 6000 (Thermo Finnigan, Milan, Italy) was used.

The column was a Varian Polaris C18 (5 um, 300 A, 250 mm 4.6 mm). The analysis were carried out at 280 and 360 nm, in gradient elution. Solvents were phosphoric acid 0.22 M (A), acetonitrile (B), and methanol (C). The gradient used for the separation and analysis was: T ¼ 0 A 96%, B 2%, C 2%; T ¼ 40 A 50%, B 25%, C 25%; T ¼ 45 A 40%, B 30%, C 30%; T ¼ 60 A 0%, B 50%, C 50%, hold: 10 min; post time: 15 min. Flow: 1 mL/min. Calibration curves were prepared across a range of concentrations of 5 to 50 mg/ mL of authentic standards of tyrosol, 3-hydroxytirosol, luteolin-7glucoside, oloeuropein, verbascoside and apigenin. All standards were purchased from SigmaeAldrich Inc. (St. Louis, MO, USA). The stock solutions of the analytes were prepared in methanol (1000 mg/mL). Intermediate stock standard solutions were prepared at 100 mg/mL in methanol by dilution of stock standard solutions. Working standard solutions were prepared in methanol and were used for qualitative and quantitative analysis.

2.6. DPPH scavenging activity as Trolox Equivalent Antioxidant Capacity (TEAC)

Phenolic extracts were obtained from 5 g of destoned olives. The olives were homogenized, added with 10 ml of methanol and vigorously stirred for 20 min. Then, the mixture was centrifuged at 4000 rpm for 25 min. DPPH-free radical scavenging capacity of phenolic extracts was evaluated as following: 200 mL of the extracts or standard (Trolox) was added to 3 ml methanol solution of DPPH radical. The mixture was shaken vigorously for 1 min by vortexing and left to stand at room temperature in the dark for 60 min. Thereafter, the absorbance for the sample was measured using a Varian Cary 50 UV-VIS spectrophotometer (Varian Inc. The Netherlands), at l¼ 517 nm, optical path 10 mm. A negative control was taken after adding DPPH solution to the respective extraction solvent.

The free radical scavenging capacity was expressed in Trolox Equivalents (TE), e.g. mmol TE/kg, and quantified against a calibration curve of Trolox (r ¼ 0.99).

2.7. Microbiological analyses

Samples of uninoculated brines were collected. Appropriate decimal dilutions were prepared and plated, in duplicate, on FH agar medium, incubated at 37 C for 72 h, in anaerobiosis, for mesophilic lactobacilli enumeration, on MEA agar medium

(Microbiol, Uta Cagliari), incubated at 25 C, in aerobiosis, for yeasts and moulds, on VRBGA medium (Microbiol), incubated at 37 C for 18e24 h, in aerobiosis for Enterobacteriaceae. Furthermore, the concentration of mesophilic lactobacilli in inoculated (SSL and SIE) brines was checked on FH agar medium. After 1, 3, 7, 10, 15, 30, 45, 60, 90 and 150 days from olives brining, samples constituted of 135 g of olives and 90 ml of fermentation brine, taking into account the olives:brine ratio (3:2) in the experimental vats, were collected and homogenized for 10 min by a BagMixer paddle blender (Interscience Corporation, Saint Nom, France). Microbial counts were performed, in duplicate, on the growth media indicated above.

Analyses were performed on 3 vats for each experimental trial (SSL, SIE and NF), and expressed as mean CFU/ml.

2.8. Texture analyses

Texture Profile Analyses (TPA) were carried out with a TA-XT plus texture analyzer (Stable Microsystems, Surrey, UK), with a 30 kg plugged load cell. The Exponent software (ver. 6.1.3.0) was used for acquiring and processing texture data. The TPA was carried out on 15 fruits for each replicate (45 fruits for each fermentation condition). The longitudinal side of olives was compressed by 15% with the P/40 aluminium cylinder. Test speed was set at 1 mm/s, time between compressions was 2 s, trigger force 0.05 N. The software calculated the following parameters: hardness, adhesiveness, cohesiveness, gumminess, chewiness and springiness, according to Szczesniak (1963) and Friedman, Whitney, and Szczesniak (1963).

2.9. Sensory analyses

Sensory analyses were performed by Quantitative Descriptive Analysis (Lawless & Heymann, 2010), using 8 trained assessors (ISO 8586, 2012) The training consisted of theoretical education, exercises in general sensory evaluation, and description of sensory attributes of natural table olives (SISS, 2012). Assessors were calibrated for the “bitterness” descriptor with reference standard (caffeine aqueous solutions), to fit the different intensities of a 10 cm unstructured scale. Commercial olives and olive pastes were also employed to set up the “bitterness” intensity scale. Samples were prepared and served according to the IOC Method for the sensory analysis of table olives (IOC, 2011). Samples were presented in a randomized order and evaluated by QDA three times (repetition), over a maximum of two sessions of 2 samples each per day. In order to establish the reaching of a commercial bitterness level, samples had been evaluated for the descriptor together with standard commercial debittered olives of the same variety (naturally processed “Tonda di Cagliari” olives). Samples were accounted as debittered when no significant differences were found between experimental samples and commercial reference.

2.10. Statistical analyses

Sensory panel performances in terms of assessor's repeatability and sample discrimination ability were monitored with the software PanelCheck (http://www.panelcheck.com), which

performed a three-way ANOVA for assessor, sample, replicate effect, assessorereplicate interaction and productereplicate interaction.

Experimental data were submitted to analysis of variance and Tukey's ($P < 0.05$) test through the Minitab® software package (version 17.1.0, Minitab Inc., Coventry, UK), in order to establish any statistical differences among SSL, SIE and NF trials.

3. Results and discussion

3.1. Physico-chemical analyses

SSL and SIE samples showed slight statistical differences in titratable acidity and pH during the fermentation process, while they were significantly different from NF samples, which showed higher pH throughout the whole fermentation process (Fig. 1) and lower titratable acidity values (Fig. 2). SIE and SSL samples underwent a rapid pH decline, which reached values <4 in 12 days, while NF samples reached pH 4.3 after 45 days, and these values were kept constant until the end of the observations. The lowering of pH < 4.0 and the increasing of acidity are essential for the preservation of the product and to avoid proliferation of harmful and spoilage bacteria (Lanza, 2013). Moreover, NF samples showed the higher ratio between volatile and titratable acidity (1.78) at the end of the sampling period. Data showed that samples inoculated with SIE or SSL reached security pH values in short times, due mainly to the conversion of sugars into organic acids. Furthermore, the diffusion of some acid phenols and the hydrolysis of oleuropein, producing elenolic acid, may contribute to pH lowering and titratable acidity rise (Kiai & Hafidi, 2014). However, the inoculum of LAB starters, given the same amount of available nutritive compounds for microbial proliferation, seem to be the main responsible for the observed physico-chemical parameters differences between samples.

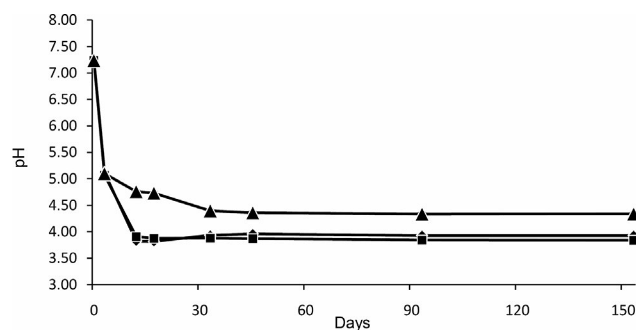

Fig. 1. pH evolution during fermentation. Confidence intervals are shown as vertical bars (n ¼ 3, P ¼ 0,05). - SIE; A SSL; NF.

3.2. Phenolic compounds evolution

According to Table 1, the main classes of phenolic compounds present in olives were phenolic acids, phenolic alcohols, flavonoids and secoiridoids. Hydroxytyrosol and tyrosol were the most abundant phenolic alcohols in olives. Verbascoside, a hydroxycinammic acid derivative, was also found in the samples, along with the flavonoid luteolin-7-glucoside, the secoiridoid oleuropein and the flavon apigenin. These results are similar to those obtained from extracts of other olive cultivars (Silva, Gomes, Leitao, Coelho, & Vilas Boas, 2006. Very few studies reported the evolution of phenolic compounds in the pulp extracts during the processing of natural table olives. Cardoso et al. (2005) quantified the main phenolic compounds in MeOH extracts of olive pulp, hydroxytyrosol being the most abundant. Marsilio et al. (2005) reported the phenolic composition of Greek style processed table olives of the var. “Ascolana tenera”

both naturally fermented and inoculated with a L. plantarum starter culture. Oleuropein and hydroxytyrosol were the most abundant phenols in unprocessed olives.

Oleuropein undergoes hydrolysis and yields several simple molecules like hydroxytyrosol and oleuropein aglycone, during maturation and during the processing. Olives showed loss of total phenols content during fermentation, due to diffusion of these compounds to the brine, but their content does not varied significantly among samples during the experiment (Table 1). The highest decrease in concentration was observed for luteolin-7-glucoside. On the other hand, at the end of sampling period (156 days), there were significant differences in hydroxytyrosol and oleuropein content among samples. NF showed the lowest levels of hydroxytyrosol and highest levels of oleuropein. This could be probably due to the higher degree of enzymatic hydrolysis, carried out by lactic acid bacteria with b-glycosidase and esterase activity, on SIE and SSL samples. This fact had implications in the palatability of processed olives, as stated hereinafter in the sensory analysis section. Moreover, a higher retention of hydroxytyrosol in SIE and SSL samples is desirable because this compound is one of the major bioavailable antioxidant compounds with radical scavenging activity present in olives, as showed by in vitro and in vivo studies (Deiana et al., 2008; D'Angelo et al., 2005; Visioli et al., 2000).

Table 1. Concentration of total and main phenolic compounds identified in pulp extracts. Means (n ¼ 3) followed by different letters at the same sampling time denotes a statistically significant difference (n ¼ 3, P ¼ 0.05).

Sample	Day	Total phenols	Hydroxy-tyrosol	Tyrosol	Verbascosid	Luteolin-7 glucoside	Apigenin	Oleuropein
Unprocessed	0	3611.68	609.28	26.11	57.74	969.65	33.77	218.00
SSL	23	2411.70a	101.78a	6.52a	20.94a	15.26a	4.65a	22.23a
SIE		2166.43a	78.36b	4.62b	38.33b	10.29a	3.23ab	45.06b
NF		2174.53a	61.84c	5.09b	37.00b	25.88b	4.49b	49.63b
SSL	78	2345.08a	109.11a	8.01a	17.55a	25.04a	4.40a	5.13a
SIE		1984.71a	91.44ab	5.33ab	35.49a	17.74ab	3.70a	44.10c
NF		2192.67a	83.87b	5.01b	21.62b	19.45b	4.33a	24.53b
SSL	107	2434.25a	114.84a	6.31ab	16.78a	22.66a	5.74a	5.94a
SIE		2177.66a	107.63a	7.20a	25.57ab	23.55b	5.40a	23.02b
NF		2506.61a	75.75b	4.57b	14.83b	20.65a	3.04b	15.85b
SSL	156	2408.42a	110.05a	4.99a	12.34a	6.89a	3.76a	2.35a
SIE		1998.08a	92.61b	8.69b	16.66b	6.89a	5.18a	5.34a
NF		2158.33a	80.91c	5.58a	3.82c	18.18b	3.96a	18.82b

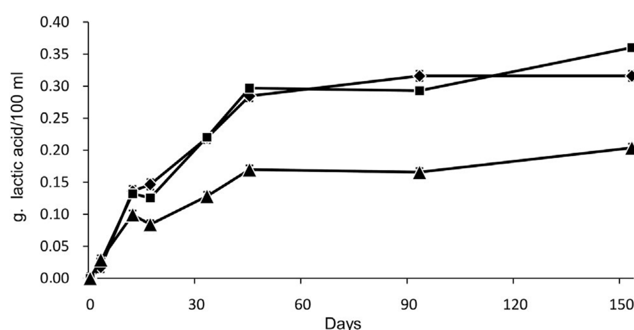

Fig. 2. Titratable acidity evolution during fermentation. Confidence intervals are shown as vertical bars (n ¼ 3, P ¼ 0.05). - SIE; A SSL; NF.

3.3. DPPH scavenging activity as Trolox Equivalent Antioxidant Capacity (TEAC)

The total antioxidant capacity and its evolution during 150 days of brining is reported in Fig. 3. The loss of antioxidant capacity (TEAC) during the processing of natural table olives could be partially correlated with the loss of total polyphenols (Fadda, Del Caro, Sanguinetti, & Piga, 2014), although specific antioxidants could exert the TEAC in the extracts obtained. TEAC was significantly higher in fruit extracts obtained from LAB inoculated samples compared to natural fermentation throughout the fermentation. SIE showed the higher values of TEAC compared to other samples. The radical scavenging activity over reactive chemical species is related to the antioxidant compounds present in the extract. Although no clear correlation could be found between total phenolic compounds and TEAC, it is worth to be noticed that SIE and SSL samples showed higher amount of hydroxytyrosol during the processing and at the end of the fermentation, compared to NF samples. Hydroxytyrosol shows a broad spectrum of biological properties due to its strong antioxidant and radicalscavenging properties (Fernandez-Bolanos, L~ opez, L opez-García, & Marset, 2012). Owen et al. (2003) tested the antioxidant potential of purified phenols from olive pericarp and found that hydroxytyrosol was the most active compound with radicalscavenging activity. The molecule is reported as one of the most active compounds against peroxyl, other free radicals and reactive nitrogen species (Deiana et al., 2008, and references therein) in olives. The higher content of hydroxytyrosol in LAB inoculated samples could partially explain the higher AC showed by the extracts obtained. Other phenols present in the extracts could exert antioxidant activity.

3.4. Microbiological analyses

Uninoculated brines showed a very low level of contamination (<100 CFU/ml), mainly yeasts, while mesophilic lactobacilli and Enterobacteriaceae were not detected. The starter cultures concentrations in SSL and SIE brines, immediately after the inoculum, were 6.82 and 7.25 log CFU/ml, respectively, but mesophilic counts decreased after 24 h (4.43 and 5.12 log CFU/ml, respectively). Yeasts were always detectable during fermentation, although their count decreased during the first 7 days from brining (from 3 to 1 log CFU/ ml), probably due to their adaptation difficulties to brine conditions. Yeasts counts showed no significant differences among samples, reaching 5 log CFU/ml at 30 days and keeping that level until the end of the experiment. Moulds were not detected in any sample.

During the early stage of fermentation, SIE showed higher mesophilic lactobacilli counts than SSL (Fig. 4). Average SSL counts reached the same log CFU/ml of SIE (6.66 and 6.84, respectively) after 15 days of fermentation. In NF vats, mesophilic lactobacilli were not detected until 30 days (1.65 log CFU/ml), and reached the maximum concentration (6.05 log CFU/ml) at 90 days, remaining constant until the end of the experiment.

During the first days from brining, the highest counts of SIE mesophilic lactobacilli could suggest a greater ability of this starter to adapt to the conditions of the substrate compared to SSL starter. This could be because SSL is a monostrain, not indigenous Lb. plantarum culture, while SIE is a mix of autochthonous Lb. pentosus strains, probably with different technological and physiological characteristics, that may alternate in the development during the fermentation.

Moreover, the SIE starter proved to be more efficient in counteracting the development of Enterobacteriaceae compared with both SSL and the microflora naturally present in NF vats.

Indeed, Enterobacteriaceae were not detected in SIE samples starting from the 10th day from brining, while in SSL and NF samples they were not detectable starting from the 30th day.

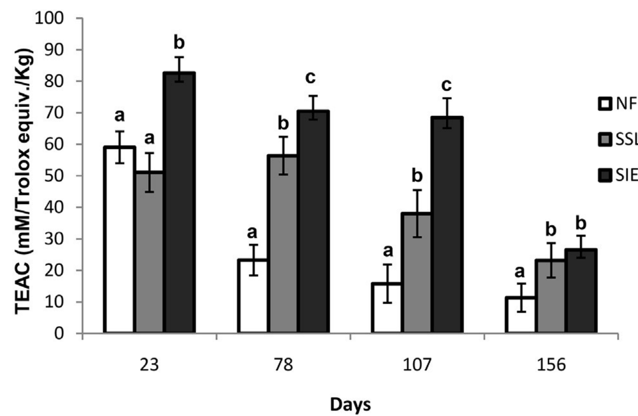

Fig. 3. Antioxidant activity as TEAC (Trolox Equivalent Antioxidant Capacity) during the processing. Confidence intervals are shown as vertical bars. Means with different letters at the same sampling time denotes a statistically significant difference (n ¼ 3, P ¼ 0.05). SIE: Dark grey; SSL: Grey; NF: White.

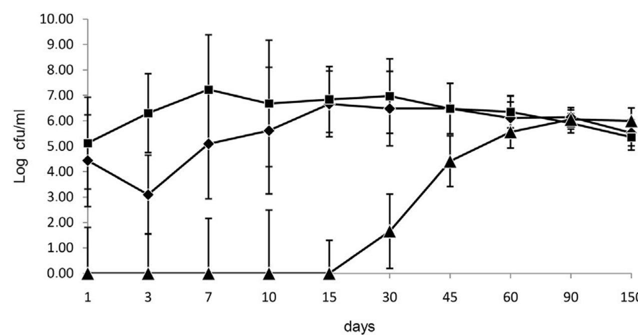

Fig. 4. Mesophilic Lactobacilli growth during fermentation. Confidence intervals are shown as vertical bars (n ¼ 3, P ¼ 0.05).- SIE; A SSL; ::NF.

3.5. Texture analyses

Results of TPA tests showed no differences among samples in "hardness" and "adhesiveness" parameters (Table 2). Statistical analyses marked a significant effect of storage time over all TPA parameters, except "adhesiveness" and "resilience". "Hardness" is measured as the maximum force at the first bite, while "adhesiveness" is measured as the negative peak registered at the end of the first compression, as the attitude of the sample to stick to the probe. NF samples showed significantly higher values in "cohesiveness", "springiness" and "resilience" at the end of the observations, compared to SIE an SSL samples. "Cohesiveness" accounts for the strength of the chemical bonds making up the olive pulp, "springiness" and "resilience" explain the retained elasticity of the product. Among the inoculated samples, SIE samples retained more elasticity and cohesiveness than SSL samples. The same results were observed for "gumminess" (the product of "hardness" and "cohesiveness") and "chewiness" (the product of "gumminess" and "springiness"). Fadda et al. (2014) reported a decrease in "hardness" during brining of "Tonda di Cagliari", and this is in accordance whit our findings.

The same authors reported a decrease in springiness, cohesiveness and related parameters (chewiness and gumminess). Textural changes can be ascribed to hydrolysis of cell wall pectic polysaccharides (Coimbra, Waldron, Delgadillo, & Selvendran, 1996) and consequent loss of structural coherence of olive tissues during brining, as observed by Servili et al. (2008).

Table 2
Texture of samples inoculated with SIE and SSL and obtained by NF. Means (n ¼ 3) followed by different letters at the same sampling time denotes a statistically significant difference (n ¼ 3, P ¼ 0.05).

		Hardness (g)	Adhesiveness (g sec)	Springiness	Cohesiveness	Gumminess	Chewiness (g mm[1])	Resilience
SSL	23	2397.72a	0.84a	0.52a	0.43a	1034.48a	541.39a	0.23a
SIE	23	2321.27a	0.60a	0.57b	0.48b	1110.99ab	629.58b	0.26b
NF	23	2292.31a	0.39a	0.61c	0.53c	1215.94b	737.38c	0.29c
SSL	78	2136.20a	0.67a	0.55a	0.462a	988.16a	550.69a	0.24a
SIE	78	2027.41a	0.33a	0.57a	0.49b	990.43a	577.31a	0.26a
NF	78	2009.42a	0.52a	0.64b	0.58c	1150.62b	722.61b	0.32b
SSL	107	2294.01 a	0.65a	0.58b	0.45a	1027.10a	593.86a	0.24a
SIE	107	2441.42 a	0.81a	0.63a	0.51b	1227.12a	778.28a	0.28a
NF	107	2467.26 a	0.51a	0.64a	0.54c	1316.85b	840.22b	0.29b
SSL	156	2046.75a	0.43a	0.56a	0.45a	918.7a	511.85a	0.24a
SIE	156	2057.8 a	0.43a	0.58a	0.49b	998.26ab	589.49ab	0.26b
NF	156	2009.45a	0.57a	0.60b	0.55c	1100.44c	665.14b	0.30c

3.6. Sensory analyses
SSL and SIE resulted debittered (i.e. resulted not significantly different from standard debittered samples) at the end of the sampling period (156 days), while NF samples retained a higher degree of bitterness, resulting significantly different from the commercial standards ($P < 0.05$). NF samples resulted debittered after 12 months. The differences between samples can be explained by b-glycosidase and esterase activity of the inoculated bacteria. As shown by microbiological analyses, inoculated bacteria rapidly colonized the substrate, becoming the predominant bacterial group, metabolizing the available carbohydrates, lowering the pH and degrading oleuropein to hydroxytirosol and other products. NF samples showed very low LAB count until 30 days, resulting in low acidity development. As a result, samples retained higher amounts of oleuropein, as stated by HPLC analyses, compared to inoculated samples, resulting more bitter at the end of the fermentation process, thus protracting the processing time.

4. Conclusions

The Lb. plantarum starter (SSL) and the Lb. pentosus strains mixed culture (SIE) were successfully used to drive the fermentation process of natural olives. SIE resulted more efficient in supplanting the spoilage microbiota (Enterobacteriaceae) compared to SSL and NF. Both starters were able to lower the pH at security levels in a shorter time compared to NF driven fermentation that resulted in higher final pH levels and lower titratable acidity. Olives processed with starter cultures retained higher amounts of hydroxytyrosol and antioxidant capacity, at the end of the processing. Texture analyses showed that NF samples retained more cohesiveness and elasticity than inoculated samples. However, SIE samples texture resulted more firm and elastic, compared to SSL texture. Microbial starters efficiently debittered the olives in 5 months, while NF samples resulted not yet debittered at the end of the sampling period. The application of LAB starters is very attractive for the industry, reducing costs (e.g. energy), fermentation

times, risk of spoilage, improvement of process control, standardization of the product and safety features, and increased shelflife. Market is lacking of commercial starters developed for natural table olives. The use autochthonous SIE could represent a cost-effective alternative to LAB commercial starters at an industrial level and could provide the basic material for the selection of complex microbial starter with positive technological characteristics.

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