



# Effect of olive leaf extract combined with *Saccharomyces cerevisiae* in the fermentation process of table olives

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**Abstract** Yeasts have a great importance in the table olives quality and have been proved more and more as starter cultures. Moreover, the addition of olive leaf extract (OLE) could enhance the nutritional value of table olives, but there are no studies in which added OLE has been combined with yeasts during fermentation. The aim of this work was to determine if the quality and functional value of table olives increases when OLE and a yeast starter are used during a Spanish-style olive fermentation process. Several combinations were used: (1) fermentations trials with OLE combined with a strain of *Saccharomyces cerevisiae*; (2) fermentations with OLE; (3) control fermentations, with no extract or starter culture. During fermentation performed with the addition of OLE and yeasts, the yeast number remained stable for most of the time, resulting in a slight decrease of yeasts by the end of the process. The phenolic profile of olive flesh and brines of the trials was analysed during the fermentation. The addition of OLE increased the concentration of phenols in olive flesh and brines at the end of the fermentation; in these fermentations, hydroxytyrosol was the most abundant, at around 1700 mg/kg in olive flesh and

3500 mg/L in brines olive flesh, whereas in the control fermentation the concentrations were around 900 mg/kg and 2500 mg/L, respectively. In spite of adding OLE, the fermentation resulted in olives without bitterness. We can conclude that yeast inoculation combined with OLE improves safety, nutritional value and other properties of the final product, without affecting its sensorial qualities.

**Keywords** Fermented table olives · Olive leaf extract (OLE) · *Saccharomyces cerevisiae* · Phenolic compounds · Food quality

## Introduction

Many investigations have been focused on increasing the quality of food products from a health perspective. Among the various potential bioactive compounds, consumers are particularly interested in phenols because of their antioxidant properties. Different researchers have increased the phenolic composition in commercial food commodities, by adding spices to virgin olive oil, using microencapsulated phenols in bread, or using nano-encapsulated phenolic extracts in wines (Castañeda-Peñalvo et al. 2016; Motilva et al. 2016; Pasrija et al. 2015), by exposing table grapes to Ultraviolet-C (Crupi et al. 2013), or even by incorporating them into the food packaging materials (Licciardello et al. 2015).

A wide range of by-products from the food industry are a potential natural source of phenolic compounds; this is the case for residues obtained from the elaboration of virgin olive oil and table olives, such as twigs and leaves. Recently, extracts obtained from olive leaves have been studied for fortifying food and beverages, so they are considered of high added value to the food industry

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(Delgado-Adámez et al. 2014). Major phenolic compounds in olive leaves include oleuropein, hydroxytyrosol, luteolin, apigenin, tyrosol, verbascoside, rutin, caffeic acid, chlorogenic acid, quercetin and epicatechin, among others (Delgado-Adámez et al. 2014; Difonzo et al. 2017; Martín-Vertedor et al. 2016). Additionally, olive leaf extracts (OLE) present potential health benefits, including anti-inflammatory and antitumoral activity, and they can act as modulators of the human immune response (Magrone et al. 2018; Martín-Vertedor et al. 2016). All these healthy qualities categorize OLE as a key functional food ingredient, in particular as a phenolic compound source.

The usage of starter cultures in food is another known strategy to improve its flavour, aroma and microbiological parameters. Traditionally, lactic acid bacteria have regularly been used in the fermentation of table olives, since these microorganisms produce lactic acid, favouring microbiological stability of the product. *Lactobacillus plantarum* and *L. pentosus* are among the most common species found in the traditional elaboration of fermented olives (Zago et al. 2013). Yeasts constitute another important microbial group often found in table olive fermentations. The most common genera of yeasts in table olives fermentation are *Aureobasidium*, *Candida*, *Cryptococcus*, *Issatchenkia*, *Pichia*, *Rhodotorula*, *Saccharomyces* and *Zygorhizula*, among others (Bevilacqua et al. 2012). Although this group is associated with olive spoilage (Hernández et al. 2018), several yeasts have been analyzed recently to narrow down their contribution to fermentation, and those which give optimal benefits have been selected (Arroyo-López et al. 2012; Hernández et al. 2007, 2008).

A few recent works in the literature have evaluated the possibility of enhancing the nutritional (El Sheikha and Hu 2018), probiotic activity and functional value (Ray et al. 2014) of foods during the fermentation process. Furthermore, other researchers have studied the addition of phenolic-rich extracts obtained from olive leaves, to improve the table olive quality (Caponio et al. 2018; Lalas et al. 2011), although no study has tested the use of OLE combined with yeasts during fermentation to date. In the studies above, OLE treatment increased polyphenol content, but did not affect the quality of the olives. Based on the above, this present study aimed to increase the concentration of phenolic compounds and the quality of table olives by using OLE during fermentation, in combination with a starter culture of yeasts. For this, OLE was obtained and characterized by HPLC in order to determine its phenolic profile. OLE was added during the process of table olive fermentation, combined with a starter culture of a *Saccharomyces cerevisiae* strain. In a second step, fermentation evolution was monitored by physico-chemical, instrumental and microbiological determinations. Finally,

the quality of olives obtained was determined by sensorial analysis.

## Materials and methods

### Plant materials

The olive fruits and leaves used in the study were obtained from an official certified ‘Carrasqueña’ cultivar. Both were collected from the experimental olive (*Olea europaea* L.) orchard in ‘Finca La Orden-Valdesequera’ (Guadajira, province of Badajoz, Spain) within the limits of the olive-growing area ‘Tierra de Barros’. After harvesting, olive fruits and leaves were immediately transported to the laboratory to be processed. Then, leaves were vacuum-packed (Gustav Müller VS 100, Germany) in plastic bags and stored at  $-80\text{ }^{\circ}\text{C}$  until they were used for the study. OLE was obtained following the method described by Martín-Vertedor et al. (2016); olive leaf samples were dried in an oven (12 min,  $120\text{ }^{\circ}\text{C}$ ), then they were grounded in a mill until obtaining particles of 0.5–3 mm; and finally, phenol compounds were extracted with hot ultrapure water (1:10 w/v, 3 h at  $60\text{--}65\text{ }^{\circ}\text{C}$ ); the extract was filtered and centrifuged at 21,036 g.

### Microbial strains

In this work, a strain of *S. cerevisiae* named 2 was used as the starter culture. This strain was obtained from an industrial olive fermentation and selected in previous work performed by our research group for its technological properties in the fermentation of table olives and its antimicrobial activity (Schaide et al. 2016).

### Table olive elaboration process and analysis

Olive fruits were submerged in a solution of 2.5% NaOH (w/v), at room temperature ( $25\text{ }^{\circ}\text{C}$ ), until the alkali reached two-third of the flesh, measured from the epidermis to the pit. After this lye treatment, the lye was removed by flushing with tap water. Finally, the treated table olives were placed in three different brines: the first one was a *control treatment* (trial C), consisting of 30 kg of table olives and 30 L of 8% NaCl (p/v) brine solution; the second treatment, with a *phenolic-rich extract* added (trial P), consisting of 30 kg of olives and 30 L of OLE in 8% NaCl (p/v) brine solution; the last fermentation, with a *phenolic-rich extract* and a *starter culture* added (trial P + S), consisting of 30 kg of olives, 30 L of OLE in 8% NaCl (p/v) brine solution and enough starter culture (*S. cerevisiae* strain 2) to reach a concentration of  $6\text{ log}_{10}\text{ cfu/mL}$ . Yeasts were counted using a Neubauer improved cell-

counting chamber. Prior to inoculation, yeasts of *S. cerevisiae* strain 2 were grown in YPD broth (Pronadisa, Lab. Conda, Spain) 18 h at 25 °C; approximately 30 mL of the culture of yeasts was obtained. Table olives were fermented for 121 days, adjusting the temperature to 18 °C; 121 days was considered enough to finish the fermentation by microorganisms and to obtain a final quality product (Caponio et al. 2018; Aponte et al. 2010). These fermentation processes were performed in duplicate. Table olives were sampled through their fermentation, and physico-chemical, microbiological, phenolic profile and antioxidant activity measures were carried out. At the end of the fermentation process, sensory analysis was performed to determine the acceptance rate of the table olives in the sample.

### Microbiological analysis

Microbiological tests were performed periodically throughout the fermentation process and in triplicate. For this, brine samples were obtained, diluted in peptone water, plated in the following culture media and incubated: YEPD Agar (yeast extract peptone dextrose agar) (Pronadisa, Lab. Conda, Spain), 48 h at 30 °C (yeasts); MRS Agar (Man, Rogosa and Sharpe agar) (Pronadisa, Lab. Conda, Spain), 48 h at 37 °C in anaerobic environment (lactic acid bacteria); *Pseudomonas* Agar, 48 h at 30 °C (*Pseudomonas sp.*) (Pronadisa, Lab. Conda, Spain); MYP Agar (mannitol egg yolk polymyxin agar) (Pronadisa, Lab. Conda, Spain), 48 h at 37 °C (*Bacillus cereus*); VRBA Agar (Violet Red Bile Agar) (Pronadisa, Lab. Conda, Spain), 48 h at 37 °C (coliforms) and VRBG Agar (violet red bile glucose agar) (Pronadisa, Lab. Conda, Spain), 48 h at 30 °C (*Enterobacteriaceae*).

### Physico-chemical analysis

#### General physico-chemical analyses

Different physico-chemical analyses were performed during the fermentation. The pH was analysed with a Crison pH meter, Model Basic 20 (Crison Instruments, Barcelona, Spain). Titratable acidity was determined by titration with sodium hydroxide (0.1 N) and expressed as g lactic acid/100 mL of brine (Garrido Fernández et al. 1997). Total chlorides were determined by AgNO<sub>3</sub> titration according to analyses following the Mohr Method, by titrating the brine with 0.1 N AgNO<sub>3</sub>, using K<sub>2</sub>CrO<sub>4</sub> as indicator. Results were expressed as g/100 g. These analyses were conducted in triplicate.

#### Texture analysis

Texture analysis was carried out periodically during the fermentation by using a texturometer (TA.TX2, Stable Microsystems, Surrey, UK) fitted with a 30 kg load cell. The puncture test was performed using a needle probe with 2 mm diameter. The test speed was 0.5 mm/s and the penetration depth was limited to 4 mm. Three parameters were taken into account to evaluate the evolution of texture during fermentation; hardness; distance and slope. The data obtained in this determination were maximum force expressed in kg, distance in mm, and the slope of the curve obtained. The data for each measurement were the mean of 30 olives.

#### Surface colour analysis

Surface colour analyses of the olives were performed periodically using a portable Minolta Chroma Meter CR-300 colorimeter (Minolta, Osaka, Japan). to determine the CIELAB space coordinates (L\*: lightness; a\*: red-green; b\*: yellow-blue). The data for each measurement were the mean of 30 olives.

### Determination of phenolic compounds

The phenolic profile determination was carried out on fresh olive fruit and on olive fruit and brine during the fermentation.

#### Extraction of polyphenols from olives

For the extraction of polyphenols, the methodology described by Cabrera-Bañegil et al. (2017) was used. A portion of 2 g triturated and homogenized fruits was weighed. To the homogenized sample, 10 mL of methanol, containing sodium fluoride (2 mM NaF) to inactivate polyphenol oxidases and prevent phenolic degradation (Tomás-Barberán et al. 2001), was added. The solution was sonicated for 30 min at 4 °C in a P-Selecta ultrasonic bath (model 513; P-Selecta S.A., Barcelona, Spain). The samples were centrifugated for 10 min at 4 °C at 10,000 rpm in a Thermo Scientific Sorvall Legend XT/XF centrifuge (Thermo Fischer Scientific, USA).

#### Reversed phase HPLC analysis

Identification and quantification were performed following the method proposed by Cabrera-Bañegil et al. (2017). An Agilent 1100 model HPLC system (Agilent Technologies, Palo Alto, CA, USA) controlled by ChemStation for LC 3D system Rev. B.03.02 software was used. Chromatographic separation was performed with a Gemini-NX C18 column

(150 × 4.6 mm i.d., 3 µm particle sizes; Phenomenex, San José, CA, USA) heated to 35 °C. The mobile phase used was composed of 0.1% aqueous TFA (A) and acetonitrile (B) in the following gradient mode: initial conditions 10% B; from 0 to 3 min 10% B; from 3 to 15 min 15% B; from 15 to 20 min the composition was kept constant at 15% B; from 20 to 25 min 18% B and from 25 to 40 min 30% B. Then, a period of 5 min was necessary for column equilibration. The flow was fixed at 0.500 mL/min for all the experiments, and the injected volume was 5 µL. Chromatographic data processing was performed using Agilent ChemStation software (Agilent Technologies, Santa Clara, CA, USA). The quantification was done by the external standard method.

Phenolic compounds were monitored and quantified by DAD as follows: benzoic acids at 280 nm, cinnamic acids at 320 nm, flavones and quercetin-3-rutin at 350 nm, and anthocyanins at 515 nm. Fluorescence detection was used for the analysis of hydroxytyrosol, tyrosol, PB1, catechin, PB2 and epicatechin.

### Sensory analysis

The sensory assessment of table olives was performed by a panel of eight expert tasters belonging to the multidisciplinary team of the University of Extremadura and CICYTEX research centre, who were trained following the International Olive Council (IOC) directives (IOC 2011). The panel evaluated olives according to an intensity scale that varied from 1 (sensory sensation not perceived) to 11 (sensory intensity extremely perceived). The samples for analysis were presented in standard tasting glasses containing three olives at the bottom of the glass in a single layer, being covered with the brine solution maintained at 28 °C ± 2 °C. Sensory analysis was performed following the standardized norm of the IOC (2011) with slight modifications. The sensory properties of the olive fruits were colour, aspect, hardness, acidity, salty taste, bitter taste, aromas, defects (off-flavours) and the global assessment. Sensory analysis was performed in triplicate.

### Statistical analysis of data

In the statistical treatment of the data, IBM SPSS version 19 for Windows software was used. Data were expressed as mean ± SD and were analysed using one-way analysis of variance (ANOVA). When the difference between the mean values was significant, a test comparison of means by the Tukey method (univariate analysis) was performed. The significance level was set at  $p < 0.05$ .

## Results and discussion

### Monitoring the fermentation process

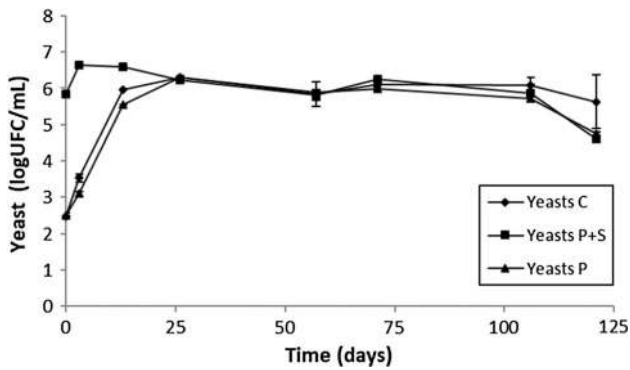
#### *Evolution of microbiota through fermentation*

It was observed that, in the inoculated trial with *S. cerevisiae* strain 2 (P + S), the concentration of yeasts was initially higher than in non-inoculated trials (C and P), as shown in Fig. 1 ( $p < 0.05$ ). In the case of the latter two trials, the yeast concentrations reached values around 2.5 log<sub>10</sub> cfu/mL in trials C and P. On the other hand, trial P + S showed a higher population ( $5.83 \pm 0.06$  log<sub>10</sub> cfu/mL) due to the addition of *S. cerevisiae* at the beginning of the fermentation ( $p < 0.05$ ). In this case, it was twice as concentrated as that of the non-inoculated trials. In trial P + S, the concentration started to change during the first week because of its highly concentrated yeasts (6 log<sub>10</sub> cfu/mL), while those trials without a starter culture needed 2 weeks to reach higher concentrations. Yeast concentrations in trial P + S maintained a stable stage during the rest of the fermentation process, showing a slight decrease from the 121st day of fermentation. On the other hand, in trial P, the yeast population was also higher than in trial C. In spite of the presence of phenolic compounds, the starter culture of *S. cerevisiae* strain 2 was not inhibited by the OLE concentration used in this study. Trials P and C showed similar behaviour until the 26th day of fermentation ( $p < 0.05$ ), with exponential yeast growth. From this day, the microorganism concentrations remained stable.

Furthermore, during the fermentation, the presence of lactic acid bacteria and different bacteria groups that could be related to the loss of quality in the final product was analyzed. Lactic acid bacteria were not detected using MRS medium. Thus, the growth of enterobacteria, coliforms, lactic acid bacteria, *Pseudomonas*, *B. cereus* and aerobic mesophilic bacteria was studied. In this study, none of these microorganisms were detected during the fermentation process. Other studies indicated the presence during fermentation of different kinds of microorganisms that can affect the final product quality, such as *Lactobacillus* (Sánchez et al. 2000), enterobacteria (Caponio et al. 2018) and *Pseudomonas* (Grounta et al. 2016). In the studies of Caponio et al. (2018), the addition of OLE had a potential antimicrobial activity that we have not appreciated in our work.

Results for the growth of yeasts during fermentation agree with earlier studies (Pereira et al. 2015). At the beginning of the fermentation, there was an exponential growth of yeasts during the first 10 days. The yeast concentration levels stabilized in all the fermentations after that period, and in the last phase of the fermentation, the





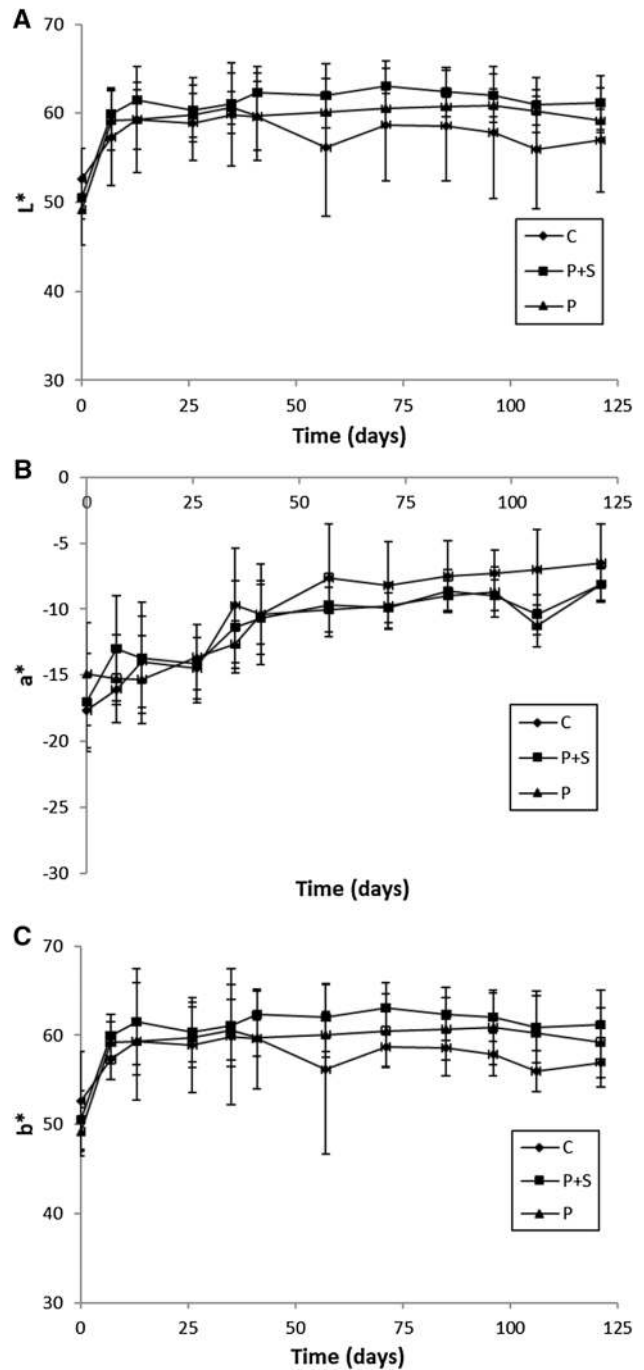
**Fig. 1** Evolution of free acidity and yeasts during fermentation. Results are expressed as the mean ± SD of the three sample replicates

yeast concentration growth slowed down. Other authors (Panagou et al. 2003) indicated that olive fermentation depends on the intrinsic microflora of raw materials, containers used for storage, and processes prior to fermentation (storage, cooking, washing, etc.). Other studies have reported the presence of different yeast species during spontaneous fermentation of table olives, such as *Saccharomyces*, *Candida*, *Zygoascus*, *Wickerhamomyces*, *Debaryomyces*, *Pichia* and *Yamadazyma* (Grounta et al. 2016; Muccilli et al. 2011).

*Evolution of physico-chemical parameters throughout fermentation*

**General physico-chemical analyses** The pH of the brines under study started adjusting to around 3.8 in all the trials (data not shown); they behaved similarly throughout the fermentation process, with slight changes during fermentation, to achieve pH 3.8 at the end of the process. The pH in the process was controlled, by adding lactic acid, in order to maintain adequate fermentation conditions.

With respect to free acidity conditions, initially all the brines started with a low acidity levels (expressed as % lactic acid); some differences ( $p < 0.05$ ) were observed between P and P + S, and the C trial, because of the addition of OLE in the first two cases (Fig. 2). These statistical differences persisted during the fermentations process, although in trial C a slight increase in acidity was observed. During the following 71 days of fermentation, the free acidity levels remained stable, although a slight decrease in acidity was observed from this point until the end of the fermentation. In other fermentation studies using mixed starter cultures of *S. cerevisiae* and *L. plantarum* in Taggiasca table olives (Pistarino et al. 2013), the process started at pH values between 6.6 and 7.4 and ended with a value of pH 4 after 100 days. They found that by using these mixed starter cultures a rapid reduction of pH



**Fig. 2** Colour evolution during the fermentation process (L\*, a\*, b\*). Results are expressed as the mean ± SD of three sample replicates

occurred during the first few days of the process, thus reducing the presence of pathogenic and spoiling microorganisms in the culture. In the study of Caponio et al. (2018), with OLE combined with a *L. plantarum* starter, rapid production of lactic acid associated with a decrease in pH was found in the inoculated fermentations. OLE addition did not influence pH significantly, nor acid lactic production. Results found by Sánchez et al. (2000)

were similar to ours; lactic acid reached 0.44% in controlled fermentations, while the value for spontaneous fermentation was 0.27%. In the latter case, the titratable acidity increased up to 0.96% after 57 days of fermentation. After that, the acidity levels remained constants until the end of the process.

The total chloride concentration present in the brine was also monitored and controlled as needed. The values remained stable during the first 71 days of fermentation, followed by a slight decrease until the end of the process (data not shown). The latter result might be due to sodium chloride absorption by the olives submerged in the brine (Ünal and Nergiz 2003).

**Texture analysis** During the fermentations, olive texture variation was measured (*maximum force*, *distance* and *slope*) (Table 1). With respect to the parameter *maximum force*, only trial C presented significant differences (unlike trials P + S and P), showing its highest value at the beginning of the process ( $p < 0.05$ ). Although the values fluctuated during the fermentation, they followed a downward trend. This parameter indicates the resistance of the skin to the penetration of the probe. Therefore, our study of yeast inoculation did not produce a clear softening of the olives, since the values of maximum force did not show significant differences in trials P + S, P and C ( $p > 0.05$ ). These results partially differ from those of previous studies. Fadda et al. (2014), by using natural fermentation on table olives, observed that the maximum force increased throughout the fermentation. Also, Romeo et al. (2009) explained that the stability of table olive texture during fermentation is due to the salt concentration used. These discrepancies could be explained by the fact that there is a large variety of olive fruits and several elaboration processes.

With respect to the parameter *distance*, only trial P presented significant differences ( $p < 0.05$ ) during the fermentation process (Table 1). All three trials behaved similarly throughout the fermentation. This implies that the skin elasticity of the olives does not undergo any significant changes, since the parameter *distance* denotes changes in the elasticity of the fruit. In addition, the trials under study did not show any significant differences ( $p > 0.05$ ) with respect to the parameter *slope* (Table 1). This parameter is related to the firmness of the pulp (that is, degree of softening), and the degree of maturity of the olives.

The concept of firmness indicates the physical anatomy of the structure of the olive tissue, including cell size, shape, wall thickness and strength, and cell-to-cell adhesion, together with turgor status (Toivonen and Brummell 2008). Previous studies (Blanco et al. 1999) explained that *S. cerevisiae* strains produce enzymes with proteolytic and

**Table 1** Evolution of texture during the fermentation process

| Time (Days) | Control            |                   |                   | P + S          |                   |                | P              |                 |                |
|-------------|--------------------|-------------------|-------------------|----------------|-------------------|----------------|----------------|-----------------|----------------|
|             | Force (kg)         | Distance (mm)     | Slope (kg/s)      | Force (kg)     | Distance (mm)     | Slope (kg/s)   | Force (kg)     | Distance (mm)   | Slope (kg/s)   |
| 0           | 0.72 ± 0.13 ns B   | 3.03 ± 0.84 ns NS | 0.11 ± 0.21 ns NS | 0.70 ± 0.17 NS | 3.18 ± 0.38 ns NS | 0.12 ± 0.04 NS | 0.69 ± 0.17 NS | 2.99 ± 0.75 A   | 0.11 ± 0.02 NS |
| 7           | 0.63 ± 0.24a A,B   | 3.2 ± 0.41 ns     | 0.1 ± 0.36 ns     | 0.68 ± 0.14a,b | 3.23 ± 0.38       | 0.11 ± 0.02    | 0.71 ± 0.16b   | 3.19 ± 0.41 A,B | 0.11 ± 0.02    |
| 13          | 0.66 ± 0.15 ns A,B | 3.31 ± 0.43 ns    | 0.1 ± 0.2 ns      | 0.74 ± 0.18    | 3.27 ± 0.48       | 0.11 ± 0.03    | 0.69 ± 0.17    | 3.21 ± 0.38 A,B | 0.11 ± 0.02    |
| 26          | 0.70 ± 0.12 ns A,B | 3.22 ± 0.36 ns    | 0.11 ± 0.2 ns     | 0.67 ± 0.17    | 3.25 ± 0.42       | 0.1 ± 0.03     | 0.66 ± 0.18    | 3.15 ± 0.39 A,B | 0.11 ± 0.02    |
| 35          | 0.66 ± 0.16a A,B   | 3.16 ± 0.4 ns     | 0.1 ± 0.21 ns     | 0.74 ± 0.27c   | 3.28 ± 0.43       | 0.11 ± 0.03    | 0.7 ± 0.19b    | 3.25 ± 0.44 B   | 0.11 ± 0.03    |
| 41          | 0.62 ± 0.22 ns A,B | 3.14 ± 0.53 ns    | 0.1 ± 0.27 ns     | 0.70 ± 0.2     | 3.18 ± 0.43       | 0.11 ± 0.03    | 0.65 ± 0.16    | 3.22 ± 0.41 A,B | 0.11 ± 0.03    |
| 57          | 0.69 ± 0.21 ns A,B | 3.18 ± 0.42 ns    | 0.11 ± 0.26 ns    | 0.71 ± 0.20    | 3.28 ± 0.43       | 0.11 ± 0.02    | 0.69 ± 0.16    | 3.27 ± 0.41 B   | 0.11 ± 0.02    |
| 71          | 0.62 ± 0.15a A,B   | 2.97 ± 0.37 ns    | 0.10 ± 0.22 ns    | 0.70 ± 0.15b   | 3.22 ± 0.42       | 0.11 ± 0.02    | 0.70 ± 0.15b   | 2.21 ± 0.43 A,B | 0.11 ± 0.02    |
| 106         | 0.59 ± 0.21a A     | 2.94 ± 0.56a      | 0.10 ± 0.29 ns    | 0.64 ± 0.16b   | 3.14 ± 0.38b      | 0.11 ± 0.02    | 0.64 ± 0.17b   | 3.24 ± 0.35b B  | 0.11 ± 0.02    |
| 121         | 0.62 ± 0.18 ns A   | 3.02 ± 0.53 ns    | 0.10 ± 0.03 ns    | 0.61 ± 0.17    | 3.14 ± 0.38       | 0.11 ± 0.03    | 0.61 ± 0.17    | 3.17 ± 0.37 A,B | 0.11 ± 0.02    |

Results are expressed as mean ± SD of the three sample replicates. Different small letters in the same row indicate significant statistical differences (Tukey's Test,  $p < 0.05$ ) among fermentations. Different capital letters in the same column indicate significant statistical differences (Tukey's Test,  $p < 0.05$ ) during the fermentation process. ns/NS: no significant differences

xylanolytic activity, that can affect olive texture. However, in this study, yeast inoculum did not have a negative effect on the olive texture. This is also consistent with previous results from our research group, since *S. cerevisiae* yeasts did not show pectolytic activity (Hernández et al. 2007).

**Colour analysis** Olive colour (CIELAB space) was also measured during the fermentation in order to determine whether the presence of yeasts and OLE affects this feature (Fig. 2). At the beginning of the fermentation, the parameter ‘L\*’ (brightness) presented values around 50 (Fig. 2a). Values increased for all the trials after the 7th day; trials P + S and P had the highest values for brightness by the end of the fermentation. According to López-López et al. (2016), parameter ‘L\*’ is related to the luminance of the surface of the olives. As a result, with respect to this parameter, olives of trials P + S and P could have better quality than the other olives under study. The differences between trial C and trials with OLE might be due to the antioxidant action of polyphenols that are present in the extract.

With respect to parameter ‘a\*’ (–a\* green hue; +a\* red hue), trials C and P + S had the lowest values at the beginning of the fermentation ( $p < 0.05$ ), with a gradual increase throughout the process (Fig. 2b). This is particularly observed in the change of table olive colour (from green to brown). In the last stages, trials P + S and P reached much higher values than in trial C ( $p < 0.05$ ). For that reason, olives fermented with OLE keep a greener colour than control olives (without OLE). Ramírez et al. (2015) produced olives with a more brownish tone compared to the olive pigmentation obtained in this work. That may be explained by the fact that the fermentation period in that study was longer (6 months) than in our study (4 months).

In relation to the parameter ‘b\*’ (–b\* blue hue; +b\* yellow hue), at the beginning of the fermentation, trials C and P showed the highest values (Fig. 2c). There was a gradual decrease in these values throughout fermentation; by the end of the process, trial C showed the greatest losses, as compared with the other trials ( $p < 0.05$ ). This implies that olives lose their yellowness (parameter +b\*) and acquire a bluer colour, inducing a brownish-tone end result in the olives. In general, there were not significant differences in the colour of the olives with the addition of starter culture; *S. cerevisiae* strain 2 did not affect the browning of olives throughout fermentation. This indicates that OLE partially protect the pigmentation of the olives, so the yeast strain used in this study did not affect the olive colour negatively, as compared with non-inoculated fruits. Rodríguez-Gómez et al. (2013), who determined the instrumental colour in Manzanilla variety, did not find differences between spontaneous fermentation and that

with *L. pentosus inocula*. They obtained similar values in all the trials (around 49.9 for L\*, 3.3 for a\* and 34.1–35.3 for b\* by the end of fermentation).

#### *Phenolic profile during the fermentation process*

The phenolic profile of olive flesh and brines during fermentation was analysed. In previous studies, it was proved that lye treatment affects the phenolic compounds in olives; all of the compounds decreased, except for hydroxytyrosol and epicatechin, whose concentrations remained constant, and tyrosol, vanillin and *p*-coumaric acid which increased significantly (Data not shown). The greatest decrease was associated with oleuropein (from 4978 to 10 mg/kg). In general, in our study, the final concentrations of all types of phenols analysed were higher in trials with OLE than in the Control trials. In olive flesh from trials with OLE, most of the phenols diminished during the fermentation, except vanillic acid, oleuropein, luteolin-7-O-g, verbascoside and *t*-ferulic acid. In brines, none of the phenols had diminished at the end of the fermentation, and the concentration of some of them (PB1, vanillic acid, epicatechin, verbascoside, *p*-coumaric acid or vanillin) increased in trials with the addition of OLE with respect to the beginning of the fermentation. In general, the main phenolic compound detected was hydroxytyrosol, followed by tyrosol, PB1, vanillic acid, luteolin-7-O-g and, *p*-coumaric acid (Tables 2 and 3). These results contrast with those of Caponio et al. (2018), for main phenolic compounds and concentrations, probably due to the different varieties of olive used in the two studies. They found that the most abundant phenolic compounds were verbascoside, luteolin, hydroxytyrosol and rutin in olive flesh; and verbascoside and hydroxytyrosol in the brine; moreover, in this study the concentrations were different to ours.

Table 2 shows the results obtained in olive flesh after lye treatment and during fermentation. Overall, at the end of the fermentation, the concentrations of most of phenolic compounds were higher in trials with OLE (P + S and P) than in trial C, with the only exception of verbascoside that did not show differences between the trials. The evolution of these compounds during the fermentation was different. From day 13 to the end of the fermentation, olives from trials P + S and P showed higher hydroxytyrosol, tyrosol, PB1, *t*-ferulic acid and oleuropein concentrations than those from trial C. In addition, overall, the evolution of vanillic acid and *p*-coumaric acid concentrations was similar in trials P + S and P. The maximum concentrations of hydroxytyrosol, PB1 and vanillic acid occurred after 13 days of fermentation for both treatments, while for *p*-coumaric acid and epicatechin they took place at day 7 of fermentation. The maximum tyrosol and oleuropein concentrations obtained in trial P were achieved after 41 and

**Table 2** Phenolic profile of olive flesh (mg/kg) during fermentation

| Day | T           | Hydroxytyrosol    | Tyrosol        | PBI          | Vanillic acid           | Epicatechin       | Oleuropein  | Luteolin      |
|-----|-------------|-------------------|----------------|--------------|-------------------------|-------------------|-------------|---------------|
| 0   | C, P + S, P | 1972 ± 52 C,D     | 110.5 ± 3 C,D  | 61 ± 2 D     | 60 ± 5 B                | 3.3 ± 0.1 C       | 10 ± 1 D    | n.d.          |
| 7   | C           | 2099 ± 52a D      | 113 ± 1 ns D   | 67 ± 2 ns D  | 36 ± 41 ns A,B          | 3.1 ± 0.2 ns C    | n.d.        | 9 ± 1b A,B    |
|     | P + S       | 2021 ± 148a A,B   | 106 ± 9 A      | 59 ± 11 B    | 53 ± 13 C               | 3.6 ± 1.6 B       | n.d.        | 5 ± 1a A      |
|     | P           | 2677 ± 423b C,D   | 128 ± 18 C     | 79 ± 14 C    | 66 ± 13 D               | 4.0 ± 1.6 B       | n.d.        | 9 ± 1b A,B    |
| 13  | C           | 1800 ± 34a B,C,D  | 114 ± 3a D     | 51 ± 1a C    | 58 ± 1 ns B             | 3.5 ± 0.1b C      | 5 ± 2a A,B  | 7 ± 1a A      |
|     | P + S       | 2990 ± 166b D     | 129 ± 9b B     | 72 ± 16b C   | 72 ± 16 D               | 2.8 ± 0.2a A,B    | 10 ± 2a,b A | 12 ± 2b B     |
|     | P           | 2916 ± 224b D     | 125 ± 9a,b C   | 87 ± 8b C    | 72 ± 8 D                | 3.0 ± 0.6a,b A,B  | 15 ± 6b B,C | 11 ± 2b A,B   |
| 26  | C           | 1532 ± 358a B     | 89 ± 21 ns B,C | 33 ± 7a B    | 27 ± 7a A               | 2.1 ± 0.5 ns B    | n.d.        | 9 ± 1a A,B    |
|     | P + S       | 2243 ± 225b B,C   | 113 ± 13 A     | 50 ± 6b A,B  | 51 ± 5b C               | 2.4 ± 0.1 A       | 12 ± 3b A,B | 14 ± 2b C     |
|     | P           | 1935 ± 258a,b A,B | 101 ± 11 A,B   | 45 ± 5a,b A  | 41 ± 8a,b B,C           | 2.6 ± 0.6 A       | 8 ± 1a A,B  | 7 ± 2a A      |
| 41  | C           | 1547 ± 121a B     | 101 ± 8a B,C,D | 29 ± 3a B    | 28 ± 3a A               | 1.7 ± 0.1a B      | 4 ± 1a A,B  | 14 ± 2a,b C,D |
|     | P + S       | 2437 ± 122b C     | 130 ± 6b B     | 60 ± 4b B    | 44 ± 3b B,C             | 2.4 ± 0.1b A      | 24 ± 2b B,C | 16 ± 2b D     |
|     | P           | 2629 ± 121b C,D   | 134 ± 4b C     | 60 ± 2b B    | 46 ± 4b C               | 2.5 ± 0.3b A      | 25 ± 2b C,D | 12 ± 2a B,C   |
| 71  | C           | 1596 ± 50a B      | 111 ± 2 ns C,D | 27 ± 2a B    | 31 ± 4 ns A             | 1.6 ± 0.1a B      | 4 ± 1a A,B  | 16 ± 1 ns D   |
|     | P + S       | 2468 ± 130b C     | 134 ± 6 B      | 53 ± 4b A,B  | 40 ± 4 A,B              | 2.5 ± 0.2b A      | 32 ± 5b C   | 17 ± 1 D      |
|     | P           | 2044 ± 401a,b B   | 114 ± 19 A,B,C | 44 ± 11b A   | 32 ± 7 A,B              | 2.3 ± 0.3b A      | 33 ± 11b D  | 13 ± 4 B,C,D  |
| 85  | C           | 1163 ± 411a A     | 81 ± 28a A,B   | 19 ± 7a A    | 17 ± 1a A               | 0.8 ± 1.2a A      | 6 ± 3a B,C  | 13 ± 6 ns C,D |
|     | P + S       | 2049 ± 136b A,B   | 112 ± 6b A     | 45 ± 4b A    | 31 ± 2b A               | 2.3 ± 0.2b A      | 26 ± 6b B,C | 17 ± 2 D      |
|     | P           | 2098 ± 172b B     | 114 ± 8b A,B,C | 46 ± 2b A    | 32 ± 3b A,B             | 2.4 ± 0.1b A      | 33 ± 3b D   | 13 ± 1 B,C,D  |
| 106 | C           | 1684 ± 89a B,C    | 108 ± 5 ns C,D | 28 ± 3a B    | 26 ± 2a A               | 1.7 ± 0.1a B      | 8 ± 1a C,D  | 15 ± 1 ns D   |
|     | P + S       | 2184 ± 322b B,C   | 117 ± 17 A     | 50 ± 6b A,B  | 39 ± 6b A,B             | 2.3 ± 0.4b A      | 34 ± 2b C   | 17 ± 2 D      |
|     | P           | 2267 ± 101b B,C   | 122 ± 5 B,C    | 46 ± 5b A    | 39 ± 3b A,B,C           | 2.4 ± 0.2b A      | 32 ± 10b D  | 17 ± 4 D      |
| 121 | C           | 876 ± 82a A       | 64 ± 10a A     | 15 ± 2a A    | 14 ± 5a A               | 1.5 ± 0.1a A,B    | 3 ± 1a A    | 11 ± 1a B,C   |
|     | P + S       | 1801 ± 247b A     | 102 ± 10b A    | 46 ± 1b A    | 33 ± 4b A,B             | 2.1 ± 0.4b A      | 28 ± 5b C   | 17 ± 2b D     |
|     | P           | 1539 ± 210b A     | 97 ± 10b A     | 37 ± 6b A    | 27 ± 5b A               | 2.1 ± 0.2b A      | 31 ± 4b D   | 17 ± 2b C,D   |
| Day |             | Luteolin-7-O-g    | Apigenin-7-O-g | Verbascoside | <i>p</i> -coumaric acid | <i>r</i> -ferulic |             |               |
| 0   |             | 20 ± 3 A,B        | n.d.           | 25 ± 1 A,B   | 32 ± 2 B,C              | 1.0 ± 0.1 A       |             |               |
| 7   |             | 46 ± 9 ns D       | 7 ± 1 ns B     | 56 ± 6b B    | 67 ± 2 ns F             | 2.4 ± 0.1 ns D    |             |               |
|     |             | 56 ± 12 A,B       | 7 ± 1 A        | 35 ± 7a A,B  | 53 ± 18 D               | 2.4 ± 1.5 A       |             |               |
| 13  |             | 54 ± 15 B,C,D     | 8 ± 2 A,B      | 31 ± 5a A    | 64 ± 20 C               | 3.8 ± 1.1 A,B     |             |               |
|     |             | 30 ± 3a B,C,D     | 5 ± 1a A       | 14 ± 1a A    | 68 ± 2b F               | 1.8 ± 0.1a C      |             |               |
|     |             | 67 ± 22b A,B,C    | 9 ± 2b A,B     | 28 ± 8a A,B  | 51 ± 5a C,D             | 3.5 ± 0.2b B,C    |             |               |
|     |             | 67 ± 6b C,D       | 9 ± 1b A,B,C   | 93 ± 22b B   | 60 ± 8a,b B,C           | 4.5 ± 0.5c B,C    |             |               |



**Table 2** continued

| Day | Luteolin-7-O-g | Apigenin-7-O-g | Verbascoside   | <i>p</i> -coumaric acid | <i>t</i> -ferulic |
|-----|----------------|----------------|----------------|-------------------------|-------------------|
| 26  | 42 ± 12a D     | 6 ± 2a B       | 19 ± 11 ns A   | 42 ± 8 ns E             | 1.2 ± 0.5a B      |
|     | 109 ± 24b D    | 13 ± 2b C      | 21 ± 5 A       | 41 ± 5 A,B,C            | 3.4 ± 0.3b A,B,C  |
|     | 32 ± 10a A,B   | 7 ± 1a A       | 23 ± 9 A       | 48 ± 6 A,B              | 3.0 ± 0.6b A      |
| 41  | 37 ± 7a C,D    | 8 ± 4 ns B     | 42 ± 35 ns A,B | 38 ± 3a D,E             | 1.2 ± 0.3a B      |
|     | 84 ± 12b C     | 12 ± 3 B,C     | 59 ± 9 C       | 45 ± 3b B,C,D           | 4.2 ± 0.6b C,D    |
|     | 49 ± 10a B,C,D | 8 ± 1 A,B      | 32 ± 2 A       | 51 ± 2c A,B,C           | 5.3 ± 0.2c C      |
| 71  | 22 ± 2a A,B,C  | 5 ± 1 a A      | 8 ± 1 ns A     | 35 ± 1 ns C,D,E         | 0.7 ± 0.0a A      |
|     | 76 ± 17c B,C   | 14 ± 3c C      | 30 ± 8 A,B     | 39 ± 3 A,B              | 4.9 ± 0.8b D      |
|     | 49 ± 10b B,C,D | 9 ± 2b A,B,C   | 23 ± 19 A      | 38 ± 7 A                | 3.7 ± 0.9b A,B    |
| 85  | 34 ± 14a B,C,D | 5 ± 2a A       | 13 ± 6a A      | 24 ± 10a A,B            | 1.0 ± 0.1a A,B    |
|     | 55 ± 7a,b A,B  | 10 ± 2b A,B,C  | 33 ± 9b A,B    | 33 ± 2b A               | 4.1 ± 0.3b C,D    |
|     | 69 ± 18b D     | 11 ± 2b C      | 29 ± 12ab A    | 37 ± 2b A               | 4.4 ± 0.2b B,C    |
| 106 | 39 ± 4 ns D    | 7 ± 2 ns B     | 32 ± 2 ns A,B  | 29 ± 3a B,C             | 1.2 ± 0.2a B      |
|     | 45 ± 17 A      | 11 ± 5 A,B,C   | 32 ± 7A,B      | 33 ± 4a A               | 3.5 ± 0.3b B,C    |
|     | 26 ± 17 A      | 10 ± 3 B,C     | 38 ± 24 A      | 38 ± 2b A               | 3.5 ± 0.8b A,B    |
| 121 | 14 ± 2a A      | 4 ± 1a A       | 32 ± 31b A,B   | 19 ± 3a A               | n.d.              |
|     | 49 ± 9b A      | 8 ± 2b A       | 32 ± 32b 4 B,C | 30 ± 4b A               | 2.7 ± 0.2 ns A,B  |
|     | 45 ± 5b A,B,C  | 8 ± 1b A,B     | 38 ± 38.4a A   | 34 ± 4b A               | 3.0 ± 0.1 A       |

Results are expressed as mean ± SD of the three sample replicates. Different small letters in the same row indicate significant differences (Tukey's Test,  $p < 0.05$ ) among fermentations. Different capital letters in the same column indicate significant differences (Tukey's Test,  $p < 0.05$ ) during the fermentation process. ns/NS: no significant differences

**Table 3** Phenolic profile of brine (mg/L) during fermentation

| Day | T     | Hydroxytyrosol    | Tyrosol       | PBI                     | Vanillic acid     | Epicatechin      | Oleuropein    | Luteolin-gluc |
|-----|-------|-------------------|---------------|-------------------------|-------------------|------------------|---------------|---------------|
| 0   | C     | n.d.              | n.d.          | n.d.                    | n.d.              | n.d.             | n.d.          | n.d.          |
|     | P + S | 3209 ± 106 B      | 165 ± 2 B,C   | 60 ± 2 A                | 38 ± 4 A          | 1.6 ± 0.2 A      | 30 ± 3 A      | 26 ± 3 C      |
|     | P     | 3329 ± 155 B      | 166 ± 3 B,C   | 59 ± 1 A                | 37 ± 3 A          | 1.4 ± 0.3 A      | 32 ± 3 A      | 27 ± 3 C      |
| 7   | C     | 2226 ± 19a A      | 141 ± 1a B    | 22 ± 1a A               | 21 ± 1a A         | 0.6 ± 0.1a A     | n.d.          | 4 ± 1a B      |
|     | P + S | 3417 ± 370b B,C,D | 178 ± 8b D    | 56 ± 2b A               | 33 ± 3b A         | 1.5 ± 0.1b A     | 34 ± 1 ns A   | 28 ± 2b E     |
|     | P     | 3525 ± 106b B,C   | 185 ± 4b C    | 61 ± 1c A               | 42 ± 2c A         | 1.7 ± 0.4b A     | 49 ± 20 A,B   | 28 ± 2b C     |
| 13  | C     | 2228 ± 14a A      | 138 ± 2a A,B  | 28 ± 4a B               | 27 ± 1a B         | 0.9 ± 0.1a B     | 3 ± 1a A      | 4 ± 1a B      |
|     | P + S | 3107 ± 237b A     | 164 ± 11b B,C | 62 ± 2b A,B             | 39 ± 6b B         | 1.8 ± 0.1b B     | 48 ± 171b C   | 24 ± 2b D     |
|     | P     | 2942 ± 370b A     | 151 ± 13a,b A | 65 ± 2b A               | 39 ± 5b A         | 2.1 ± 0.1c B     | 61 ± 16b B    | 24 ± 4b B     |
| 26  | C     | 2302 ± 30a A      | 139 ± 4a A,B  | 31 ± 1a C               | 28 ± 1a B,C       | 0.9 ± 0.1a B     | 5 ± 1a B      | 3 ± 2a A,B    |
|     | P + S | 3697 ± 164b E     | 179 ± 5b D    | 70 ± 1b A,B,C           | 53 ± 2b C         | 1.6 ± 0.4b A     | 35 ± 6b A,B   | 25 ± 2b D     |
|     | P     | 3647 ± 361b C     | 169 ± 18b B   | 71 ± 9b B               | 55 ± 6b B         | 2.0 ± 0.2c B     | 42 ± 8b A     | 29 ± 3c C     |
| 41  | C     | 2463 ± 9a B       | 139 ± 1a A,B  | 35 ± 1a D               | 31 ± 1a C         | 0.9 ± 0.1a B     | 5 ± 1a B      | 1 ± 2a A      |
|     | P + S | 3555 ± 75b C,D,E  | 169 ± 2b C    | 76 ± 2b B,C             | 43 ± 7b B         | 2.1 ± 0.1b C     | 46 ± 3b B,C   | 22 ± 2b C     |
|     | P     | 3633 ± 72b B,C    | 170 ± 2b B    | 77 ± 2b C               | 53 ± 2c B         | 2.3 ± 0.1c B,C,D | 53 ± 6c A,B   | 24 ± 2c B     |
| 71  | C     | 2596 ± 85a C      | 143 ± 7a B    | 42 ± 1a E               | 37 ± 1a E         | 1.1 ± 0.1a C     | 9 ± 2a C      | 3 ± 1a A,B    |
|     | P + S | 3648 ± 183b D,E   | 166 ± 7b C    | 84 ± 6b C               | 54 ± 2b C         | 2.4 ± 0.2b D     | 49 ± 7b C     | 23 ± 2b C,D   |
|     | P     | 3697 ± 79b C      | 167 ± 6b B    | 84 ± 2b D               | 59 ± 2c C         | 2.5 ± 0.1b D     | 53 ± 8b A,B   | 22 ± 2b B     |
| 85  | C     | 2406 ± 148a A     | 136 ± 2a A    | 40 ± 1a E               | 37 ± 2a E         | 1.1 ± 0.1a C     | 9 ± 3a C      | 3 ± 1a A,B    |
|     | P + S | 3186 ± 82b A,B    | 152 ± 6b A    | 76 ± 1b B,C             | 50 ± 2b C         | 2.1 ± 0.1b C     | 40 ± 4b A,B,C | 18 ± 2b A     |
|     | P     | 3363 ± 48c B      | 159 ± 6b A,B  | 79 ± 3c C               | 54 ± 1c B         | 2.1 ± 0.4b B,C   | 46 ± 7b A     | 19 ± 2b A     |
| 106 | C     | 2469 ± 30a B      | 138 ± 4a A,B  | 40 ± 1a E               | 39 ± 5a E         | 1.1 ± 0.1a C     | 10 ± 2a C     | 4 ± 2a A,B    |
|     | P + S | 3355 ± 94b B,C    | 158 ± 7b A,B  | 64 ± 30a,b A,B          | 54 ± 2b C         | 2.1 ± 0.1b C     | 37 ± 11b A,B  | 20 ± 2b B     |
|     | P     | 3476 ± 149b B,C   | 162 ± 6b A,B  | 79 ± 2c C               | 59 ± 2c C         | 2.3 ± 0.1c C,D   | 44 ± 6b A     | 19 ± 4b A     |
| Day |       | Apigenin-7-gluc   | Verbascoside  | <i>p</i> -coumaric acid | <i>t</i> -ferulic | Vainillin        |               |               |
| 0   |       | n.d.              | n.d.          | n.d.                    | n.d.              | n.d.             | n.d.          | n.d.          |
|     |       | 6.6 ± 0.4 C       | 17 ± 1 A      | 5.6 ± 0.6 A             | 2.4 ± 0.2 B       | 4.1 ± 0.5 C      |               |               |
|     |       | 6.3 ± 0.5 C       | 16 ± 2 A      | 5.4 ± 0.5 A             | 2.5 ± 0.4 B,C     | 4.4 ± 0.5 C      |               |               |
| 7   |       | 0.6 ± 0.3a A,B    | 3 ± 1a A      | 3.8 ± 0.5a NS           | 0.2 ± 0.1a NS     | 1.1 ± 0.1a A     |               |               |
|     |       | 6.7 ± 0.3b C      | 18 ± 1b A     | 4.7 ± 0.3b A            | 2.3 ± 0.2b B      | 4.3 ± 0.7b C     |               |               |
|     |       | 6.5 ± 0.3b D      | 17 ± 2b A     | 8.9 ± 0.7c A,B,C        | 2.5 ± 0.3b C      | 3.9 ± 0.5b C     |               |               |
| 13  |       | 0.9 ± 0.1a A,B,C  | 3 ± 1a A      | 8.1 ± 0.9b              | 0.4 ± 0.1a        | 1.3 ± 0.1a B     |               |               |
|     |       | 6.1 ± 1.0b C      | 18 ± 1c A,B   | 5.9 ± 1.3a A            | 1.3 ± 0.1b A      | 3.1 ± 0.4c A     |               |               |
|     |       | 5.3 ± 0.7b B,C    | 16 ± 2b A     | 6.8 ± 1.3a,b A          | 1.1 ± 0.1b A      | 2.7 ± 0.2b A     |               |               |

Table 3 continued

| Day | Apigenin-7-gluc                                  | Verbascoside                             | p-coumaric acid                                 | t-ferulic  | Vainillin  |
|-----|--|--|---|--|--|
| 26  | 0.9 ± 0.2a A,B,C<br>5.2 ± 0.7b C<br>5.8 ± 0.6b C | 4 ± 1a A,B<br>18 ± 3b A,B<br>18 ± 3b A,B | 6.0 ± 1.2 ns<br>6.0 ± 0.8 A,B<br>7.2 ± 0.6 A,B  | 0.3 ± 0.1a<br>2.3 ± 0.2b B<br>2.2 ± 0.3b B             | 1.2 ± 0.2a A,B<br>3.9 ± 0.2b B,C<br>3.9 ± 0.4b C |
| 41  | 0.6 ± 0.5a A<br>5.4 ± 0.3b B<br>5.6 ± 0.7b B,C   | 3 ± 3a A<br>18 ± 1b A,B<br>18 ± 2b A,B   | 6.0 ± 0.3a<br>6.3 ± 2a A,B<br>10.5 ± 0.6b A,B,C | 0.2 ± 0.2a<br>2.2 ± 0.2b B<br>2.1 ± 0.1b B             | 1.3 ± 0.2a B<br>4.5 ± 0.2b C,D<br>4.3 ± 0.3b D   |
| 71  | 1.2 ± 0.2a C<br>5.4 ± 0.4b B<br>5.0 ± 0.3b B     | 6 ± 2a B<br>21 ± 1b C<br>20 ± 3b B       | 8.2 ± 3.9 ns<br>8.9 ± 2.2 B<br>11.7 ± 4.7 C     | 0.4 ± 0.4a<br>1.8 ± 0.2b B<br>1.8 ± 0.3b B             | 1.6 ± 0.2a C<br>4.8 ± 0.4c D<br>4 ± 0.1b C,D     |
| 85  | 1.1 ± 0.2a B,C<br>4.3 ± 0.4b A<br>4.3 ± 0.3b A   | 6 ± 2a B<br>20 ± 2b B,C<br>21 ± 4b B     | 6.7 ± 3.8 ns<br>5.5 ± 3, A<br>10.4 ± 2.9 A,B,C  | 0.5 ± 0.2 <sup>a</sup><br>1.8 ± 0.3b B<br>1.9 ± 0.3b B | 1.5 ± 0.3a C<br>4.5 ± 0.5b C,D<br>4.1 ± 0.3b C,D |
| 106 | 1.1 ± 0.2a B,C<br>4.5 ± 0.2b A<br>4.4 ± 0.4b A   | 7 ± 2a B<br>21 ± 1b C<br>21 ± 3b B       | 8.2 ± 3.3 ns<br>7.5 ± 2.5 A,B<br>11.2 ± 5.2 B,C | 0.4 ± 0.1a<br>1.8 ± 0.4b B<br>2.0 ± 0.6b B             | 1.6 ± 0.1a C<br>3.8 ± 0.3c B<br>3.4 ± 0.2b B     |

Results are expressed as mean ± SD of the three sample replicates. Different small letters in the same row indicate significant statistical differences (Tukey's Test,  $p < 0.05$ ) among fermentations. Different capital letters in the same column indicate significant statistical differences (Tukey's Test,  $p < 0.05$ ) during the fermentation process. ns/NS: no significant differences

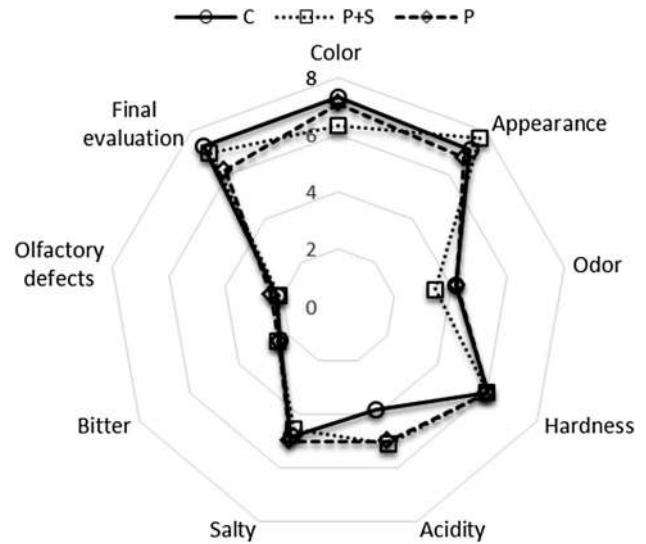


Fig. 3 Sensory analysis of table olives after the fermentation process. Results are expressed as the mean of three sample replicates

71 days respectively. In trial P + S, the luteolin-7-glucoside concentration increased, with a maximum at 26 days of fermentation. Apigenin-7-glucoside concentrations in trials P + S and P showed their maximum at 71 and 85 days of those fermentations, respectively. In trial P + S, the concentration of luteolin reached a maximum on day 41 of the fermentation.

On the other hand, phenolic profile was analysed in brine throughout the fermentations (Table 3). The concentrations of most of the brine phenolic compounds of the two trials with OLE (P + S and P) were significantly higher than for the control throughout the fermentation. p-coumaric acid was the unique phenolic compound whose concentrations did not show significant differences compared to the control. Hydroxytyrosol and vainillin concentrations showed small differences during the fermentation. PB1, vanillic acid, epicatechin and oleuropein showed a similar behaviour, with an increase from the start to 71 days of fermentation, and then the concentrations kept constant. On the other hand, overall, tyrosol, luteolin-7-glucoside, apigenin-7-glucoside and t-ferulic acid concentrations decreased throughout the fermentation.

Caponio et al. (2018), in fermentations inoculated with *L. plantarum* found a significant increase in the amount of hydroxytyrosol and verbascoside in olive flesh. Hydroxytyrosol has been associated with oleuropein degradation and the diffusion of phenols from olive fruit to brine. In our study, yeast inoculation could have produced the hydrolysis of other phenols. However, the results in brine were very different; the hydroxytyrosol and tyrosol concentrations increased in brines during the fermentation. Results obtained by Lalas et al. (2011) are comparable to ours; they found an increase of the oleuropein and hydroxytyrosol

content in olive flesh in table olives with OLE. Indeed, these compounds, oleuropein and hydroxytyrosol are related to healthy effects. In the studies of Martín-Vertedor et al. (2016), OLE showed in vitro antioxidant, antimicrobial and antitumoral activities after simulated digestion. These results suggest that the consumption of OLE may be useful for preventive and/or therapeutic purposes.

### Sensorial analysis

Results of the sensory analysis did not show significant differences between the table olives obtained (Fig. 3). This is a good result since no differences in bitterness were detected by tasters in spite of OLE being used during fermentation. The values found were  $2.33 \pm 1.51$  for trial C,  $2.44 \pm 1.23$  for trial P + S and  $2.4 \pm 0.84$  for trial P. In trial P, an increase in the bitterness of the fruit would be expected, due to phenolic compounds, fact which was not reflected in the study. Several authors have indicated that the bitter taste of food is related to phenolic compounds, such as flavonoids, mainly flavanols and flavonols phenols (Lalas et al. 2011). These authors found differences in the bitterness of treated table olives with an OLE; but this fact did not affect the acceptability of the product. This is consistent with our results; the addition of a phenolic extract did not affect negatively to the sensory quality of the product. Indeed, other investigations have attributed to yeasts a role in contributing to the sensorial characteristics of table olives (Aponte et al. 2010). The inoculation of selected yeast strains would ensure the homogeneity of olives during the process and would allow the acquisition of green table olives with a high quality.

### Conclusion

Inoculation of table olives fermentations inoculated with a *S. cerevisiae* strain combined with OLE improved some properties of the final product, including colour and sensorial qualities, and did not affect the table olive texture. Furthermore, the yeast strain inoculated is suitable for industrial scale for obtaining a standardized-quality safe product. Also, the fermentation process enriched in phenols increased the content of these compounds in the final product, without affecting its bitterness. In fact, all processed table olives presented a high sensory quality. In light of these results, the consumption of this product with higher amounts of oleuropein and hydroxytyrosol would allow acquisition of healthy effects thanks to their antioxidant properties, among others. In this sense, our results confirm and complement those obtained in previous studies; however, further studies in vitro and/or in vivo are required to prove the health properties of our table olives.

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### Compliance with ethical standards

**Conflict of interest** The authors declare they have no conflict of interest.

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