LWT - Food Science and Technology 90 (2018) 381–388

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

# Use of starter cultures for table olives fermentation as possibility to improve the quality of thermally stabilized olive-based paste

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ARTICLE INFO

*Keywords:*
Fermentation
Olive-based paste
Quality improvement
Starter cultures
Thermal stabilization

ABSTRACT

The objective of this work was to improve the quality of thermally stabilized olive-based paste (ObP), obtained from table olives fermented with different starter cultures. Four different trials were performed: conventional fermentation by indigenous bacteria and yeasts (Treatment 1); fermentation using commercial *Lactobacillus plantarum* as starter (Treatment 2); fermentation using commercial *L. plantarum* and selected autochthonous yeast *Wickerhamomyces anomalus* DiSSPA73 (Treatment 3); fermentation using commercial *L. plantarum*, *W. anomalus* DiSSPA73, *L. plantarum* DiSSPA1A7, and *Lactobacillus pentosus* DiSSPA7 (Treatment 4) on which microbiological, physicochemical, and sensory analyses were carried out. The results showed no significant influence of starter cultures on colorimetric indices. From the olives fermented using yeasts, both with and without association of selected lactic bacteria, we obtained an olive-based paste richer in phenol compounds, carotenoids, and free amino acids, which had a better volatile and sensory profile, characterized by lower amounts of the volatile compounds associable to sensory defects.

## 1. Introduction

Table olives are one of the most popular agro-fermented traditional food products in Mediterranean countries – mainly Spain, Greece, and Italy – which together supply almost 30% of annual world olive production (IOC, 2016). Table olives are mainly composed of monounsaturated fatty acids and their consumption can prevent and reduce the risk of cardiovascular diseases (Kastorini, Milionis, Goudevenos, & Panagiotakos, 2010). In addition, other minor constituents, like tocopherols and phenolic compounds, have antioxidant and antimicrobial properties (Malheiro, Sousa, Casal, Bento, & Pereira, 2011).

One of the most important steps in the production of table olives is degradation of their excessively high levels of polyphenolic compounds. Total or partial depletion of these compounds is fundamental to make olives fit for human consumption (Difonzo et al., 2017). Most fermentation processes of table olives are still performed following artisanal practices, with a moderate degree of technological innovation. The process starts spontaneously and is strongly influenced by the olive cultivar itself, its indigenous microbiota and methodological factors, such as fermentation temperature and the salt concentration of brines (Hurtado, Reguant, Bordons, & Rozès, 2012). The presence of a variety of contaminating microorganisms from different sources, as well as the state of drums and storage conditions, may pose problems regarding food safety and also sensory and health quality (De Faveri, Aliakbarian, Avogadro, Perego, & Converti, 2008). In order to have better control of the process, most authors recommend inoculation of brine with suitable starter cultures, of which lactic bacteria acid (LAB) preparations are the most used; these usually consist of *Lactobacillus plantarum* and *Lactobacillus pentosus* or both (Sánchez, Rejano, Montano, & de Castro, 2001; Panagou, Schillinger, Franz, & Nychas, 2008), although in recent years the use of yeasts (Arroyo-López et al., 2012a,b; Bevilacqua et al., 2015), which together with LAB contribute to the organoleptic quality and shelf-life of table olives, has also been suggested (Bevilacqua et al., 2015; De Angelis et al., 2015).

Table olive consumption has increased mainly due to marketing by manufacturers, who have essentially aimed to introduce new products that satisfy growing consumer awareness of their health benefits. Most studies in literature are focused on table olives consumed as such, but the uniqueness of fermented olives has made them an inevitable ingredient of gourmet dishes and gastronomic excellences. These include green and black olive pastes, which are traditional foods made with finely crushed olive flesh conserved with extra virgin olive oil, and have been introduced onto the global market. The olive pastes can include additional ingredients, such as herbs, capers, anchovies, sun-dried tomatoes, artichokes, nuts or even truffles (Anniva & Tsimidou, 2009). Preparation of olive-based paste (ObP) entails preliminary operations,

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https://doi.org/10.1016/j.lwt.2017.12.061
Received 13 July 2017; Received in revised form 16 December 2017; Accepted 25 December 2017
Available online 26 December 2017
0023-6438/ 

such as sorting, washing, stoning and blanching, according to the ingredients added. Subsequently, all ingredients are homogenized, and the mixture is packed and thermally stabilized to ensure a shelf life of about 2 years. The quality of this type of product essentially depends on the quality of the most important ingredients - table olives (green or black) and olive oil - as well as on changes that can occur during the preparation and storage (Lanza, Di Serio, Giansante, Di Loreto, Russi, & Di Giacinto, 2013). These changes can include the impact of pasteurization on chemical composition, which may reduce quality in terms of color due to the Maillard reaction, affecting the flavor with the development of a cooked flavor that affects the sensory properties of the final product (Cosmai, Summo, Caponio, Paradiso, & Gomes, 2013; Cosmai et al., 2017).

In a previous paper, De Angelis et al. (2015) evaluated and demonstrated the ability of selected lactobacilli and yeast strains to provide a more controlled fermentation, with positive effects on the overall quality of table olives (higher amounts of free amino acids, phenolic and volatile organic compounds, and a better sensory profile). On the basis of these results, the aim of this work was to investigate whether the observed difference in quality of fermented table olives was also present in thermally stabilized olive-based paste, considering that the thermal treatment is known to affect sensory and nutritional quality. The study also investigated the main physical and chemical parameters, as well as the sensory implications of these products.

## 2. Materials and methods

### 2.1. Table olives and olive-based paste preparation

Fermented green table olives were produced as reported in a previous paper (De Angelis et al., 2015). The strains used to ferment table olives were previously selected based on their ability to grow in olive brine, to hydrolyze oleuropein and antimicrobial activity (De Angelis et al., 2015). Before processing, olives with mechanical or insect damage were discarded. Sorted and washed olives of the Italian cv. *Bella di Cerignola* – from the 2015/2016 crop and provided by a local farm (Puglia Conserve srl) sited in Modugno (Apulia, Italy) – were fermented in the laboratory according to the traditional Apulia region "natural-style" method in plastic containers with 25 kg of olives and 25 L of brine (7% of NaCl) at room temperature (18–25 °C). Olive-based paste (ObP) was prepared in the same way and all the treatments (Trs) were different from the use of different starter cultures. Overall, four different trials were performed: conventional fermentation by indigenous bacteria and yeasts, used as control (Tr1); fermentation by commercial *Lactobacillus plantarum* (Sacco srl, Como, Italy) as starter (Tr2); fermentation by commercial *L. plantarum* and selected autochthonous yeast *Wickerhamomyces anomalus* DiSSPA73 (Tr3); and fermentation by commercial *L. plantarum*, *W. anomalus* DiSSPA73, *L. plantarum* DiSSPA1A7, and *Lactobacillus pentosus* DiSSPA7 (Tr4), isolated from table olives (cv. Bella di Cerignola) and genetically identified (De Angelis et al., 2015). Each strain was inoculated in the container of olives at a final cell density of ca. 7 log CFU $mL^{-1}$ of brine. Ingredient composition of ObP was the following: 770 g $kg^{-1}$ of pitted fermented table olives (three sub-lots of about 1 kg for fermentation trial to make three replicates for trial), 155 g $kg^{-1}$ cv. *Coratina* extra virgin olive oil, and 75 g $kg^{-1}$ of grilled zucchini. All ingredients were mixed by a homogenizer (WFP16SE, Waring Commercial, Torrington, USA) for 5 min until obtaining a homogeneous creamy paste, packed in transparent glass jars, having the capacity of 80 mL, and subjected to a bain-marie thermal stabilization treatment (85 °C × 60 min) to be then cooled to room temperature. Overall, four kinds of ObP were obtained on which microbiological, physicochemical and sensory analyses were carried out.

### 2.2. Microbiological analyses

ObP samples (5 g) were diluted with 45 mL of sterilized physiological solution and homogenized using a Stomacher 400 lab blender (Seward Medical, London) for 3 min. Cell densities of presumptive bacteria, yeasts and moulds were determined according to Cosmai et al. (2017).

### 2.3. Physicochemical analyses on the olive-based paste

The measurement of pH, colorimetric indices and hardness were carried out according to Cosmai et al. (2017). The concentration of organic acids, ethanol, and free amino acids (FAA) was determined on water-soluble extracts obtained by centrifugation of ten grams of sample at 1756 *g* $min^{-1}$ for 10 min for removing oily fraction. The pellet was diluted with perchloric acid 1:1 (w/w) and incubated overnight at 4 °C. The suspension obtained was filtered to remove solid fraction and the liquid suspension was centrifuged at 15805 *g* $min^{-1}$ for 10 min. The supernatant was filtered through a Millex-HA 0.22 mm pore-size filter (Millipore Co., Bedford, MA). Analysis of lactic and acetic acids, and ethanol was carried out through HPLC (Zeppa, Conterno, & Gerbi, 2001). The concentration of FAA was determined using a Biochrom 30 Amino Acid Analyser (Biochrom LTD, Cambridge Science Park, England) (De Angelis et al., 2007). A mixture of amino acids at known concentration was used as external standard.

The extraction of phenolic compounds form the ObP was carried by liquid-liquid extraction, and the identification by HPLC analysis following the procedure reported in De Angelis et al. (2015). The extracts for HPLC analysis were prepared with the same procedure but with the addition of the internal standard (0.25 mL of gallic acid at the concentration of 100 mg $L^{-1}$).

For the volatile compounds determination, the sample (1.00 g ± 0.05) was weighed into 20-mL vials, sealed with a screw top aluminum cap and pierceable butyl rubber septa, and submitted to the SPME/GC-MS analysis using a 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (Supelco, Bellefonte, USA) in the conditions reported in Cosmai et al. (2017). The volatile compounds were identified by comparison with the mass spectra present in the NIST and Wiley libraries, and quantified by standardizing the peak areas of compounds of interest with the peak area of 1-propanol (100 μL of a solution at 160 mg $L^{-1}$).

### 2.4. Sensory analysis

Sensory analysis provided a group of eight trained panelists, from 25 to 50 years old, recruited among researchers and technicians of the Food Science and Technology Unit of Bari University. The identification of the descriptors was carried out by considering those usually reported in the literature for olive paste (Aka-Kayguluoglu, Akpinar-Bayizit, & Sahin-Cebeci, 2014; Alvarenga et al., 2012). Five descriptors were selected to be inserted in the evaluation sheet: one for visual evaluation (color homogeneity); two for taste (bitter and acid) and flavor (olive and off-flavor). The descriptor intensity was expressed by panelists on a 10-cm non-structured line scale (IOC, 2011; De Angelis et al., 2015). The panelists determined the intensities of the attributes listed in the profile sheet by using a ruler to measure the segment running from the origin of the scale to the mark made by the taster. In the panel session, the samples were coded by three-digit numbers, served in plastic cups, and tap water was provided between samples to cleanse the palate.

### 2.5. Analytical determinations on the lipid fraction

On the lipid fraction, previously extracted by washing with petroleum ether, were carried out the determinations of total carotenoids and tocopherols according to Caponio et al. (2014).