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Effect of starter cultures on fermentation of naturally and alkali-treated *cv.* Conservolea green olives

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ABSTRACT

The effect of starter cultures, i.e. a commercial (CM, based on *Lactobacillus pentosus* appropriate for olives fermentation) and an autochthonous (OSC, developed by oleuropeinolytic strains belonging to the *Lactobacillus plantarum* group, isolated from olives) on the microbiological profile and physicochemical traits during the fermentation of naturally (untreated) and alkali-treated *cv.* Conservolea green olives was studied in comparison to uninoculated spontaneous fermentation (SP, control). In addition, the reduction of the natural bitterness of the product during processing was assessed. Lactic acid bacteria in OSC treatment were dominated faster and grown in higher populations than CM or SP during the first ten days of the fermentation, resulting in a more intense drop of the brine pH leading to a faster fermentation. Fruit phenolic compounds and antioxidant capacity decreased more in alkali-treated than in naturally processed olives. In alkali-treated olives the bitter taste was not detectable regardless the fermentation process applied. However, in the naturally processed olives the OSC treatment resulted in a medium-low while CM or SP in medium to medium-high bitter taste, indicating that the use of OSC might be a promising tool for the efficiency of processing of naturally green olives.

1. Introduction

Table olives are an important fermented product in particular in producer countries. The world demand for fermented table olives is steadily increasing (IOC, 2017) because of their nutritional and palatable characteristics. The olive fruit is appreciated for its phenolic content and the associated antioxidant activity (Landete, Curiel, Rodrígues, de las Rivas, & Muñoz, 2008). 'Conservolea' is the most widespread cultivar in Greece processed as green olives by Spanish-style and naturally black olives by Greek-style depending on maturity and the period of harvesting of the fruits. The primary purpose of table olive fermentation is to achieve a preservation effect and enhance the organoleptic attributes. In addition, the elimination of the natural bitterness is of importance to make the fruit palatable. The phenolic compounds in the raw fruit, particularly the bitter glucoside oleuropein, requires the sodium hydroxide treatment for the debittering of Spanish-style green olives in a short period of time. On the other hand, natural green olives, which are not treated with alkali, lose their bitterness slowly for months or even a year (Ramirez, Brenes, De Castro, Romero, & Medina, 2017).

The Spanish-style processing includes: harvesting, sorting, washing, lye treatment (1.8–2.0 g NaOH/100 ml H_2O) to hydrolyse the bitter constituent oleuropein, washing to remove the excess of alkali and finally brining (6–8 g NaCl/100 ml H_2O); the olives during storage in brine undergo a spontaneous fermentation mainly by lactic acid bacteria (LAB) (Aponte et al., 2012). Acidification of the brine with CO_2 and lactic/HCl acids has been reported to result in successful lactic fermentation processes (Vergara, Blana, Mallouchos, Stamatiou, & Panagou, 2013. The main drawback of Spanish-style processing is a dramatic loss of total phenolics from olive flesh, while natural olive processing favours a higher retention of biophenols (Marsilio et al., 2005). Moreover, a high volume of wastewater is generated in order to remove alkali. The interest to remove the bitterness of the olives without the use of a NaOH solution and to provide a desirable product for consumers has increased in recent years. This can be achieved by starter-driven fermentations performed inoculating a starter formulation able to hydrolyse oleuropein and dominate the fermentation process.

The LAB strains which can be used in table olive fermentation as starter culture consist of *L. plantarum* (Marsilio et al., 2005; Sabatini, Mucciarella, & Marsilio, 2008) or *L. pentosus* (Panagou & Tassou, 2006; Panagou, Schillinger, Franz, & Nychas, 2008). To achieve dominance over the indigenous microbiota, starter cultures are selected according to criteria that include homo- and hetero-fermentation, organic acid

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production, salt tolerance, acid tolerance, flavour development, temperature range, oleuropein-splitting capability and bacteriocin production (Corsetti, Perpetuini, Schirone, Tofalo, & Suzzi, 2012; Kotzekidou & Tsakalidou, 2006). In addition, starter cultures are recommended to reduce the probability of spoilage and avoid any deviation of typical organoleptic quality during table olive industrial process. *L. plantarum* and *L. pentosus* strains which provide high LAB counts during fermentation, reduce the survival time of Enterobacteriaceae and decrease the pH to desirable levels have been used (Aponte et al., 2012; Hurtado, Reguant, Bordons, & Rozès, 2012; Randazzo et al., 2011; Ruiz-Barba & Jiménez-Díaz, 2012). The predominance of a potential probiotic starter culture in large-scale industrial fermentations of green Spanish-style olives is favored by inoculation immediately after brining to prevent wild initial microbiota growth; followed by re-inoculation 24 h later to improve competitiveness (Rodríguez-Gómez et al., 2017).

Commercial starter cultures are already available on the market. Although the interest in their application is increasing steadily, their use is still not common. The reason is that the microbiological control of the process cannot be achieved because the strains used have not been optimized for this particular fermentation (Ruiz-Barba & Jiménez-Díaz, 2012). Well-defined autochthonous bacteria are adapted and competitive in olive fruit and can be used as starter culture (Tataridou & Kotzekidou, 2015). *L. plantarum* is the species frequently used to ferment plant food products where phenolic compounds are abundant as this bacterium possesses relevant enzymatic activities to obtain bioactive compounds. Complex phenolics are hydrolyzed by *L. plantarum* to simpler and more biologically active compounds leading to a functional food. In addition, the adaptative behavior of *L. plantarum* under stress induced by phenolics also modulates traits beneficial for its gastrointestinal survival (Rodríguez et al., 2009). *L. plantarum* strains which contribute to degradation of phenolic compounds can lead to the development of an autochthonous starter culture in order to achieve the biological debittering of olives (Kaltsa, Papaliaga, Papaioannou, & Kotzekidou, 2015).

The aim of the present work was to determine the effect and evaluate the efficiency of a commercial and an autochthonous starter culture (composed of five oleuropeinolytic strains described in detail by Kaltsa et al., 2015) compared to spontaneous fermentation on the microbiological profile and physicochemical parameters during the fermentation of *cv*. Conservolea green olives. The effect of starter cultures on total phenolics, antioxidant capacity and reduction of bitterness was evaluated in naturally (without any debittering pre-treatment) and alkali treated green olives compared to uninoculated spontaneous process.

2. Materials and methods

2.1. Preparation of starter cultures

The commercial culture (CM) preparation 'Vege-Start 60' (purchased by Chr. Hansen A/S, Copenhagen, Denmark) was used according to the instructions of the manufacturer. 'Vege-Start 60' is a culture based on pure freeze-dried *Lactobacillus pentosus* appropriate for olives fermentation.

Autochthonous oleuropeinolytic starter culture (OSC) was prepared as described in detail by Tataridou and Kotzekidou (2015) and consists of equal populations of each one of the following *L. plantarum* strains: Lp 15, Lp 20, Lp 28, Lp 40 and Lp 48. The characteristics and the oleuropeinolytic potential of the strains have been described elsewhere (Kaltsa et al., 2015). All strains were isolated from olives and retained in the collection of our laboratory. The selected strains were propagated in 100 ml MRS broth and incubated at 30 °C for 24 h. Cells were harvested by centrifugation (10,000 × g, 10 min), washed twice in saline water (0.85 g NaCl/100 ml), and finally resuspended in 10 ml sterile saline water. The cell density was determined by total viable count after plating the serial dilutions (in a diluent containing peptone from casein (Scharlau) 0.1 g/100 ml and NaCl 0.85 g/100 ml) on MRS agar (MRSA, Merck, Darmstadt, Germany) followed by incubation at 30 °C for 2 days. Finally, the volume calculated to obtain the desired population of each culture in the mixture and inoculated into the brine as wet inoculum.

Table 1
Characteristics of an autochthonous (consisted of oleuropeinolytic *Lactobacillus plantarum* strains) and a commercial (based on *Lactobacillus pentosus*) starter culture.

Characteristics	Starter culture	
	Autochthonous (OSC)[a]	Commercial (CM)[b]
Temperature growth range	15-40 °C	15-40 °C
Growth in pH range	3 < pH < 8	3.7 < pH < 8
Tolerance in NaCl	8%	4-8%
1% Oleuropein biodegradation (in MRS broth)	> 97%	n.r.

n.r.: not reported.
[a] Consists of five oleuropeinolytic *Lactobacillus plantarum* strains as reported in detail by Kaltsa et al. (2015) and Tataridou and Kotzekidou (2015).
[b] "Vege Start-60" appropriate for olives fermentation purchased by Chr. Hansen A/S, Copenhagen, Denmark.

Some characteristics of both autochthonous and commercial starter culture are presented in Table 1.

2.2. Olive processing

'Conservolea' green olives were harvested in October from the University farm at Thessaloniki from olive trees of uniform load in random to minimize the maturation and environmental effects. Fruits were hand screened for various defects such as blemishes, defects, and insect damage and uniform colour. The olives were divided in two lots and subjected to processing as naturally (untreated) olives or alkali-treated elaboration (i.e. olives immersed in a solution containing 1.8 g NaOH/100 ml H_2O for approximately 8 h at room temperature until the alkali reached 2/3 of the flesh as measured from the epidermis to the pit, checking the penetration using the phenolphthalein indicator, followed by rinsing of olives to remove excess alkali) in our laboratory. Olives of each lot were further divided into three sublots each; three replications of 1 kg per sublot, placed in 2 L PVC fermentation vessels and covered with 1 L brine consisting of 6 g NaCl/100 ml H_2O. The olives remained in brine for equilibrium for 24 h at room temperature.

Naturally and alkali-treated olives were subjected to the following treatments: In the first treatment the fermentation was carried out by the spontaneous microbiota (SP – control treatment); in the second treatment the brine was inoculated with 1×10^8 CFU/ml of the commercial culture Vege-Start 60 (CM); and in the third treatment the brine was inoculated with 1.1×10^8 CFU/ml of the autochthonous starter culture (OSC). Fermentation vessels were kept at room temperature. In all cases, brines were periodically mixed and salt concentration maintained fixed at the initial level of 6 g NaCl/100 ml H_2O by addition of dry salt. The experimental trials were carried out in triplicate and all measurements were performed in three independent samples.

2.3. Microbiological analysis

At specific time intervals, brine samples (10 ml) were withdrawn and aseptically transferred to 90 ml sterile 1/4 Ringer's solution. Decimal dilutions in the same Ringer's solution were prepared and duplicate 1 or 0.1 ml samples of the appropriate dilutions were inoculated on the following agar media: Man Rogosa Sharpe agar supplemented with 200 ppm cyclohexamide and 0.02% w/v sodium azide grown anaerobically (GasPak, BBL, Cockeysville, MD) for counting lactic acid bacteria population, after incubation at 30 °C for 72 h; Potato