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Volatile compounds in uninoculated and inoculated table olives with *Lactobacillus plantarum* (*Olea europaea* L., cv. Moresca and Kalamata)

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Abstract

Table olives' flavour plays an important role in consumer's acceptability and it depends on various factors such as varieties, intrinsic characteristics, ripening of fruit and processing technologies. Flavour biogenesis is also influenced by addition in brine medium of lactic acid bacteria as inoculants, which reduce spoilage risks and improve sensory characteristics. In this work, flavour profiles of uninoculated and inoculated table olives with *Lactobacillus plantarum* (cv. Moresca and Kalamata) have been analytically characterised and compared. Twenty-one volatile compounds comprise alcohols, aldehydes, ketones, esters as well as acids formed during Greek-style olive fermentation (3 months brining after) have been characterised by gas chromatography and GC/mass spectrometry. Very high contents of ethanol and appreciable amounts of ethyl acetate, isobutanol, 2-butanone, 1-propanol and 1-hexanol were revealed in all samples with a significant increase in inoculated samples with respect to uninoculated ones. Also 1-butanol, 3-pentanol, 3-hydroxy-2-butanone, *cis*-3-hexen-1-ol and 2-butanol which were present in lower amounts, disclosed a meaningful increase in inoculated samples of both varieties, especially in Moresca inoculated sample. Acetic acid, isopentanol, 2-pentanol, propyl acetate, ethyl propanoate and 4-penten-1-ol showed a significant increase in inoculated Kalamata sample. These results showed that inoculation of brine medium with lactic acid bacteria starters significantly influenced aroma profiles of both varieties, in particular an increase in concentration of various flavour compounds has been revealed in inoculated table olives.
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Keywords: *Olea europaea* L.; Table olives; Volatile compounds; *Lactobacillus plantarum*

1. Introduction

Table olives are currently the most important fermented vegetable products in the developed world. Worldwide production is around 1.5 million tons, of which nearly half is produced in European Union, predominantly in Spain, Greece, Italy and Portugal (IOOC, 2005). For table olive consumption, the fruits are opportunely processed and served as an appetizer or as a complement to salads, pasta, pizza and other foods (Marsilio, Russi, Iannucci, & Sabatini, 2008). The main purposes of table olives fermentation are to improve the preservation and the organoleptic properties of the final product. An important criterion to determine the effectiveness of fermentation is the concentration of lactic acid. However, the production of other end products of microbial metabolism, such as volatile compounds present in large or trace concentrations, may affect the sensory properties of table olives, especially flavour and aroma (Panagou & Tassou, 2006). Table olives' well-odours test a good fermentation and quality of the end product. The production of volatile flavour components tends to be the first mechanism considered for the development of a specific flavour to a particular fermented food (McFeeters, 2004). Flavour is tight connected with the quali-quantitative composition of volatile compounds playing an important role in consumer's acceptability (Koprivnjak, Conte, & Totis, 2002; Sabatini & Marsilio, 2008). Volatile compounds are not produced in significant amounts during fruit growth but increase during the climacteric stage of ripening and during fermentation process (Kalua et al., 2007). Fermenting olives are typically very complex ecosystems with active

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enzyme systems from the raw materials interacting with the metabolic activities of microorganisms (McFeeters, 2004). Fermented foods are popular throughout the world and the production of fermented food products is important in many countries in providing income and employment. Fermentation is a technique that has been employed for generations to preserve food for consumption at a later date and to improve food security. The lowering of the pH operated from lactic acid bacteria inhibits the growth of food spoiling or poisoning bacteria and destroys certain pathogens (Hammes & Tichaczek, 1994).

Furthermore, fermentation can improve the flavour and appearance of food. In food fermentations, the by-products play a beneficial role in preserving and changing the texture and flavour of the food substrate. For example, acetic acid is the by-product of the fermentations of some fruits. This acid not only affects the flavour of the final product, but more importantly has a preservative effect on the food. Several alcohol-fermented foods are preceded by an acid fermentation and in the presence of oxygen and *Acetobacter*, alcohol can be fermented to produce acetic acid. *Lactobacillus plantarum* has been known to play a preponderant role in olive fermentation. This species has been extensively studied also with the aim of its use as *starter cultures*. Lactic acid bacteria are employed in olive fermentation to enhance the olive preservation due to a progressive acidification of the fermenting brine with a consequent pH decrease and the production of antimicrobial substances and bacteriocins (Marsilio et al., 2005; Ruiz et al., 2005; Ruiz-Barba, Cathcart, Warner, & Jiménez-Díaz, 1994). Also, the use of lactic acid bacteria as inoculants during olive fermentation is a practice that improves the current processing technologies. The inhibitory effect of lactic acid bacteria is due to the accumulation of main primary metabolites (lactic and acetic acids, ethanol and carbon dioxide) as well as to the production of other antimicrobial compounds, such as formic and benzoic acids, hydrogen peroxide, diacetyl, acetoin and bacteriocins (Delgado, Brito, Fevereiro, Peres, & Figueiredo-Marques, 2001). Starter cultures also prevent contamination of secondary microflora responsible for the gas pocket in the olives (Leal-Sánchez, Jiménez-Díaz, Maldonado-Barragán, Garrido-Fernández, & Ruiz-Barba, 2002). They also improve the aroma and flavour characteristics of the product (Borcakli, Özay, & Alperden, 1995), so it is of a great interest to study the effect of starter cultures on the quali-quantitative composition of volatile compounds in most important industrial varieties of table olives. Until today, the determination of volatile compounds in treated green olives fermentation has attracted little attention, mainly by Spanish researches to detects spoilage incidents (García-García, Romero-Barranco, Durán-Quintana, & Garrido-Fernández, 2004; Montaño, De Castro, & Rejano, 1992; Montaño, Sánchez, & De Castro, 1993; Montaño, Sánchez, & Rejano, 1990), whereas no data are available in literature for Greek table olives (Panagou & Tassou, 2006). In this work, *Greek-style* table olives' flavour profiles of two different cultivars (Moresca and Kalamata) have been compared. In addition, the effects of a selected lactic acid bacteria starter culture on the olive fermentation and volatile compounds biogenesis have been assessed.

2. Materials and methods

2.1. Plant material and processing

Olive fruits of Moresca and Kalamata cultivar from Brindisi area (Italy) were used in this study. Olives, hand harvested at the black ripening stage, were processed by Greek method, according to the Unified Qualitative Standard applying to Table Olives in International Trade (International Olive Oil Council, 2004). Olives were washed twice with water and directly put into a brine solution made up of 7% w/v NaCl and fermented at room temperature (Greek method).

A selected oleuropeinolytic *L. plantarum* bacterial strain (LAB B1-2001) was used as inoculant. The bacterial strain was propagated in MRS broth (Oxoid) for 18 h at 30 °C, then the culture was inoculated into MRS broth and incubated until the exponential phase of growth was reached. Cells were pelleted by centrifugation at 10,000×*g* for 15 min at 4 °C, washed twice with sterile water, and suspended at a concentration of ca. 10^{10} CFU/mL. The culture was then inoculated into the fermenting brine in a ratio of 40 ml/L. All the fermentation processes were carried out at ambient temperatures. The experimental trials were carried out in triplicate. Fig. 1 shows the experimental set up.

2.2. Volatile compounds extraction

Dynamic headspace method (Solinas, Marsilio, & Angerosa, 1987), largely used to analyze quality and quantity flavour molecules of olive oil has been updated and used in this work to extract volatile compounds. Chemical−physical characteristics of table olives are different from that of olive oil. Fermented

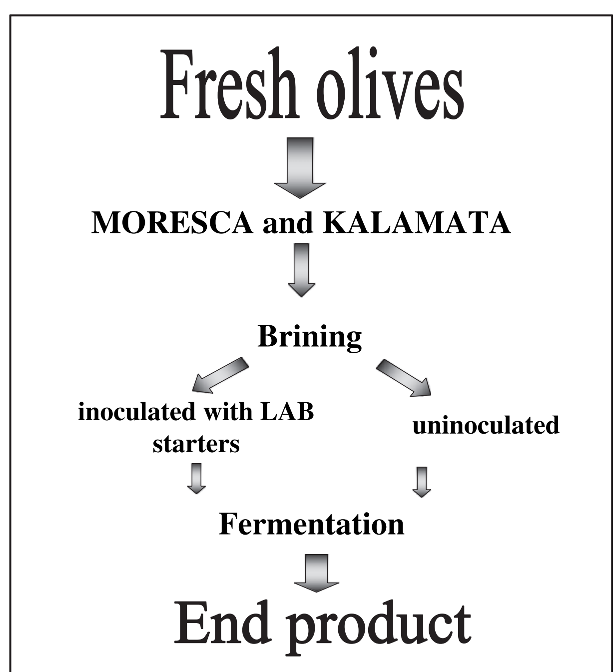

Fig. 1. Experimental set up.

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