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An investigation on molecular partition of aroma compounds in fruit matrix and brine medium of fermented table olives

Nadia Sabatini [a,*], Enzo Perri [b], Vincenzo Marsilio [a]

[a] CRA-Centro di Ricerca per l'Olivicoltura e l'Industria Olearia Sede Scientifica di Pescara, Viale Petruzzi 75, 65013 Citta' S. Angelo (PE), Italy
[b] CRA-Centro di Ricerca per l'Olivicoltura e l'Industria Olearia, C.da li Rocchi, 87036, Rende (CS), Italy

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ABSTRACT

Aroma is considered as a quality index of olive products. Fermented olives aroma compounds are present both in the fruit matrix and in brine medium. The partition of aroma compounds between fruit matrix and brine medium is deeply different for the two and depends on several factors such as carbon chain length and/or branching, number of polar groups, sodium salt concentration, temperature, etc. In this work, an investigation on volatile compounds quali-quantitative partition in fruit matrix and in brine medium of Greek-style *Carolea* and *Nocellara del Belice* table olives has been assessed. Volatile compounds have been extracted by using headspace method for olive fruit and by solvent extraction and distillation for brine medium. Twenty-three volatile compounds in fruit matrix and fifteen aroma molecules in brine medium have been identified by Gas Chromatography and GC/Mass Spectrometry. Results showed that most volatile organic compounds had a major affinity for the fruit matrix depending both on the chemical characteristics of the molecules (chain length and branching, polar or no polar groups, etc.) and on the "salting out" effect due to high NaCl concentration of the brine, which brought aroma compounds to hydrophobic phase of the olive fruit.

Industrial relevance: The study could be potentially helpful to develop analytical methods in order to estimate the quail-quantitative composition during the fermentation process. So that it would be possible to implement automatic analytical procedures in the currently used plants for the industrial production for table olives. Furthermore, this study allow us to identify off-flavours formed by anomalous fermentation process, in order to reveal them (both in the fruit and in the brine) in premature times (just in small traces), so to obtain the fermentation process recovery.

1. Introduction

Olive product, essentially olive oil, but also table olives, are the most principle sources of unsaturated lipids of Mediterranean diet. The interest of scientific research about table olives is strongly arisen in last years, above all for the minor compounds. For table olives consumption, 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). There are three basic commercial preparations in the international market, namely Spanish style green olives, naturally olives (Greek-style) and black ripe olives (Californian style), for which elaboration processes are well-established in the literature (Panagou, Tassou, 2006; Garrido-Fernández, Fernández Dìez, & Adams, 1997). In general, any processing method aims to remove the natural bitterness of the fruit, caused by the glucoside oleuropein. Microorganism plays an important role in the generation of natural compounds, particularly in the field of food aromas (Medeiros et al., 2001).

The primary purpose of table olive fermentation is to achieve a preservation effect and to enhance the organoleptic properties of the final product (Panagou and Tassou, 2006). Flavour is a very complex sensation composed primarily of aroma and taste but also complemented by tactile and temperature response (Heath, 1981). Flavour is tight connected with the quali-quantitative composition of volatile compounds and it is considered as a quality index of olive products, playing an important role in consumer's acceptability (Koprivnjak, Conte & Totis, 2002; Kalua et al., 2007). Food fermentation processes often result in profound changes in flavour relative to the starting ingredients. The changes that occur during fermentation of foods are the result of enzymatic activity. Fermenting olives represent a very complex ecosystem with active enzymes system from the ingredient material interacting with the metabolic activities of microorganisms (McFeeters, 2004). During fermentation, flavour compounds can be formed by indigenous lactic acid bacteria and yeasts, together with other contaminating microorganism, which produce volatile compounds

* Corresponding author. Tel.: +39 85 95212; fax: +39 085 959518.
E-mail address: nadiasabatini@libero.it (N. Sabatini).

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from major fruit constituents through various biochemical pathways (Sabatini & Marsilio, 2008).

During olive fermentation acids such as lactic and acetic acid are produced, increasing the acidity level of the brine. The combination of high salt and low pH greatly reduces the risk of microbial spoilage of the olives (Zervakis, 2006). Moreover alcohols, esters, aldehydes, and ketons as well as other acids are known to be formed by microorganisms which are competitive with lactic acid bacteria in brined olives (Fleming, Etchells, & Bell, 1969). The lactic acid bacteria belong to two main groups the homofermenters and the heterofermenters. Homofermenters produce mainly lactic acid, via the glycolytic (Embden–Meyerhof) pathway. Heterofermenters produce lactic acid plus appreciable amounts of ethanol, acetate and carbon dioxide, via the 6-phosphoglucanate/phosphoketolase pathway. Yeasts and yeasts-like fungi are widely distributed in nature. Like bacteria, yeasts can have beneficial and non-beneficial effects in foods. The fermentation is usually initiated by yeasts which break down glucose into ethanol with the liberation of carbon dioxide gas. Following on from the yeasts, *Acetobacter* oxidises the alcohol to acetic acid and water (FAO corporate document repository). Thus, volatile compounds are produced both in the brine by fermentation process operated by microorganisms and in the fruit matrix by the action of endogenous enzymes (like lipooxygenases) and exogenous enzymes (produced by lactic bacteria, yeast, etc.) by lipids, proteins and amino acids metabolism. The partition of aroma compounds between fruit matrix and brine medium is deeply different for the two and depends on several factors such as carbon chain length and/or branching, polar groups, sodium salt concentration, temperature, etc.

So, the study of well and off flavours produced (in the brine and in the fruit) during table olive making process, results of a great utility for completely understanding fermentation process so to avoid spoilage risks. In this work a comparative study on volatile compound quali-quantitative partition in fruit matrix and in brine medium during Greek-style fermentation of *Carolea* and *Nocellara del Belice* table olives has been assessed. Furthermore, by using various references the biogenesis and metabolism of volatiles produced during fermentation process have been explained.

2. Experimental

2.1. Plant material and processing

Olive fruits of Nocellara del Belice cultivar from Castelvetrano area (Sicily—Italy) and Carolea cultivar from Città S. Angelo (PE) (Abruzzo—Italy) were used in this study. Olives, hand harvested at the green 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). In the Greek method the olives were directly brined without any treatment in a 7% w/v NaCl solution and naturally fermented at room temperature.

2.2. Reagents

Ethyl acetate, 2-butanone, 3-methyl-1-butanal, ethanol, ethyl propanoate, propyl acetate, 2-butanol, 1-propanol, n-propyl propanoate, isobutanol, 3-pentanol, 2-pentanol, 1-butanol, isopentanol, 1-pentanol, 1-hexanol, cis-3-esen-1-ol, styrene, ethylbenzene, o-xylene, hexanal, acetic acid, propionic acid and 1-nonanol were purchased from Sigma-Aldrich (St Louis, MO, USA). Activated charcoal (0.5–0.85 mm;20–35 mesh ASTM) was from Merck (Stuttgart, Germany).

2.3. Volatile compound extraction from fruit matrix

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 olive is a solid phase made up by hydrophilic and hydrophobic portions, while olive oil is only a hydrophobic liquid phase. For this reason it has been necessary to change some parameters linked with the technique described for olive oil. Temperature of extraction was diminished to 30–33 °C (37 °C for olive oil). So sixty grams of stoned olive fruits were put into a 120 mL Drechsel gas washing bottle. Volatiles were stripped with N_2 (1.0 $dm^3 min^{-1}$) at 33 °C for 2 h, trapped on 100 mg of activated charcoal and then eluted with 1 mL of diethyl ether (Sabatini & Marsilio, 2008).

2.4. Volatile compound extraction from brine

Volatile compounds have been extracted by using 1:10 rate of diethyl-ether/brine medium and distilled at 50 °C for 2 h. NaCl content of brine medium was brought from 7 to 15% w/v to enhance the "salting out" effect of the volatile organic compounds from aqueous phase to organic phase. The volatile fraction distilled has been added with Na_2SO_4 anhydrous and filtered to remove water excess.

2.5. GC analysis

Gas chromatography was carried out with a Carlo Erba (Milan, Italy) 5160 Mega series instrument equipped with a flame ionization detector (FID) and a Supelcowax-10 capillary column (Supelco, Sigma-Aldrich) 60 m × 0.3 mm (id), 0,1 μm film thickness was used, with Hydrogen as carrier gas at 40 kPa. The column temperature was programmed as follows: at 35 °C for 10 min, from 35 to 45 °C at 0.8 °C/

Table 1
Volatile compounds extracted from fruit matrix by GC–GC/MS expressed as $\mu g_{(compound)}/kg_{(fruit)} \pm$ S.D.

Compound	*Nocellara del Belice*	*Carolea*	Compound	*Nocellara del Belice*	*Carolea*
1 n-Octane	9.0* ± 0.7	3.0* ± 0.4	13 3-Pentanol	7.0 ± 0.4	n.d.
2 Ethyl-acetate	71.0 ± 3.5	67.0 ± 5.0	14 2-Pentanol	10.0 ± 0.1	n.d.
3 2-Butanone	82.0 ± 5.0	89.0 ± 6.0	15 1-Butanol	8.0 ± 0.6	7.5 ± 0.4
4 Methanol	n.d.	5.0 ± 0.3	16 Isopentanol	475.0 ± 38.0	579.0 ± 40.0
5 3-Methyl-1-butanal	n.d.	11.0 ± 1.0	17 1-Pentanol	13.0 * ± 0.1	2.0 * ± 0.1
6 Ethanol	1.262.0 ± 100.0	4.600.0 ± 400	18 4-Penten-1-ol	1.5 ± 0.1	2.0 ± 0.2
7 Propyl-acetate	6.0 ± 0.4	n.d.	19 3-Hydroxy-2-butanone	35.0 ± 2.0	50.0 ± 4.0
8 Ethyl-propanoate	2.5 ± 0.1	n.d.	20 1-Hexanol	96.0* ± 4.0	18.0* ± 1.0
9 2-Butanol	2.717.0* ± 210.0	22.0* ± 2.0	21 Cis-3-hexen-1-ol	86.0* ± 6.0	23.0* ± 3.0
10 1-Propanol	121.0* ± 10	33.0* ± 2.0	22 1-Nonanal	27.0* ± 1.5	6.0* ± 0.4
11 1-Hexanal	6.0* ± 0.5	1.5* ± 0.1	23 Acetic acid	8.114.0* ± 750	609.0* ± 35.0
12 Isobutanol	25.0 ± 2.0	214.0 ± 15.0			

Each value is the mean of triplicate analyses expressed in $\mu g_{(compound)}/kg$(olive fruit) ± S.D. (* = $p<0.05$ statistical significance *Nocellara del Belice vs Carolea*). n.d.: not determined.