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Volatile compounds in table olives (*Olea Europaea* L., *Nocellara del Belice* cultivar)

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Abstract

Table olive quality depends on a number of factors, including fruit characteristics, ripening and processing technologies. Volatile and semi-volatile organic compounds, present both in the sample matrix and in the headspace aroma, are responsible for the olive fruit flavour influencing the consumer's preference.

In this study, volatile compounds, in Spanish-style, Greek-style and Castelvetrano-style green olives of the *Nocellara del Belice* cultivar, were analytically characterized. Twenty-two compounds, comprising alcohols, aldehydes, ketones, esters as well as acids, formed during olive fermentation (after six, seven and eight months of brining), were extracted by the dynamic headspace technique and identified by gas chromatography and GC/mass spectrometry. Results suggested that different processing technologies significantly affected the volatile compounds of samples. In particular, meaningful differences in aromatic profiles could cause qualitative and quantitative differences in quality. Thus, a deepened knowledge of chemical features, together with an insight of the biosynthetic pathways of table olive flavour compounds, may be very useful for design and production of a success product.

Keywords: *Olea europaea* L.; Table olives; Volatile compounds; Headspace analysis

1. Introduction

High-quality table olives, the most popular fermented agro-food in the Mediterranean countries, are characterized by a pleasant fruit flavour.

Aroma compound composition in table olives depends on several factors, such as genetics, ripening degree of fruits, and processing conditions (Garrido Fernandez, Fernandez Diez, & Adams, 1997; Ruiz et al., 2005).

A pleasant fragrance derives from the equilibrium of a number of volatile substances, such as hydrocarbons, alcohols, aldehydes, ketones, esters and other compounds.

Processing technology greatly influences the chemico-physical composition of table olives and, consequently the final product. Flavour is closely 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). Changes in olive aroma allow us to compare different cultivars and processing methods, as well as to follow the evolution of quality during processing and to check off-flavours produced during fruit storage. Volatile compounds are not produced in significant amounts during fruit growth but arise during the climacteric stage of ripening (Kalua et al., 2007). Although, in the literature, a lot is known about aroma compounds in olive oil, very little is known about the quali-quantitative composition of volatile compounds in table olives, whose matrix is profoundly different from that of olive oil.

Olive oil volatile compounds are produced by enzymatic pathways during the milling process. Thus, in a good quality olive oil, good flavours are produced only by physical processes which activate endogenous enzymes, and not by a fermentative process as in table olives.

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Flavour compound formation in table olives is a dynamic process that develops mainly during the olive fermentation by indigenous lactic acid bacteria and yeasts, together with a variety of contaminating microorganisms, which produce volatile compounds from major fruit constituents through various biochemical pathways. Fermenting olives are typically very complex ecosystems with active enzyme systems from the ingredient material affecting the metabolic activities of microorganisms (McFeeters, 2004). Lye treatment and fermentation, both commonly used in most table olive preparations, cause chemical and physical changes (Bianchi, 2003; Jimenez, Guillen, Fernandez, & Heredia, 1997; Marsilio, Lanza, & Pozzi, 1996).

Lactic acid bacteria directly influence the flavour of fruits, contributing to the development of organoleptic characteristics of fermented olives, but there are less direct ways by which microorganisms affect flavour (McFeeters, 2004). Moreover alcohols, esters, aldehydes, and ketones, as well as acids, are known to be formed by microorganisms which are competitive with lactic acid bacteria in brined olives (Fleming, Etchells, & Bell, 1969). Thus, it is important, not only to investigate olive fermentation from a microbiological point of view, but also to determine volatile and semi-volatile compound contents and changes of their qualitative and quantitative profiles during the entire production process and consumption. In this study, the dynamic headspace method (Solinas, Marsilio, & Angerosa, 1987), largely used to analyze flavour molecules of olive oil, has been updated in relation to the chemical–physical characteristics of table olives and used to extract volatile compounds. Volatile compounds in Spanish-style, Greek-style and Castelvetrano-style green olives of the *Nocellara del Belice* cultivar were analyzed by gas chromatography and mass spectrometry.

2. Materials and methods

2.1. Plant material and processing

Olive fruits of the *Nocellara del Belice* cultivar from the Castelvetrano area (Sicily – Italy) were used in this study. Olives, hand-harvested at the green ripening stage, were processed by Spanish, Greek and Castelvetrano methods, according to the Unified Qualitative Standard applying to Table Olives in International Trade (International Olive Oil Council (IOOC), 2004). Briefly, the olives were lye-treated to remove the natural bitterness, washed with water to remove the excess of alkali and then placed in brine where they underwent a spontaneous lactic fermentation (Spanish-style method) at room temperature. In the Greek method, the olives were directly brined while, in the Castelvetrano method the olives were treated with a mixture of lye and solid salt (NaCl). Also, in the Greek and Castelvetrano methods, olives were spontaneously fermented at room temperature.

2.2. Reagents

Ethyl acetate, 2-butanone, 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, 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

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. The apparatus used for the volatile extraction is schematically depicted in Fig. 1.

2.4. 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/min, from 45 to 200 °C at 5.5 °C min^{-1} and then held there for 20 min. The temperature of the detector was 240 °C. The sample (1 μl) was injected by "on-column" mode. Quantitative analysis was obtained by peak area integration with a Carlo Erba Mega series integrator.

2.5. GC/mass spectrometry analysis

GC/mass spectrometry analysis was carried out with a Thermo Finnigan (San Jose, CA., USA) gas chromatograph coupled to a Polaris Q quadrupole mass-selective spectrometer.

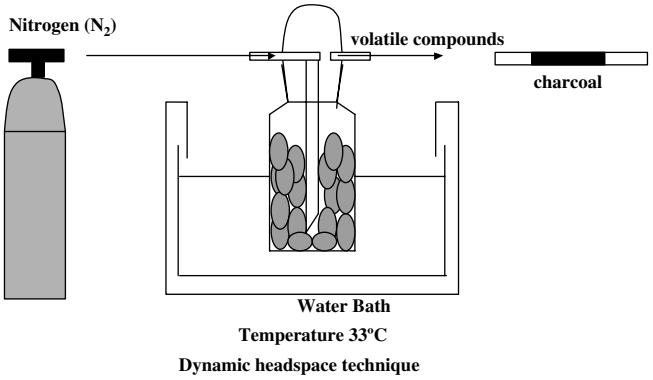

Fig. 1. Description of dynamic headspace technique for olive fruits.

Sample aliquots of 2 μl were injected. Analysis was provided with a split-less injection port.

The GC injector temperature was 200 °C with 50 ml/min split flow and the transfer line temperature was 210 °C. Helium, at 100 kPa, was employed as the carrier gas. The oven temperature programme was run at 39 °C for 10 min, then raised at 5 °C min^{-1} to 200 °C, and held there for 30 min. The ion source temperature of Polaris Q was 250 °C and electron impact mass spectra were recorded at 70 eV.

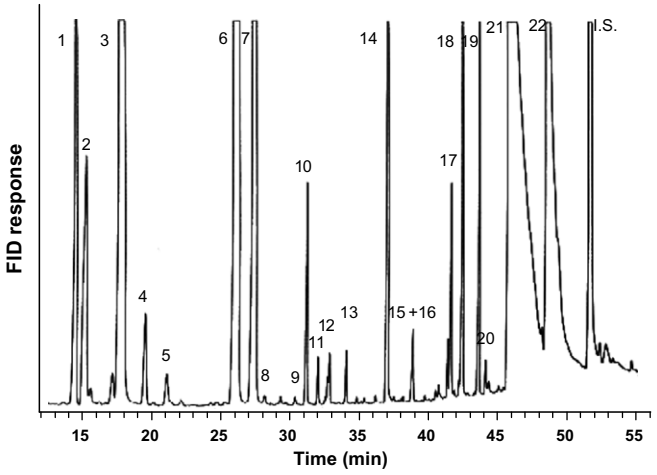

Fig. 2. Typical GC chromatogram of flavour compounds in Greek-style sample. See Table 1 for each number's description.

2.6. Qualitative and quantitative analysis

Compounds were identified by comparison of their mass spectra and retention times with those of standards compounds. 1-Nonanol was used as standard. Each value was the mean of triplicate analyses expressed in $\mu g_{(compound)}/kg_{(olive\ fruit)} \pm SD$.

2.7. Microbiological assays

At given fermentation times, brine samples were withdrawn from the containers and serial dilutions prepared in sterile distilled water for microbiological counts by the standard plate method from each dilution; 0.1 ml was spread on the media plates. Lactic acid bacteria were enumerated on MRS agar (Oxoid) at 30 °C for 72 h and yeasts on malt extract agar (Oxoid) at 28 °C for 72 h. Microorganism enumeration, in each solution, was done in duplicate. Colony forming units per ml of brine (CFU/ml brine) were calculated.

Table 1
Volatile compounds amounts of *Nocellara del Belice* cultivar, after six, seven, eight months of brining extracted by dynamic headspace technique and expressed in $\mu g_{(compound)}/kg_{(olive\ fruit)}$

	Compound	After six month brining			After seven month brining			After eight month brining		
		Greek-style	Spanish-style	Castelvetrano-style	Greek-style	Spanish	Castelvetrano-style	Greek-style	Spanish-style	Castelvetrano-style
1	Ethyl-acetate	26.7 ± 1	45 ± 3.4	40 ± 2.5	315 ± 15	37 ± 1.5	240 ± 1.5	61 ± 3.6	14 ± 0.8	57 ± 3
2	2-Butanone	30.6 ± 2	91 ± 6.1	182 ± 15	152 ± 10	50 ± 3	440 ± 17	117 ± 1.4	32 ± 1.4	107 ± 5
3	Ethanol	474 ± 30	701 ± 20	1119 ± 69	2326 ± 105	369 ± 18	2020 ± 89	740 ± 5.2	200 ± 10	580 ± 22
4	Propyl-acetate	0.8 ± 0.07	83 ± 15	3.3 ± 0.2	17 ± 1.1	53 ± 2.7	25 ± 1.4	17 ± 0.9	33 ± 1	9 ± 0.4
5	Ethyl propanoate	2.2 ± 0.15	130 ± 11	2.2 ± 0.1	49 ± 2.3	77 ± 5.5	0.9 ± 0.03	53 ± 1	43 ± 1	5 ± 0.1
6	2-Butanol	1020 ± 70	2408 ± 90	1496 ± 99	6208 ± 220	1270 ± 68	3375 ± 115	2133 ± 65	670 ± 21	1220 ± 70
7	1-Propanol	45.3 ± 3	1527 ± 11	109 ± 8	1153 ± 74	825 ± 16	323 ± 19	30 ± 1.7	534 ± 2	110 ± 6
8	Propyl-propanoate	n.d.	73 ± 4	1.1 ± 0.08	n.d.	58 ± 3	n.d.	3.6 ± 0.15	42 ± 1.4	n.d.
9	1-Hexanal	2.2 ± 0.3	0.8 ± 0.02	1.7 ± 0.05	2.2 ± 0.1	n.d.	n.d.	1.1 ± 0.6	0.3 ± 0.01	0.5 ± 0.05
10	Isobutanol	9.4 ± 0.4	36 ± 2.3	7.1 ± 0.65	68 ± 4.2	8.3 ± 3	16 ± 0.6	26 ± 1.2	2.2 ± 0.1	6 ± 0.04
11	3-Pentanol	2.5 ± 0.14	14 ± 1.2	14 ± 0.12	12 ± 0.9	8.3 ± 4.5	28 ± 1	7.4 ± 0.3	4 ± 0.1	10 ± 0.8
12	2-Pentanol	1.7 ± 0.15	10.5 ± 0.9	12 ± 0.9	16 ± 1.2	6.6 ± 2.2	25 ± 0.7	10 ± 0.2	3.3 ± 0.08	9 ± 0.09
13	1-Butanol	3 ± 0.1	25 ± 1.3	9.3 ± 0.4	15 ± 0.9	17 ± 1	19 ± 0.8	5.2 ± 2.3	12 ± 0.06	31 ± 2
14	Isopentanol	178 ± 11	138 ± 11	41 ± 0.02	692 ± 26	194 ± 13	94 ± 3	507 ± 25	96 ± 4	34 ± 3
15	1-Pentanol	4.7 ± 0.5	0.5 ± 0.01	6.9 ± 0.04	0.8 ± 0.04	0.5 ± 0.01	11 ± 0.9	0.8 ± 0.02	1.7 ± 0.08	3.6 ± 0.1
16	4-Penten-1-ol	0.5 ± 0.04	18.5 ± 1.8	17 ± 0.13	25 ± 1.8	14 ± 1.2	36 ± 1.6	15 ± 0.8	4.4 ± 0.14	10 ± 1
17	3-Hydroxy-2-butanone	13.2 ± 1.4	1.7 ± 1.1	15 ± 0.09	3 ± 2.5	n.d.	37 ± 2.3	2.2 ± 0.08	0.5 ± 0.03	7 ± 0.2
18	1-Hexanol	36 ± 2.5	84.5 ± 5	21 ± 0.13	165 ± 9	61 ± 3.2	34 ± 1.2	8 ± 0.3	24 ± 1.5	10 ± 0.9
19	cis-3-Hexen-1-ol	32.3 ± 2	124 ± 9	154 ± 12	113 ± 11	94 ± 5.1	262 ± 1.2	65 ± 3.2	34 ± 1.4	74 ± 3
20	Nonanal	10 ± 0.8	3 ± 0.01	3 ± 0.06	12 ± 0.6	7 ± 2.1	12 ± 0.8	9 ± 0.4	3 ± 0.1	4 ± 0.2
21	Acetic acid	3050 ± 150	6940 ± 195	2688 ± 165	13700 ± 660	6500 ± 165	3953 ± 150	4561 ± 250	3347 ± 120	1192 ± 56
22	Propionic acid	39.5 ± 3	3442 ± 230	111 ± 8.5	560 ± 24	3645 ± 1.5	159 ± 9	817 ± 35	1944 ± 54	120 ± 6

Each value is the mean of triplicate analyses expressed in $\mu g_{(compound)}/kg_{(olive\ fruit)} \pm SD$.
n.d.: not determined.

3. Results

The volatile compounds in Spanish-style, Greek-style and Castelvetrano-style green olives of the *Nocellara del Belice* cultivar, after six, seven and eight months of brining, were analyzed by gas chromatography and mass spectrometry.

Fig. 2 shows a typical GC chromatogram of olive fruit flavour compounds obtained by the dynamic headspace technique. Details of peak identities and volatile compound amounts, expressed in $\mu g_{(compound)}/kg_{(olive\ fruit)}$, are shown in Table 1. Among identified organic compounds there are alcohols, aldehydes, ketones, esters as well as acids. In Fig. 3 the molecular structures of volatiles identified by gas chromatography and GC/mass spectrometry are represented.

Fig. 4 (data from Table 1) displays the meaningful differences in volatile composition of three olive samples found after six months of brining. High contents of ethanol, acetic acid and 2-butanol were detected in all samples. 1-Propanol, propyl-acetate, ethyl propanoate and propionic acid were found at higher levels in Spanish-style olives. Good amounts of propyl-propanoate were detected only in this commodity. Ethyl acetate was found in all samples but particularly in Spanish-style olives. Cis-3-hexen-1-ol and 2-butanone were detected, above all in Castelvetrano-style olives, while 1-hexanol and isopentanol were mainly found in Spanish and Greek-style olives. Major levels of 2-pentanol and 3-pentanol were found in Castelvetrano and Spanish-style olives. Furthermore, a lower content of 3-hydroxy-2-butanone was revealed in Spanish-style olives.

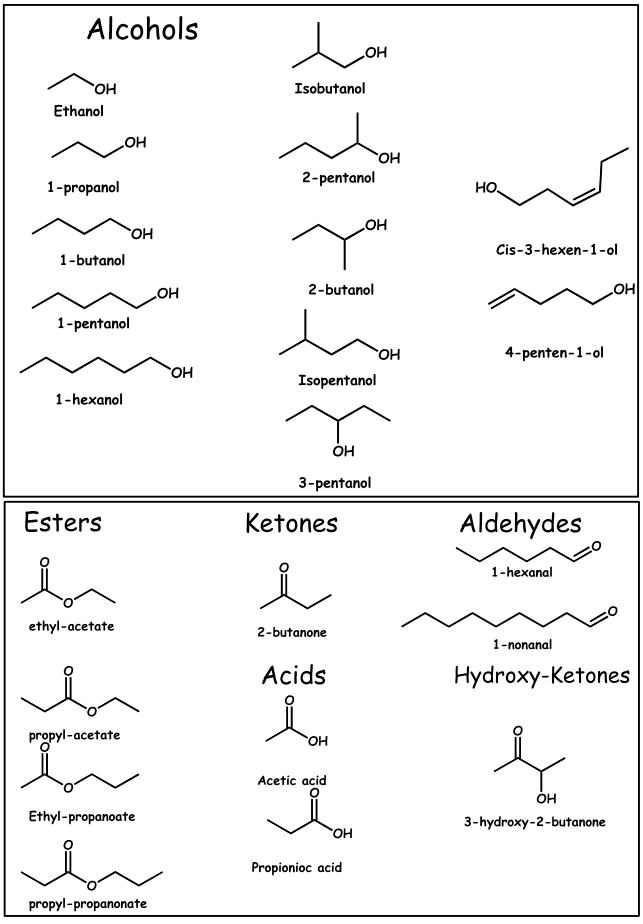

Fig. 3. Molecular structures of volatile compounds of Spanish-Greek-and Castelvetrano-style green olives of the *Nocellara del Belice* cultivar identified by gas chromatography and GC/mass spectrometry.

Fig. 5 (data from Table 1) shows the time evolution of some volatile compounds found in Greek-and Spanish-style olives after six, seven and eight months of brining. In Greek-style samples there was a meaningful increase over time of ethyl propanoate, 2-butanone and propionic acid contents. Spanish-style samples showed meaningful decreases of 2-butanol, ethanol and cis-3-hexen-1-ol. Castelvetrano-type olives (see Table 1) show an increase in the contents of most volatile compounds at the seventh month of processing and a decrease thereafter.

4. Discussion

Olive fermentation involves the breaking down of complex organic substances into simpler ones. Pyruvate is metabolized to various compounds, namely lactate in homolactic fermentation, ethanol and carbon dioxide in alcoholic fermentation and lactate, as well as other acids and alcohols, in heterolactic fermentation. Ethanol is an important flavour component end-product of the Embden–Meyerhof–Parnas glycolytic pathway, whose main function is the production of energy. The biosynthesis of higher alcohols is considered to be linked to the deamination process of amino acids (Herrero, Garcia, & Diaz, 2006). Acetic acid is produced by bacteria such as *Acetobacter* species, *Clostridium acetobutylicum* and other microorganisms, e.g. yeasts, by oxidation of ethanol. These bacteria are commonly found in foodstuffs, water and soil. The acetyl group, derived from acetic acid when bound to coenzyme A, is central to the metabolism of carbohydrates and fats. Propionic acid is produced biologically by *Propionibacterium* species as the product of the metabolic breakdown of fatty acids and/or some amino acids (en.wikipedia.org/wiki). Our results suggest that, during olive processing, all three samples underwent alcoholic, heterolactic and propionic fermentation, which led to the production of great amounts of ethanol, acetic acid, propionic acid and other alcohols and esters. After six months of processing, Castelvetrano-style olives showed higher contents of ethanol and lower amounts of acids, suggesting a major alcoholic fermentation, while Spanish-style olives showed higher contents of acids, such as acetic acid and propionic acid, suggesting a probably major proliferation of *Acetobacter* and *Propionibacterium* (PAB) in brine-medium. This evidence was confirmed by higher contents of propionic and acetate esters. In addition, such results were in agreement with microbiological analyses, which evidenced the presence of higher concentrations of yeasts in Castelvetrano samples (1.7×10^9 CFU/ml brine), while a

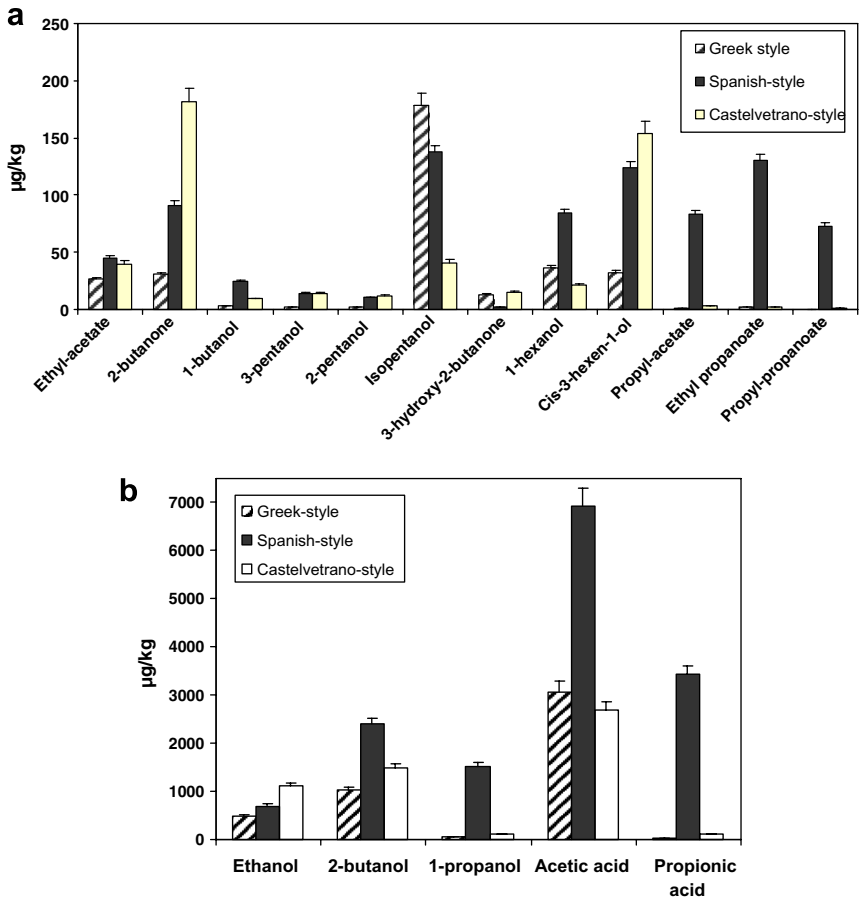

Fig. 4. Meaningful differences (data from 1) in volatile composition of Spanish-Greek-and Castelvetrano-style green olives of the *Nocellara del Belice* cultivar after six months of processing. (a) Ethyl-acetate; 2-butanone; 1-butanol; 3-pentanol; 2-pentanol; isopentanol; 3-hydroxy-2-butanone; 1-hexanol; cis-3-hexen-1-ol; propyl-acetate; ethyl-propanoate; propyl-propanoate. (b) Ethanol; 2-butanol; 1-propanol; acetic acid; propionic acid.

very low value (4×10^6 CFU/ml brine) was detected in Spanish-style samples. Volatiles esters are major components (to different extents) of the aroma of all fruits, sometimes being the compounds mainly responsible for the good flavour appreciated by consumers (Sanz, Olias, & Perez, 1997; Seimour, 1993). It is well known that acetate esters, are synthesized by an enzyme, alcohol-acyl-transferase, which catalyses the esterification of volatile alcohols with acetyl CoA molecules to produce volatile esters and free CoA-SH (Myers, Issenberg, & Wick, 1970; Salas, 2004; Tressl & Drawert, 1973). In the same way propanoate esters could be synthesized by esterification of volatile alcohols with propionyl CoA molecules.

From these results and the cited references, a proposed pathway for esters biosynthesis in table olives is presented in Fig. 6.

Although there are clearly different biogenesis pathways of volatile compounds between table olives and olive oil, it is indeed important to consider some enzymatic pathways which occur in olive oil flavour compound biosynthesis. C_5 and C_6 aldehydes and alcohols, and their corresponding esters, are the most volatile compounds present in olive oils and are produced enzymatically by polyunsaturated fatty acids through the "lipoxygenase pathway" (Gardner, 1991; Williams & Harwood, 2000).Volatile compounds responsible for green attributes of virgin olive oils have been proved to be produced by the enzymatic oxidation of linolenic and linoleic acids (Hatanaka, 1993; Olias & Pérez, 1993). The metabolism of 13-hydroperoxides of linolenic acid gives rise to cis-3-hexanal which can isomerize to trans-2-hexenal or it is quickly enzymatically reduced to cis-3-hexen-1-ol. 13-Hydroperoxides of linoleic acid are cleaved by hydroperoxide lyses, producing hexanal, which is reduced to 1-hexanol by alcohol dehydrogenases (Angerosa, 2002; Angerosa, Basti, & Vito, 1999; Angerosa, Mostallino, Basti, & Vito 2000; Feussner & Wasternack, 2002; Kiritsakis, 1998; Ridolfi, Terenziani, Patumi, & Fonatanazza, 2002; Salas, 2004; Salas et al., 2000). We can also hypothesize a lypoxygenase pathway for the samples, as shown by the presence of 1-hexanol, 1-hexanal and cis-3-hexen-1-ol. In particular, after six months of brining, higher levels of 1-hexanol in Spanish-style olives revealed a probably prevalent linoleic acid oxidation pathway, while higher amounts of cis-3-hexen-1-ol showed a probably prevalent linolenic acid oxidation pathway in Castelvetrano-style olives. On the other hand, in the Greek-style sample, there seems to be a balance between linolenic and linoleic oxidation. However, we could also consider a lypoxygenas-

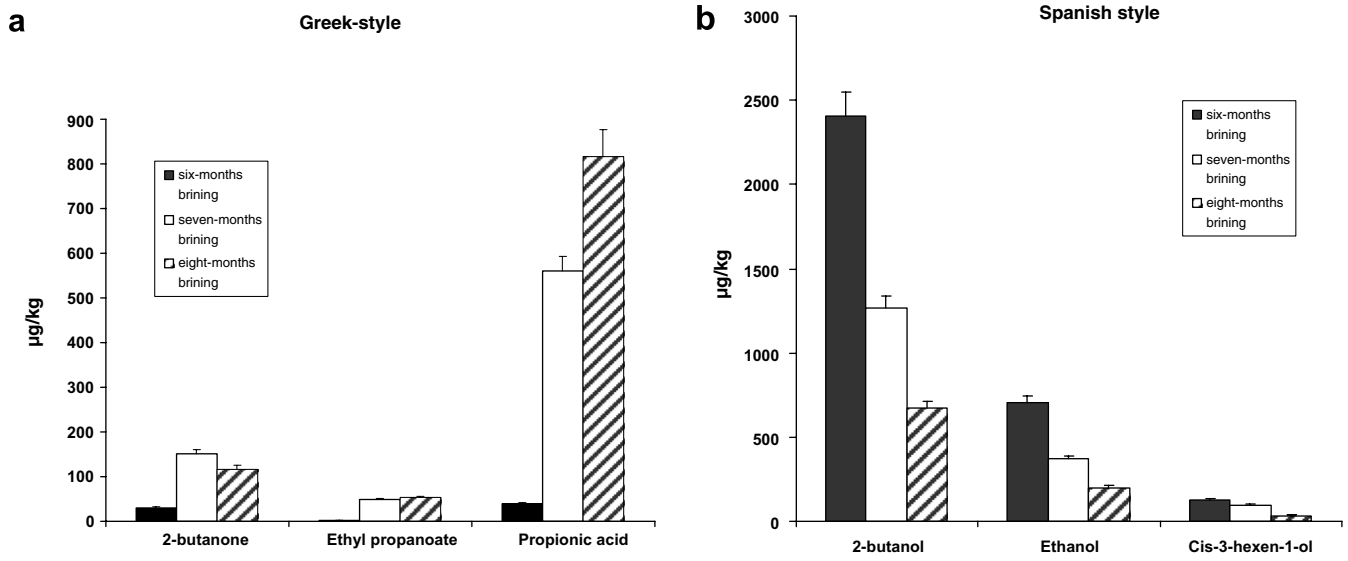

Fig. 5. Evolution over time, after six, seven and eight months of brining (data from Table 1) of some volatile compounds contained in Greek-and Spanish-style green olives of the *Nocellara del Belice* cultivar. (a) 2-Butanone; ethyl-propanoate; propionic acid. (b) 2-Butanol; ethanol; cis-3-hexen-1-ol.

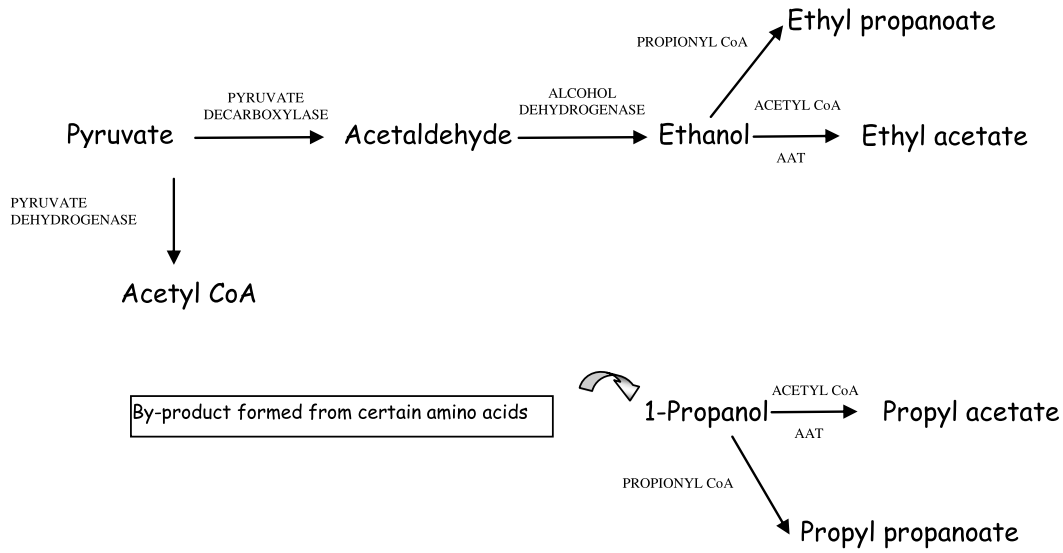

Fig. 6. Proposed pathway for ester biosynthesis in table olives. AAT = alcohol acetyl transferase.

es-like metabolism of polyunsaturated fatty acids, affected by enzymes produced in the brine medium by lactic acid bacteria and yeasts, together with other contaminating microorganisms.

Furthermore, the strong differences observed between six, seven, eight months of brining are explained by the time evolution at room temperature, which changes the dynamics of proliferative activity of microorganisms and consequently the volatile compound metabolic pathways.

The first results of this research show that processing technology significantly affects the volatile compounds of table olives. Therefore, quali-quantitative evaluation of flavour compounds is important in order to determine a quality index of the final products.

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