Proceedings

Elucidation of the Volatilome of Packaged Spanish-Style Green Olives of Conservolea and Halkidiki Varieties Using SPME-GC/MS †

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Abstract: Olives are characterized by a wide variety of volatile compounds which are primarily products of microbial metabolism that contribute to the organoleptic characteristics of the final product and especially to its flavor. The volatilome in Spanish-style processed green olives of Conservolea and Halkidiki cultivars were analytically characterized. Solid phase micro-extraction (SPME) technique was used for the extraction of volatile components from the olive samples that were further identified and quantified by gas chromatography coupled to mass spectrometry (GC–MS). Eighty-eight (88) compounds were identified, including several aldehydes, ketones, acids, terpenes, but mainly esters and alcohols. Results showed that there were no significant differences in the qualitative composition of the volatile profiles between the two varieties. Acetic and propanoic acids, thymol, ethanol, 2-butanol, 1 propanol, ethyl acetate as well as ethyl propanoate were the most dominant compounds found in both cultivars. However, some quantitative differences were spotted between the two varieties regarding some of the identified volatile compounds. The quantity of 2-butanol was higher in Halkidiki variety, while propanoic acid ethyl ester was found in higher amounts in Conservolea variety. Furthermore, differences in the quantities of some volatile compounds over time were observed. Most of the identified compounds presented an increasing trend during storage.

Keywords: volatilome; Spanish-style fermentation; green table olives; Halkidiki variety; Conservolea variety; SPME/GC-MS

1. Introduction

Table olives are for Greece and also for the wider Mediterranean region, a traditional crop of great economic and social importance [1]. Spanish-style fermentation is implemented by the Greek table olive industry using primarily two traditional table olive varieties, namely Conservolea and Halkidiki [2]. As a fermented product, table olives are characterized by a wide variety of volatile compounds which are mainly products of microbial metabolism. The flavor and aroma of olives is directly related to the qualitative and quantitative composition of their volatilome which determines both quality and shelf life of the final product, thus playing an important role in its acceptance by the consumer [3]. The purpose of the present study was to monitor and characterize the volatile profile

of the aforementioned varieties processed by the Spanish method and packaged in multi-laminated pouches under modified atmospheres for a period of 12 months.

2. Materials and Methods

2.1. Olive samples and Preparation

The samples of Conservolea and Halkidiki varieties were obtained from a company located in Northern Greece. The olives were initially processed using the Spanish method and afterwards they were packaged in multi-laminated pouches, into which a gas mixture was injected to create a modified atmosphere (70% N_2 and 30% CO_2). The pouches were stored at room temperature for a period of 12 months and analyzed every month for the determination of their volatile profile using SPME Gas Chromatography—Mass Spectrometry (GC—MS) as described elsewhere [4,5]. A total of 24 analytical samples were analyzed from each variety.

2.2. Volatile Compound Extraction Using Solid Phase Microextraction (SPME)

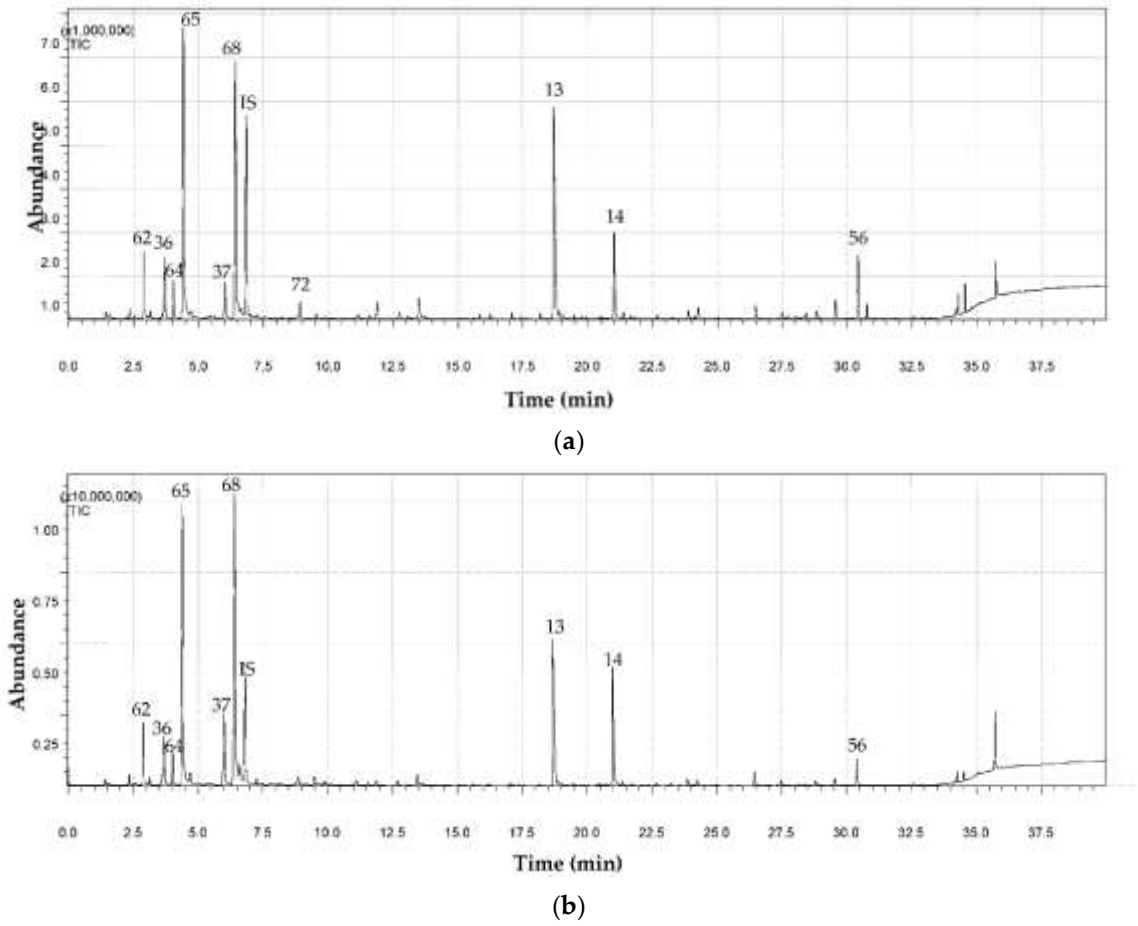
An amount of olives (5–10 g) from each pouch was snap-frozen in liquid nitrogen, ground into olive powder and stored in the freezer. An SPME Agilent Technologies fiber was used to isolate volatiles from powdered olive samples [6,7]. SPME was performed in 20 mL vials, sealed with PTFE-Silicone septa, containing 0.5 g of the homogenized olive powder and 1 μL of 1,4-dioxane as internal standard. After 15 min of heating in a water bath at 40 °C, the fiber was introduced into the vial for additional 30 min for the extraction of the volatiles from the headspace.

2.3. Gas Chromatography—Mass Spectrometry

Gas Chromatography-Mass Spectrometry (GC/MS) analysis was carried out using a Shimadzu GCMS QP-2010 Ultra system operated with the accompanied GCMS Solution software. The volatile components adsorbed on the SPME fiber were desorbed in the split-splitless inlet at 240 °C (split ratio 1/10) for 5 min and separated on a DB-WAX capillary column (30 × 0.25 mm, d.f. 0.25 μm). Helium was employed as the carrier gas at a constant linear velocity of 36 cm/s. The initial oven temperature was set at 40 °C for 5 min, then it was increased at 5 °C/min to 180 °C, at 30 °C/min to 240 °C and held there for 5 min. The temperature of ion source and interface was set at 230 °C and 240 °C, respectively. The mass spectrometer was operated in electron ionization mode with the electron energy set at 70 eV and a scan range of 40–300 m/z [4,8]. Identification of the compounds was accomplished by comparing the: (i) retention indices (RI) based on the homologous series of C8-C24 n-alkanes with those of authentic compounds (when available) and those of NIST14 library (NIST, USA); (ii) MS data with those of reference compounds and MS data obtained from NIST14. GCMS Solution (ver. 4.30; Shimadzu), AMDIS (ver. 2.72; NIST) and NIST MS Search (ver. 2.2; NIST) software were used in the identification process. The reliability of identification (RID) was set at 3 levels. A-level: agreement of RI and MS spectra with those of an authentic compound analyzed under identical experimental conditions; B-level: agreement of RI ($\Delta RI < 20$) and MS (match > 900); C-level: at least $\Delta RI < 20$ or MS similarity match > 800. The content of volatile compounds was expressed relative to internal standard.

3. Results and Discussion

The volatile compounds in Spanish-style green olives of Conservolea and Halkidiki varieties were analytically characterized by GC/MS. A typical total ion chromatogram of the volatile profile for each variety is shown in Figure 1. The concentrations of each volatile compound at 0, 6, and 12 months of storage are presented in Table S1. The eighty-eight (88) compounds identified included esters, alcohols, aldehydes, ketones, acids, as well as terpenes.



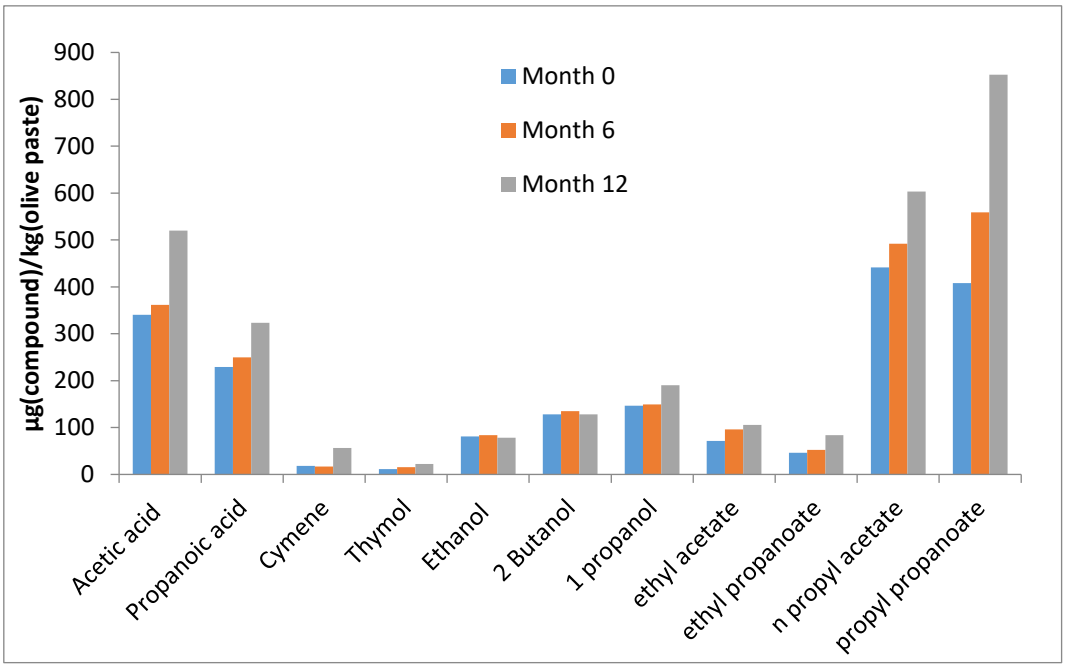
(**a**)

(**b**)

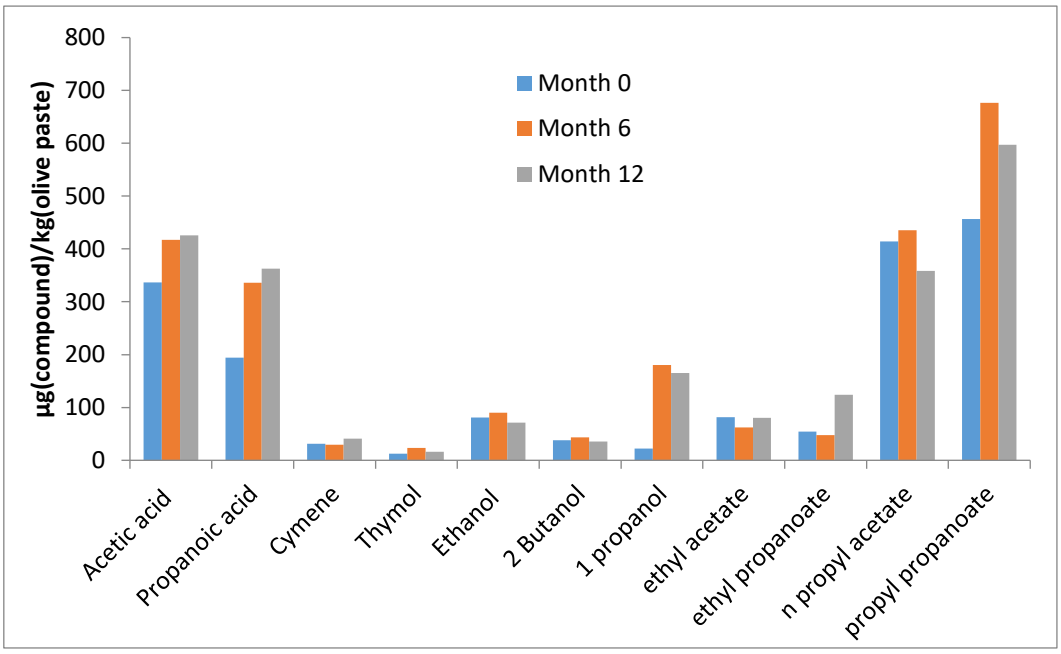
Figure 1. Typical GC chromatograms of the volatile compounds obtained by the SPME technique for (**a**) Conservolea and (**b**) Halkidiki varieties. The numbers correspond to major volatile compounds indicated in Table S1.

High contents of acids, esters and alcohols were detected in all samples. More specifically, major levels of acetic acid, propanoic acid, propyl acetate and propyl propanoate were detected in both varieties [8,9]. Furthermore, both varieties presented lower contents of ketones and aldehydes. There were no significant differences in the volatile composition of the two varieties, as seen also in their GC chromatograms. However, 2-butanol was found at higher levels in Halkidiki variety, while p-methylguaiacol and ethyl propanoate were detected in greater amounts in Concervolea variety.

The evolution of the most important volatile compounds in terms of storage time (0, 6, and 12 months) is presented in Figure 2 and Table S1. A mild increase was noticed in the contents of propanoic acid, ethyl propanoate, ethyl acetate, acetic acid, cymene and thymol, in both table olive varieties. Ethanol, 2-butanol and 1-propanol presented a small increase until the sixth month and a reduction thereafter in all tested samples. In Halkidikis variety, there was a considerable increase over time of propyl acetate and propyl propanoate, while in Conservolea variety, whereas in Conservolea variety, those compounds were significantly decreased during the last six months of storage.



(**a**)



(**b**)

Figure 2. Evolution of the most important volatile compounds over time of (**a**) cv. Halkidiki and (**b**) cv. Conservolea green olives packaged in multi-laminated pouches under modified atmospheres.

During fermentation, olives metabolize pyruvate into other compounds. Thus in the homofermentative process, lactate is the major end product, while the heterofermentative process produces mainly organic acids and alcohols. Ethanol and carbon dioxide derive also from alcoholic fermentation [8]. Based on the volatile compounds detected in this study, it is suggested that all samples underwent alcoholic and lactic fermentation which explains the high contents of acetic acid and ethanol. Bacteria such as *Acetobacter* spp. and yeasts, commonly found in food matrices, produce acetic acid as well. Propanoic acid is also produced by bacteria such as *Propionibacterium* spp. through their metabolism. The presence of the above mentioned bacteria can be also confirmed by the high amounts of propanoate and acetate esters [10,11]. The volatile profile of the table olive varieties

reported in the present study lines up with already existing publications on the volatilome of different table olive cultivars [12–16].

4. Conclusions

The present study investigated the volatile profile of fermented Spanish-style green olives of Conservolea and Halkidiki varieties during storage in multi-laminated pouches under modified atmosphere for 12 months at ambient temperature. High contents of acetic and propanoic acids, as well as propyl acetate and propyl propanoate along with ethanol were detected in all samples but no significant differences were observed among them regarding their volatilomes.

Supplementary Materials: The following is available online at www.mdpi.com/xxx/s1, Table S1: Volatile organic compounds (VOCs) expressed as μg/kg (relative to internal standard) of Conservolea and Halkidiki varieties during storage in multi-laminated pouches under modified atmospheres at 0, 6, and 12 months.

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Conflicts of Interest: The authors declare no conflict of interest.

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