

Volatile Compound Profiles by HS GC-MS for the Evaluation of Postharvest Conditions of a Peach Cultivar

Livia Malorni¹, Antonella Martignetti¹ and Rosaria Cozzolino^{1*}¹Institute of Food Science, National Council of Research, Italy

Article Information

Received date: Sep 18, 2015

Accepted date: Sep 30, 2015

Published date: Oct 07, 2015

*Corresponding author

Rosaria Cozzolino, National Council of Research, Institute of Food Science, Italy, Tel: +39 0825 299381; Fax: +39 0825 781585; Email: rcozzolino@isa.cnr.it

Distributed under Creative Commons CC-BY 4.0

Abstract

Volatile Organic Compounds (VOCs) profile of foods obtained by Gas Chromatography/Mass Spectrometry (GC/MS) can be considered a potent tool of food products quality changes occurring as a result of different processing, such as ripening and deterioration. The aim of the present study was the evaluation of volatiles profiles of peaches (cv Springcrest) during their storage in conditions similar to those of long distance transport that normally these products undergo before being placed on market. We investigated control sample (no stored fruit) and peaches stored in cardboard boxes wrapped in heat-sealed HD polythene bags, both in normal and modified atmosphere (0% and 23% CO₂) after 1 and 8 days of storage at 4°C. GC/MS analysis of these samples allowed the identification of a total of 115 VOCs.

The comparison of the VOCs profile of the three peach samples (control, normal atmosphere and 23% CO₂) shows that fruits packaged in normal atmosphere released a greater amount of esters of medium chain fatty acids, such as ethyl nonanoate and ethyl dodecanoate. On the other hand, fruits stored in normal atmosphere and modified atmosphere after 8 days of storage (increased concentration of CO₂ in packs) released a greater amount of esters of long chain fatty acids, such as ethyl hexadecanoate.

Introduction

The evaluation of volatile Organic Compounds (VOCs) profile of food obtained by Gas Chromatography/Mass Spectrometry (GC/MS) can play a key role in studies of food traceability, origin and safety. Volatiles, in fact, can be considered potential biomarkers of quality changes of a food product occurring as a result of different processing, such as ripening, deterioration, etc. In the case of transformed food items, VOCs profile can characterize not only the final product, but also the raw material, the transformation and preservation processes and the distribution of the product.

The analysis of headspace volatile compounds by using GC/MS is rapid and non-destructive to the product, allowing it to be used at an industrial level as a rapid on-line unit monitoring of the volatiles profile, and at the consumer level with the development of active food labels that would respond to biomarker changes. As certain compounds are emitted as signals of metabolic changes, these on-line units or food labels could indicate the early detection of quality loss changes on a production line or to the consumer. This quality-monitoring method could also be applied to test and optimise the processing and packaging of ready-to-use food products in shelf-life trials, with a view to extending their shelf-life.

Peach (*Prunus persica* L.) is an economically important fruit with an expanding world production situated at 20 million tons in 2010 [1]. One straightforward way to enhance peach consumption would appear to be the improvement of fruit quality. According to consumers, aroma, along with fruit firmness and colour, is the most important factor that contributes to peach quality [2]. VOCs define fruit aroma and, in combination with sugars and organic acids, contribute to the overall peach flavor.

Peach volatiles have been studied intensively and around 100 volatiles, including alcohols, aldehydes, esters, terpenoids, ketones and lactones have been described to date [3]. Peaches have a short shelf-life, because they quickly soften at room temperature; low-temperature storage is the most common current method for delaying ripening after harvest and extending their commercial life.

The aim of the present study was to investigate the evolution of volatile compounds profiles of peaches (cv Springcrest) during ripening. Particularly, we were interested in determining the VOCs profile of peaches stored in conditions similar to those of long distance transport that normally these products undergo before being placed on market.

For this purpose, we used a five-litre glass reactor with a hermetic cap. The reactor was closed for two hours with a precise amount of fruit, previously stored at 4°C in appropriately packed

Table 1: Volatile metabolites identified in peach samples by using GC-MS.

| Metabolite | ID ^a | Composition (%) ^b | | | |
|--|-----------------|------------------------------|--------------------|---------------------|---------------------|
| | | Control | 0% CO ₂ | 23% CO ₂ | 30% CO ₂ |
| ACIDS | | | | | |
| Acetic acid | RI/MS/S | 0.74 | 0.05 | 0.02 | 0.20 |
| Butanoic acid | RI/MS/S | 0.06 | - | - | - |
| Butanoic acid 3-methyl | RI/MS | 0.37 | - | - | - |
| Pentanoic acid | RI/MS/S | 0.07 | - | 0.01 | - |
| Hexanoic acid | RI/MS/S | 0.38 | - | 0.11 | - |
| Heptanoic acid | RI/MS/S | - | 0.07 | - | - |
| Octanoic acid | RI/MS/S | 0.29 | 0.71 | - | - |
| Nonanoic acid | RI/MS/S | 0.48 | 0.95 | 0.08 | - |
| <i>n</i> -Decanoic acid | RI/MS/S | 0.15 | 0.72 | - | - |
| Dodecanoic acid | RI/MS/S | - | 1.20 | - | - |
| <i>n</i> -Hexadecanoic acid | RI/MS | - | - | - | 1.37 |
| Benzene carboxylic acid | RI/MS | - | 0.36 | - | - |
| Benzoic acid | RI/MS | - | 0.49 | - | - |
| ESTERS | | | | | |
| Ethyl acetate | RI/MS/S | 0.04 | 0.44 | 1.27 | 0.86 |
| 1-Butanol-3-methyl acetate | RI/MS | 0.26 | 0.04 | 0.03 | 0.06 |
| Acetic acid pentyl ester | RI/MS | 0.07 | - | - | 0.10 |
| Ethyl tiglate | RI/MS/S | 0.04 | - | 0.07 | 0.18 |
| Acetic acid. hexyl ester | RI/MS | 0.40 | - | - | - |
| Ethyl hexanoate | RI/MS/S | 0.41 | 1.96 | 0.51 | 2.64 |
| iso-Amyl isovalerate | RI/MS | 1.57 | - | - | - |
| Acetic acid heptyl ester | RI/MS | 0.08 | - | - | - |
| <i>n</i> -Amyl isovalerate | RI/MS | 1.35 | - | - | - |
| Ethyl Octanoate | RI/MS/S | 13.49 | 22.47 | 14.04 | 19.38 |
| Ethyl Benzoate | RI/MS/S | 0.73 | 0.87 | 0.58 | 0.43 |
| <i>cis</i> -3-Hexanyl isovalerate | RI/MS | 2.08 | - | - | - |
| Benzoic acid 2-hydroxy methyl ester | RI/MS | 0.14 | - | - | - |
| Pentanoic acid 2-ethyl hexyl ester | RI/MS | 1.12 | - | - | - |
| Ethyl trans 4 decenoate | RI/MS | 1.63 | 14.71 | 12.68 | 17.80 |
| Pentyl octanoate | RI/MS/S | 0.46 | - | - | 0.44 |
| Ethyl Hexadecanoate | RI/MS/S | 7.43 | - | 7.18 | - |
| Ethyl 2 Butenoate | RI/MS | - | 0.01 | 0.07 | 0.01 |
| Ethyl Heptanoate | RI/MS/S | - | 6.41 | 2.94 | 7.54 |
| Ethyl Benzoate | RI/MS/S | 0.73 | 0.87 | 0.58 | 0.43 |
| Ethyl 2-Octenoate | RI/MS | - | 4.05 | 1.69 | - |
| Ethyl Nonanoate | RI/MS/S | - | 2.78 | 3.17 | 4.61 |
| 3 methyl buthyl Octanoate | RI/MS | - | 0.69 | - | 0.31 |
| Ethyl Decanoate | RI/MS/S | - | 6.36 | 12.58 | 5.29 |
| 3 hydroxy ethyl Butanoate | RI/MS | - | - | 0.72 | Tr |
| Ethyl 5 methylhexanoate | RI/MS | - | - | 0.07 | - |
| Benzene acetic acid ethyl ester | RI/MS | - | - | 0.41 | - |
| 3 phenyl ethyl 2 Propenoate | RI/MS | - | - | 0.59 | - |
| 3 methyl ethyl Butanoate | RI/MS | - | 0.09 | - | 0.17 |
| 3 hydroxy ethyl Butanoate | RI/MS | - | - | 0.72 | 0.04 |
| Ethyl 3 Hexenoate | RI/MS | - | - | - | 0.40 |
| N-Propyl Acetate | RI/MS | - | 0.04 | - | 0.04 |
| Ethyl Undecanoate | RI/MS/S | - | - | 0.57 | 0.36 |
| Formamide N, N diethyl | RI/MS | 0.06 | - | - | - |
| HYDROCARBONS | | | | | |
| 4 Methyl 1,3 pentadiene | RI/MS/S | 0.02 | - | - | - |
| Cyclopentane, nonyl | RI/MS | - | 1.07 | 0.93 | - |
| Cyclobutane 13, butadienyl | RI/MS | 0.07 | - | - | - |
| Decane 4 methylene | RI/MS | 0.02 | 0.06 | 0.03 | - |
| Undecane | RI/MS/S | 0.79 | 1.11 | 1.57 | 2.18 |
| Decane 3,6 dimethyl | RI/MS | 0.05 | - | - | - |
| 5 Methyldecane | RI/MS | 0.19 | - | - | - |
| Undecane 3 methyl | RI/MS | 0.26 | - | 0.31 | - |
| Tridecane | RI/MS/S | 0.85 | 1.49 | 1.33 | 1.56 |
| Tridecane 2 methyl | RI/MS | 0.22 | - | - | - |
| 3,5 Dimethyldodecane | RI/MS | 0.15 | - | - | - |
| 7 Tetradecene | RI/MS | 0.07 | - | - | - |
| 2 Tetradecene | RI/MS | 3.28 | - | - | - |
| Octadecane 2 methyl | RI/MS | 0.07 | - | - | - |
| Tetradecane 4 methyl | RI/MS | 0.22 | - | 0.07 | - |
| Nonadecane 2 methyl | RI/MS | 0.14 | - | - | - |
| Hexadecane 2,6,10,14 tetramethyl | RI/MS | 0.28 | - | 0.21 | - |
| Pentadecane | RI/MS/S | 10.89 | 4.66 | 1.50 | 2.21 |
| Hexadecane | RI/MS/S | 2.20 | 1.17 | 0.28 | 0.87 |
| Decanolactone | RI/MS | 1.63 | - | - | - |
| Heptadecane | RI/MS/S | 8.12 | 3.88 | 2.78 | 1.77 |
| Pentadecane 2,6,10,14 tetramethyl | RI/MS | 0.83 | 0.43 | 0.51 | 0.57 |
| Benzene 1,2,3 trimethyl | RI/MS | - | 0.06 | 0.01 | - |
| Decane 3,8 dimethyl | RI/MS | - | 0.16 | - | - |
| Undecane 3 methylene | RI/MS | - | 0.44 | 0.70 | - |
| Dodecane | RI/MS/S | - | 0.78 | - | 1.06 |
| Tetradecane 4 methyl | RI/MS | 0.22 | - | 0.07 | - |
| Cyclopentane, nonyl | RI/MS | - | 1.07 | 0.93 | - |
| 2 Octene 3,7 dimethyl | RI/MS | - | - | 0.21 | - |
| Decane 5 methyl 6 methylene | RI/MS | - | - | 0.38 | - |
| Cyclohexane 1,2,3 trimethyl | RI/MS | - | - | 0.27 | 0.26 |
| Undecane 2 methyl | RI/MS | - | - | 0.32 | - |
| Cyclopentane, pentyl | RI/MS | - | 0.44 | 0.64 | 0.81 |
| 5 Tetradecene | RI/MS | - | - | 0.60 | - |
| Tridecane 3 methylene | RI/MS | - | 0.89 | 0.62 | 0.78 |
| Tetradecane | RI/MS/S | - | 1.59 | 1.20 | 1.34 |
| Tridecane 3 ethyl | RI/MS | - | 0.26 | 0.23 | - |
| Cyclobutane 1 butyl 2ethyl | RI/MS | - | - | 0.16 | - |
| Eicosane | RI/MS/S | - | - | 0.56 | - |
| Dodecane 4,9 dipropyl | RI/MS | - | - | 0.15 | - |
| ALCOHOL | | | | | |
| 3 Octanol | RI/MS/S | 0.07 | - | - | - |
| 2 Hexen 1-olo-acetate | RI/MS/S | 2.29 | 1.08 | 1.03 | 1.75 |
| 1 Hexanol 2 ethyl | RI/MS/S | 1.43 | 3.54 | 0.72 | 1.08 |
| Benzyl Alcohol | RI/MS/S | 0.04 | - | 0.18 | 0.16 |
| 1 Butanol 3 methyl acetate | RI/MS/S | 0.26 | 0.04 | 0.03 | 0.06 |
| 2 Hexen 1ol acetate | RI/MS/S | 2.29 | 1.08 | 1.03 | 1.75 |
| 1-Pentanol | RI/MS/S | - | - | - | 0.14 |
| KETONES | | | | | |
| Butyrolactone | RI/MS/S | - | - | 0.07 | - |
| Acetophenone 4'-tert-butyl | RI/MS | - | 0.16 | - | - |
| Decanolactone | RI/MS | 1.63 | - | - | - |
| α -methyl- γ -Crotonolactone | RI/MS | - | 0.37 | - | - |
| TERPENES | | | | | |
| Hotrienol | RI/MS/S | 0.33 | - | - | - |
| Linalol | RI/MS/S | 16.24 | 1.17 | 0.72 | - |
| Limonene | RI/MS/S | 0.09 | 0.13 | - | - |
| Ocymene | RI/MS/S | 0.05 | 0.09 | 0.01 | 0.06 |
| Menthol | RI/MS/S | 0.10 | - | - | - |
| 3,7 Octadiene 2,6 diol 2,6 dimethyl | RI/MS | 0.30 | - | - | - |
| α -farnesene | RI/MS/S | 0.48 | - | - | - |
| o-Cymene | RI/MS/S | - | 0.06 | - | - |
| Geranyl acetone | RI/MS/S | - | 0.54 | - | - |
| ALDHEIDES | | | | | |
| Hexanal | RI/MS/S | 0.05 | 0.04 | 0.01 | 0.01 |
| Decanal | RI/MS/S | 1.12 | 1.86 | 1.10 | 1.04 |
| Octanal | RI/MS/S | 0.24 | 0.25 | 0.19 | 0.25 |
| Hexanal dimethyl acetal | RI/MS | - | 0.33 | 0.16 | 0.32 |
| Benzaldehyde | RI/MS/S | - | 0.43 | - | - |
| Benzaldehyde 2 hydroxy | RI/MS | - | - | - | 0.05 |
| Benzaldehyde 2 methyl | RI/MS | - | - | - | 0.16 |
| OTHERS | | | | | |
| Benzothiazole | RI/MS/S | - | 0.62 | 0.31 | - |

^aIdentification method as indicated by the following: RI: Kovats retention index on a on HP-Innowax column; MS: NIST and Wiley libraries spectra; S: co-injection with authentic standard compounds on the HP-Innowax column.

^bRelative composition of volatile metabolites calculated as the percent ratio of the respective peak area relative to the total peak area data; Tr: trace (<0.1%).

with normal or modified atmosphere. The reactor had the function to concentrate fruit VOCs, that were sampled at scheduled times through a glass cartridge containing tenax as adsorbing substance and using He as a carrier gas. The glass cartridge with the metabolites absorbed was manually put in the Thermal Desorption Unit (TDU), mounted directly onto the Cooled Injection System (CIS) of a quadrupole linear GC/MS, in order that the volatiles were directly desorbed and extracted in the injector of the instrument.

Experimental Procedure

Peaches (cv Springcrest) were delivered in our laboratory packaged in cardboard boxes wrapped in heat-sealed HD polythene bags packaged both in normal and modified atmosphere (0% and 23% CO₂). Once arrived, the boxes were stored at 4°C. At scheduled intervals (1 and 8 days), 1 kilogram of peaches was removed from the storage pack and placed in the five-liter glass reactor with a hermetic cap. VOCs were sampled on a glass cartridge containing tenax as adsorbing substance by flushing it inside the glass reactor for two hours. In order to desorb and extract the volatile metabolites, the glass cartridge was manually inserted in the Thermal Desorption Unit (TDU) mounted directly onto the Cooled Injection System (CIS) of a quadrupole linear gas chromatograph device (model GC 7890A, Agilent Technologies, Santa Clara, USA) coupled to a mass spectrometer (5975 C Agilent). In this way, the metabolites were thermally desorbed and transferred directly to a capillary column HP-Innowax (30m×0.25 mm×0.5µm Agilent J&W) and analyzed. The oven temperature program was initially set at 40°C for 5 min, ramped to 220°C at 5°C min⁻¹ and held at 220°C for 14 min. The temperature of the ion source and the quadrupole were kept at 230°C and 150°C, respectively; helium was used as carrier gas with a flow of 1.5 ml/min; injector temperature was held at 240°C and the pulsed split less mode was used for the analysis.

Results and Discussion

VOCs profiles of peaches (cv Springcrest)

GC/MS analysis of control sample (no packaged fruit) and of peaches packaged in cardboard boxes wrapped in heat-sealed HD polythene bags, both in normal and modified atmosphere (0% and 23% CO₂) after 1 and 8 days of storage at 4°C allowed the identification of a total of 115 VOCs, belonging to different chemical classes, namely: hydrocarbons (40), alcohols (7), terpenes (9), ketones (4), aldehydes (7) esters (34), acids (13) and others (1). Specifically, Figure 1 shows typical GC/MS profiles obtained from control (A), and samples stored in normal (0% CO₂) (B) and modified atmosphere (23% CO₂) (C) after 8 days of storage at 4°C. Metabolites identification was achieved by (i) comparison of the GC retention time and mass spectra with those, when available, of the pure standard compounds; by (ii) comparison of the MS spectra for each putative compound with those of the data system libraries (NIST 2005 and Wiley 2007); and (iii) by the determination of the Kovats indices using a C₈-C₂₀ *n*-alkanes series and matching the experimental values with those reported in the literature for similar chromatographic columns [4].

Table 1 reports the identified 115 volatiles components, the identification methods and the relative composition of volatile metabolites, calculated as the percent ratio of the respective peak area relative to the total peak area (RPA%) gained by GC/MS analysis.

Volatiles present in all samples were: ethyl acetate, acetic acid, hexanal, furfural, heptanal, octanal, ethyl hexanoate, 2-ethyl-1-hexanol, undecane, ethyl octanoate, tridecane, nonanoic acid, pentadecane, heptadecane, while, VOCs found only in packed samples but not in the control were: ethyl-2-butenate, *p*-xylene, 3,7-dimethyl-1,3,6-octadiene, ethyl heptanoate, decanal, naphthalene, ethyl 2,4 decadienoate.

The most intense peaks of the GC/MS chromatogram after 1 day of storage at 4°C of control samples (no packaged peaches) are those assigned to linalool and to ethyl octanoate, while those of peaches packaged in normal atmosphere are assigned to ethyl trans-4-decenoate and ethyl decanoate. Besides these metabolites, the GC/MS chromatogram of peaches packaged in modified atmosphere (23% CO₂) revealed the presence of three other signals that were assigned to ethyl octanoate, ethyl hexadecanoate and ethyl dodecanoate, on the basis of their MS spectra and by the GC/MS analysis of the corresponding standard compounds.

As already reported by Aubert et al. [5], the levels of linalool significantly decreased with increasing duration of storage. This was in total agreement with the results of the current investigation, since, after 8 days of storage the concentrations of linalool were under the limits of detection.

Compounds detected in the GC/MS chromatogram of peaches packaged in normal atmosphere stored at 4°C after 8 days were the same as those found in samples packaged in modified atmosphere (23% CO₂) after 1 day of storage. This finding can be due to fact that after 8 days of storage, the CO₂ concentration increased from 0% to 23% because of the respiratory activity of the fruits.

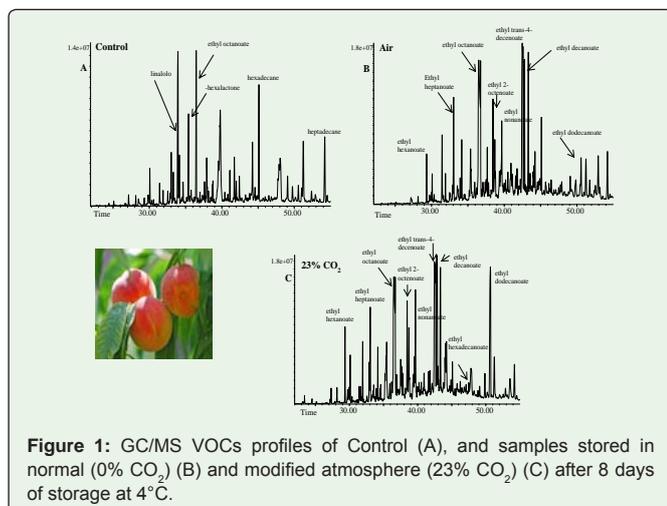
For peaches packaged in modified atmosphere (23% CO₂) analysed after 8 days of storage at 4°C, results show that the most intense peaks of the GC/MS chromatogram are ethyl octanoate, ethyl-trans-4-decenoate and ethyl dodecanoate. Also in these packs, after 8 days of storage, the CO₂ concentration raised from 23% to 36% because of the respiratory activity of the fruits.

Comparing the volatile metabolome of the three peach samples (control, normal atmosphere and 23% CO₂) results indicate that fruits packaged in normal atmosphere release a greater amount of esters of medium chain fatty acids, such as ethyl nonanoate and ethyl dodecanoate. On the other hand, fruits packaged in normal atmosphere and modified atmosphere after 8 days of storage (increased concentration of CO₂ in packs) released a greater amount of esters of long chain fatty acids, such as ethyl hexadecanoate.

Fatty acid pathway plays an important role in determining the characteristic aroma profile during postharvest peach fruit ripening. Lipoxygenase (LOX) and Hydroperoxide Lyase (HPL) convert linoleic and linolenic acids to hexanal and hexenal, respectively, via 9- and 13-hydroperoxide isomers [6]. The aldehydes can then be reduced to the corresponding C₆ alcohols by Alcohol Dehydrogenase (ADH). The aroma esters are produced through Alcohol Acyltransferase (AAT) catalyzing the final linkage of an acyl moiety and an alcohol [6]. On the other hand, Bellincontro et al [7] reported that the formation of aroma characteristics of peach is closely associated with ethylene biosynthesis.

The major fatty acid component in peach fruit at harvest was palmitic acid (44-62%), followed by linoleic acid (29-34%), linolenic acid (6-8%), stearic acid (4-6%) and oleic acid (1-3%) [8].

Concentrations of stearic and oleic acid were relatively unchanged in the fruit ripening process [8], while polyunsaturated linoleic and



linolenic acids and the saturated palmitic acid accumulate through the ripening period, as they are the main substrates for Lipoygenase (LOX)-catalyzed oxidation, leading to the formation of long chain ester compounds. This could explain our results about the greater amount of esters of long chain fatty acid released by the samples during storage.

References

1. FAO. 2010.
2. Bruhn CM. Consumer and retailer satisfaction with the quality and size of california peaches and nectarines. *J Food Qual.* 1995; 18: 241-256.
3. Wang Y, Yang C, Li S, Yang L, Wang Y, Zhaod J, et al. Volatile characteristics of 50 peaches and nectarines evaluated by HP-SPME with GC-MS. *Food Chem.* 2009; 116: 356-364.
4. Kovats E. Gaz-chromatographische Charakterisierung organischer Verbindungen. Teil 1: Retentionsindices aliphatischer Halogenide, Alkohole, Aldehyde und Ketone. *Helvetica Chimica Acta.* 1958; 41: 1915-1932.
5. Aubert C, Bony P, Chalot G, Landry P, Lurol S. Effects of Storage Temperature, Storage Duration, and Subsequent Ripening on the Physicochemical Characteristics, Volatile Compounds, and Phytochemicals of Western Red Nectarine (*Prunus persica* L. Batsch). *J Agric Food Chem.* 2014. 62: 4707-4724.
6. Schwab W, Davidovich-Rikanati R, Lewinsohn E. Biosynthesis of plant-derived flavor compounds. *The Plant Journal.* 2008; 54: 712-732.
7. Bellincontro A, Morganti F, DeSantis D, Botondi R, Mencarelli F. Inhibition of ethylene via different ways affects LOX and ADH activities, and related volatiles compounds in peach (cv. 'Royal Gem'). *Acta Hortic.* 2005; 682: 445-452.
8. Zhang B, Shen J-y, Wei W-w, Xi W-p, Xu C-J, Ferguson I, et al. Expression of Genes Associated with Aroma Formation Derived from the Fatty Acid Pathway during Peach Fruit Ripening. *J Agric Food Chem.* 2010; 58: 6157-6165.