Research Article

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# PTR-TOF-MS analysis of volatile compounds in olive fruits

**Elisa Masi,[a*] Annalisa Romani,[b] Camilla Pandolfi,[a] Daniela Heimler[a] and Stefano Mancuso[a]**

## Abstract

**BACKGROUND: Volatile compounds of Cellina di Nardò and Ogliarola Barese, two typical Italian olive varieties, have been characterised at different ripening stages. Proton transfer reaction–time-of-flight–mass spectrometry (PTR-TOF-MS) was used for the first time on these fruits with the aim of characterising the volatile profile and, in the case of Ogliarola, the changes which may occur during the maturation process.**

**RESULTS: PTR-TOF-MS does not involve any sample pre-treatment, and allows high-resolution measurements, large spectra and small fragmentation of the volatiles. Therefore it allows both compound identification and data statistical treatments. In the present work, about 40 compounds that contribute to the discrimination between samples of the two varieties have been identified.**

**CONCLUSIONS: Three groups of compounds were identified: (1) compounds that are typical of mature fruits of Ogliarola, (2) compounds that tend to decrease during the change from green to mature fruits, and (3) compounds that increase during the maturation process.**


**Keywords:** *Olea europaea*; olive fruit; proton transfer reaction–time-of-flight–mass spectrometry (PTR-TOF-MS); volatile organic compounds (VOCs); principal component analysis (PCA)

## INTRODUCTION

The Italian region of Apulia is characterised by a large production of virgin and extra virgin oil; in Italy, about 80% of olive oil production is located in southern regions,[1] and Apulia represents the most important area. The traditional olive growing designs are evolving in two different directions: on the one hand there are super-high planting density models,[2] and the other hand, the use of mono-varietal oils from particular geographical areas[3] is proposed. Two typical Italian olive varieties from the Apulia region are Cellina di Nardò and Ogliarola Barese; the secondary metabolites of the first one have been reported recently,[4,5] while Ogliarola Barese has never been studied.

The volatile composition of olive fruits and the consequent flavours and aromas of olive oil are influenced by many factors, such as olive variety, geographical region of cultivation, fruit ripeness at harvest and processing methods. Among these, the relationship between oil qualities with respect to the degree of maturation has become a topic of increasing interest of study, starting from the early work of Morales and co-workers.[6] Their study was concerned with the concentration of volatile compounds responsible for the green sensory notes in virgin olive oils obtained from different varieties at different stages of ripeness. In contrast, in the case of different varieties harvested at the same ripening degree, it is the varietal parameter which plays the main role in the volatile mixture.[7] In addition, the distribution of aroma components, mainly $C_5$ and $C_6$ compounds, between pulp and seeds has been examined considering four Spanish varieties.[8]

The use of proton transfer reaction (PTR), coupled with a mass spectrometer (MS), was proposed by Lindinger and colleagues[9] for the *in vivo* analysis of volatile organic compounds (VOCs). As a result of the proton transfer reaction, the volatile molecules are chemically ionised by hydronium ions. The system has an efficient implementation when coupled with a time-of-flight (TOF)-MS analyser, which allows the detection of VOCs at low concentrations and with fast response times.[10,11] Therefore PTR-TOF-MS is a non-invasive technique that allows the determination of whole mass spectra with a time of resolution less than 1 s and the detection of high molecular weight molecules with a high resolution power (M/dM ~ 4000). Consequently, unambiguous determination of chemical formulae due to a better interpretation of mass spectra is achieved.[12]

PTR-TOF-Ms has been applied in various fields, such as in the characterisation of Iberian dry cured ham,[13,14] the study of volatile compounds from Trentigrana cheese,[11] for the investigation of volatile compounds from apple post-harvest storage as related

* *Correspondence to: Elisa Masi, Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, University of Florence, P.le delle Cascine 18, 50144 Florence, Italy, E-mail: elisa.masi@unfi.it*

a *Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente (DISPAA), University of Florence, P.le delle Cascine 18, 50144 Florence, Italy*

b *Dipartimento di Statistica, Informatica, Applicazioni "G. Parenti" (DISIA), University of Florence, Viale Morgagni 59, 50134 Florence, Italy*

 

to apple ripening,[14] in the determination of volatile compounds from cereal bars,[15] and as a control tool for coffee roasting.[16] The technique allows the use of *in vivo* analysis without a previous pre-treatment of the sample.

Therefore, this technique, combining high sensitivity, good precision, accuracy and very poor fragmentation of the volatile molecules, can be used for fingerprinting purposes as well as for detailed investigation of single compounds.

In the present study, PTR-TOF-MS has been used to perform an *in vivo* analysis of VOCs in olive fruit samples, with the aim of showing its potential in discriminating among different varieties and stage of ripeness and in an attempt to identify which compounds are involved in the ripening process.

## EXPERIMENTAL

### Plant material

Olives used in our study belong to two different Apulia varieties of *Olea europaea* L.: Cellina di Nardò and Ogliarola Barese. Fruits with the widest range of skin colours were hand-picked at the end of October 2012 from trees of Antica Masseria Le Lamie (Brindisi, Apulia, Italy), and stored at refrigeration temperature (5–10°C) until analysis. They were classified according to the International Olive Council (IOC, 1984) into three different colour levels: green, green to red and red in the case of Ogliarola, while for Cellina only mature (red) fruits were selected.

### PTR-TOF-MS

VOCs emitted from olive fruit samples were analysed with a PTR-TOF-MS 8000 (Ionicon Analytik GmbH, Innsbruck, Austria) using drift voltage 600 V, temperature 110°C, pressure 2.25 mbar, and $H_3O^+$ as reagent ion. All mass spectra ranging between $m/z = 17.03$ and $m/z = 239.18$ were simultaneously detected with 1 s as integration time. For a detailed explanation of the system, see Lindinger *et al.*[9] and Brilli *et al.*[17]

Intact fruits could not be used since their volatile emission is negligible. Small pieces cut from many representative fruits were weighed and put immediately in a 20 mL glass bottle, topped with a special glass cap that allowed access of the capillary of the instrument and an inlet Teflon tube connected to a zero-air generator. For each fruit type, four replicates/recordings were obtained measuring the headspace mix for at least 5 min; bottles were kept at fixed temperature (28°C ± 1°C). The headspace of an empty bottle, stored at the same temperature, was measured and used for background subtraction.

Raw data (count rate of the analytes recorded in number of counts per second, cps) were acquired with TofDaq software (Tofwerk AG, Innsbruck, Switzerland), using a dead time of 20 ns for the Poisson correction. In order to guarantee high mass accuracy throughout the analysis study, the mass scale was calibrated following the peaks of known components, present in the spectra at any time ($NO^+$ peak, $m/z = 29.9974$ and the main isotope of acetone, $C_3H_7O^+$, $m/z = 59.0497$).[12] For each replicate, we obtained average spectra considering 50 recorded spectra (corresponding to 50 s of analysis) and, for a better comparison between samples, data were normalised to sample mass (expressed in grams).

Principal component analysis (PCA) was used as an unsupervised multivariate technique to represent and explore samples and variables correlations. For the PCA analysis, the average spectra of the four replicates of each olive fruit sample were used. PCA is a mathematical tool used to reduce the variability of complex data set, generating a relative small number of new descriptors (principal components, PCs) according to the correlation between the original variables. Hierarchical cluster analysis (HCA) was also used to show similarity between samples. Further considerations have been performed on some VOCs whose variability among samples resulted particularly interesting.

In order to better investigate the relationship between samples and variables (namely, VOCs), we assigned each variable to possible specific molecules, and focused our attention particularly on that already published[18–37] observing their abundance and variation among samples. We also attributed a possible identity to other interesting/discriminant compounds with the help of known standard compounds as well as with available proton affinities and fragmentation patterns of pure standard.[18–22] Tentative identification was based on literature concerning olive fruit[23–29] or, when not available, concerning olive oil[30–37] and other food and non-food categories.[10,17,38–41]

## RESULTS AND DISCUSSION

Once the fruit is damaged by cutting, the lipoxygenase pathway is triggered, producing many $C_5$ and $C_6$ compounds. Such compounds, produced also during malaxation, represent the main volatiles in olive oil and, incorporated into the oil phase, confer its characteristic aroma. Our results must be regarded under this aspect: they do not represent either the volatile emission of intact fruits or that of olive homogenate; despite this occurrence they allow sample comparison.

The analysis performed on the PTR-TOF-MS mass spectra of the headspace of the four classes under study allowed the compilation of a table of 309 mass peaks (masses with intensity equal to zero cps in all the samples were ignored).

The first two components obtained with PCA explained more than 72% of the total variability, and derivation of a two-dimensional scatter plot allowed each different sample to be clearly visualised, grouping in four distinct units (Fig. 1A).

Mature olives belonging to two different varieties and green olives of Ogliarola showed the bigger spatial distance in the scatter plot; replicates of Ogliarola olives at intermediate stage of ripeness, though distinct from the rest of the samples, formed a less compact group; moreover, their position in the two-dimensional plot suggests that the volatile profile of this type of fruit may be closer to that of green fruits of the same variety as well as to that of mature ones belonging to Cellina, a different variety. This result may reflect the fact that, during olives maturation, some compounds decrease or disappear, other are formed *ex novo*, and consequently it may happen that green fruits share poor similarity with mature ones (see the following discussion for a better explanation).

Considering also the third new factor, PC3, the percentage of variability explained did not increased much (87% vs. 72%); nevertheless, the biplot representation using PC1 and PC3 helped to find some more interesting differences between green olive and fruit at intermediate stage of ripeness (Fig. 1B). Interestingly, such analysis also highlighted some analogies between green fruits of Ogliarola and mature fruits of Cellina.

Hierarchical cluster analysis is used to give an alternative interpretation of complex data. Groups are formed calculating the similarity among samples based on Pearson coefficients of correlation (Table 1) and mean linkage clustering is used to draw a dendrogram (Fig. 2). Through this analysis, we could not significantly distinguish between green and green to mature olive fruits, while

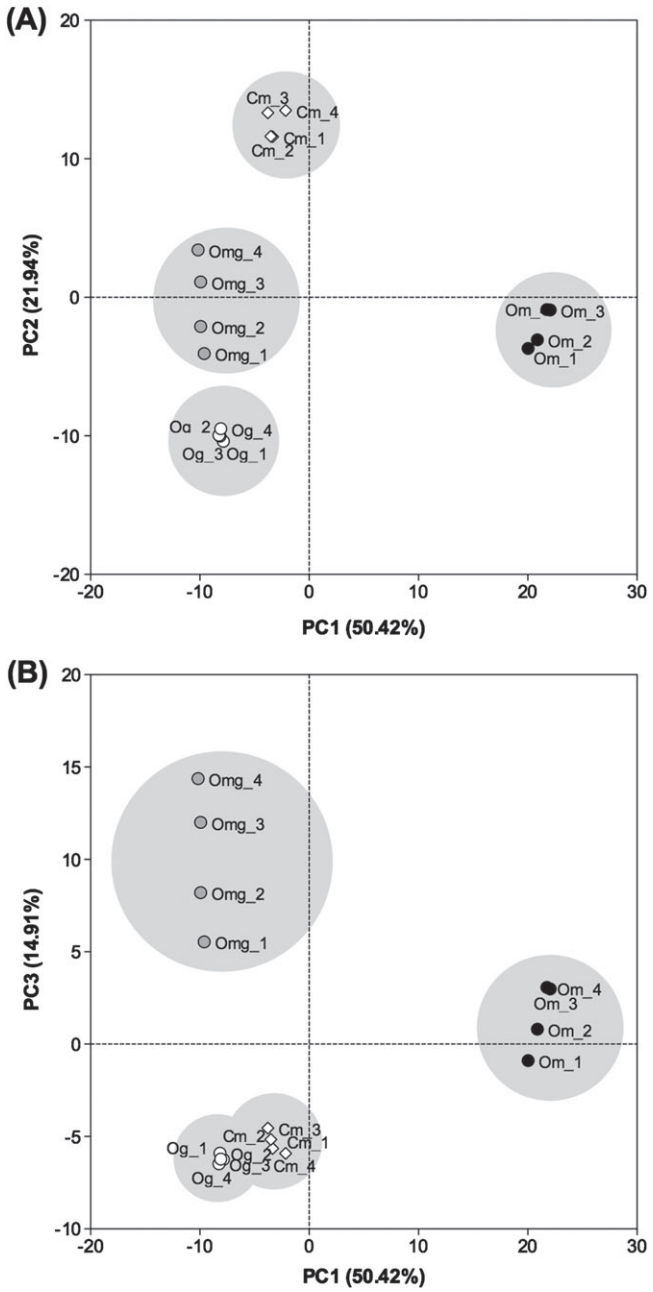


**Figure 1.** PCA scatter plot of the two first factors (A) and of the first and third factors (B) of the samples of olive fruits obtained analysing 309 mass peaks detected with PTR-MS-TOF technique (Og, Ogliarola green; Omg, Ogliarola green to mature; Om, Ogliarola mature; Cm, Cellina mature).

these samples were clearly separated from mature fruit of both varieties. Results therefore confirmed what observed with PCA, with the latter one showing a greater ability to investigate on samples characteristics.

The contributions of each original variable to those generated with the PCA are reported in Table 2. The values of measured and theoretical protonated masses, as well as their tentative identification, are indicated. The accuracy of the measured masses was slightly affected by the internal calibration performed with relatively small molecule compounds;[12] differences between expected and measured masses tended to increase for bigger mass sizes, with an average value of 0.07 Da. Nevertheless,

**Table 1.** Pearson's coefficient of correlation performed on 309 mass peaks detected with PTR-TOF-MS technique

| Sample | Ogliarola, green fruit | Ogliarola, green to mature fruit | Ogliarola, mature fruit | Cellina, mature fruit |
|---|---|---|---|---|
| Ogliarola, green fruit | **1** | **0.976** | 0.326 | 0.305 |
| Ogliarola, green to mature fruit | **0.976** | **1** | 0.288 | 0.266 |
| Ogliarola, mature fruit | 0.326 | 0.288 | **1** | 0.717 |
| Cellina, mature fruit | 0.305 | 0.266 | 0.717 | **1** |

Values in bold type, excluding the diagonal, indicate significant correlations for $P < 0.0001$.

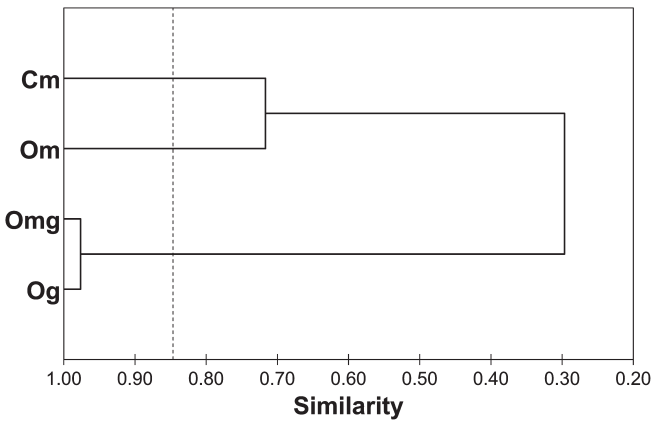


**Figure 2.** Hierarchical cluster analysis dendrogram obtained analysing 309 mass peaks detected with PTR-MSTOF technique (Og, Ogliarola green; Omg, Ogliarola green to mature; Om, Ogliarola mature; Cm, Cellina mature).

this occurrence did not affect the tentative identification of compounds and the comparison among samples.

Compounds that have the greater role in the definition of the first component were quite abundant in Ogliarola mature olives, whose position in the bi-factors plot was in the positive range of PC1 (Fig. 1A). Many of these compounds link to typical undesirable flavours in olive oil. For example, methyl and ethyl esters, whose amount increases with inappropriate practices in fruit harvesting and storing before oil extraction, should be avoided since they are precursor, by esterification, of fatty acid alkyl esters (FAAEs), which contribute to oil fermentative sensory defects.[28] In addition, compounds such as propyl propanoate, 1-propanol and acetic acid, which were found in fruits in advanced stage of anaerobic fermentation,[28] contribute to undesirable aromas in oil.

Concerning the second principal component (PC2), the compounds that mainly participated to its definition were mostly present in Cellina samples, which are in the positive range of this PC. The separate positions of green and green to mature Ogliarola fruit samples in the plot (Fig. 1A) suggest that compounds, which most contribute to PC2, increase their amount during the whole ripening process (e.g. decanol, octanal, $\alpha$-dimethyl-*p*-styrene)[25] or, at least, during the first stages, such as heptenal[24,25] and butanal.

**Table 2.** Compounds detected with PTR-MS-TOF technique that mainly contribute to the definition of PC1, PC2 and PC3, tentative identification and references; tentative identification of masses has been performed with the aid of known standard compounds as well as with available proton affinities and fragmentation patterns of pure standard, and confirmed by previous studies (references are listed in the table)

| Contribution to PCA (%) | Measured protonated mass (*m/z*) | Theoretical protonated mass (*m/z*) | Protonated chemical formula | Tentative identification | References |
|---|---|---|---|---|---|
| **PC1** | | | | | |
| 0.633 | 117.0910 | 117.1657 | $C_6H_{13}O_2^+$ | Propyl propanoate | 23 |
| 0.630 | 117.1274 | 117.2096 | $C_7H_{17}O^+$ | Heptanol | 25, 31, 33 |
| 0.621 | 89.0597 | 89.11214 | $C_4H_9O_2^+$ | Ethyl acetate/3-hydroxyl-2-butanone/3-methyl propanoic acid/butanoic acid | 23, 25–26, 28, 31, 33–34, 36–38, 41 |
| 0.620 | 61.0648 | 61.1024 | $C_3H_9O^+$ | 1-Propanol | 23, 28 |
| 0.620 | 61.0284 | 61.0585 | $C_2H_5O_2^+$ | Acetic acid | 23, 26, 28, 30–33, 36–38, 41 |
| 0.618 | 89.0961 | 89.1560 | $C_5H_{13}O^+$ | 2-Pentanol/1-pentanol/iso/3-methyl butanol | 23, 25, 30, 31, 34, 37 |
| 0.617 | 75.0441 | 75.0553 | $C_3H_7O_2^+$ | Propionic acid/methyl acetate | 23, 28, 30, 38, 41 |
| 0.617 | 103.0754 | 103.1389 | $C_5H_{11}O_2^+$ | Propyl acetate/ethyl propanoate/3-methyl butanoic acid | 23, 25, 28, 30, 31, 34 |
| 0.614 | 103.1117 | 103.1828 | $C_6H_{15}O^+$ | 1-Hexanol | 23, 25, 28, 30–32, 34, 36, 37 |
| 0.609 | 93.0699 | 93.1479 | $C_7H_9^+$ | Toluene | 10, 24, 25, 31, 36, 37 |
| 0.600 | 107.0491 | 107.1303 | $C_7H_7O^+$ | Benzaldehyde | 24, 25, 28, 37, 41 |
| 0.592 | 105.0621 | 105.1588 | $C8H9^+$ | Styrene | 10, 28, 31, 36 |
| 0.558 | 99.1168 | 99.1949 | $C_7H_{15}^+$ | Cycloheptane/heptene/methyl cyclohexene/1-heptene | 24 |
| 0.540 | 99.0804 | 99.1511 | $C_6H_{11}O^+$ | Cyclohexanone/mesityl oxide/cis-3-hexenal | 26, 28–31, 35–37 |
| 0.535 | 113.0597 | 113.1340 | $C_6H_9O_2^+$ | Sorbic acid | * |
| 0.495 | 101.0961 | 101.1669 | $C_6H_{13}O^+$ | *cis*-3-Hexen-1-ol/hexanal fragmentation of *trans*-2-hexen-1-ol | 23–26, 28–31, 33, 36–38, 41 |
| 0.477 | 83.0491 | 83.1084 | $C_5H_7O^+$ | 2-Methyl furan/pyran | 33–35, 41 |
| 0.452 | 105.0369 | 105.1722 | $C_4H_9OS^+$ | Methional | 25 |
| 0.441 | 137.1325 | 137.2435 | $C_{10}H_{17}^+$ | Terpenes | 24, 25, 28, 29, 31, 36–41 |
| 0.425 | 69.0335 | 69.0816 | $C_4H_5O^+$ | Furan | 10, 28, 30, 41 |
| 0.419 | 145.1223 | 145.2193 | $C_8H_{17}O_2^+$ | Ethyl hexanoate/octanoid acid | 31, 37, 38 |
| 0.399 | 63.0263 | 63.1364 | $C_2H_7S^+$ | Dimethyl sulfide | 36, 38, 41 |
| 0.396 | 85.0648 | 85.1243 | $C_5H_9O^+$ | Cyclopentanone | * |
| 0.376 | 87.0804 | 87.1401 | $C_5H_{11}O^+$ | 4-Penten-1-ol/2-methyl butanal/3-methyl butanal | 23, 25, 28, 30, 31, 33, 35, 37, 38 |
| 0.360 | 163.0754 | 163.1936 | $C_{10}H_{11}O_2^+$ | Safrol/isosafrol | * |
| 0.308 | 133.0648 | 133.1681 | $C_9H_9O^+$ | Cinnamaldehyde | 28 |
| 0.153 | 205.1573 | 205.3616 | $C_{15}H_{25}^+$ | *β*-Caryophyllene/copaene | 25, 28, 31, 37 |
| **PC2** | | | | | |
| 1.172 | 159.1743 | 159.2899 | $C_{10}H_{23}O^+$ | Decanol | 25 |
| 1.015 | 129.1274 | 129.2206 | $C_8H_{17}O^+$ | Octanal | 23–25, 28, 30, 31, 33, 36, 41 |
| 0.973 | 73.0648 | 73.1133 | $C_4H_9O^+$ | Butanal/butanone | 23, 36, 38, 41 |
| 0.891 | 113.0961 | 113.1778 | $C_7H_{13}O^+$ | Heptenal | 24, 25, 28, 30, 31, 34, 36 |
| 0.873 | 133.1012 | 133.2118 | $C_{10}H_{13}^+$ | *α*-Dimethyl-*p*-styrene | 25 |
| 0.844 | 47.0491 | 47.0756 | $C_2H_7O^+$ | Ethanol | 25, 28, 31, 33, 34, 36, 37 |
| 0.690 | 109.1012 | 109.1900 | $C_8H_{13}^+$ | Methyl norbornene | * |
| **PC3** | | | | | |
| 0.850 | 45.0335 | 45.0598 | $C_2H_5O^+$ | Acetaldehyde | 17, 28, 34, 38, 41 |
| 0.623 | 153.1274 | 153.2437 | $C_{10}H_{17}O^+$ | 2,4-Decadienal | 24, 25, 28, 30, 31, 34 |
| 0.596 | 57.0335 | 57.0707 | $C_3H_5O^+$ | 2-Propenal | 17, 31, 38 |
| 0.457 | 121.0648 | 121.1571 | $C_8H_9O^+$ | Phenyl acetaldehyde | 25, 28 |

*No literature has been found to confirm the identification.

Other compounds, such as acethaldehyde, 2,4-decadienal, 2-propenal and phenyl acetaldehyde, whose abundance increases during ripening,[24,25] which were not underlined by the principal component analysis involving the two first new components, were highlighted when considering the third one (Fig. 1B and Table 2). Some of the compounds that increased during the ripening process are responsible for unpleasant aromatic characteristics of olive oils.[28]

As expected, the PTR-TOF-MS analysis showed many VOCs with similar abundance in all the samples and, indeed, the presence of many compounds was shared between green fruits and fruits at intermediate ripening stage. Nevertheless, higher resolution

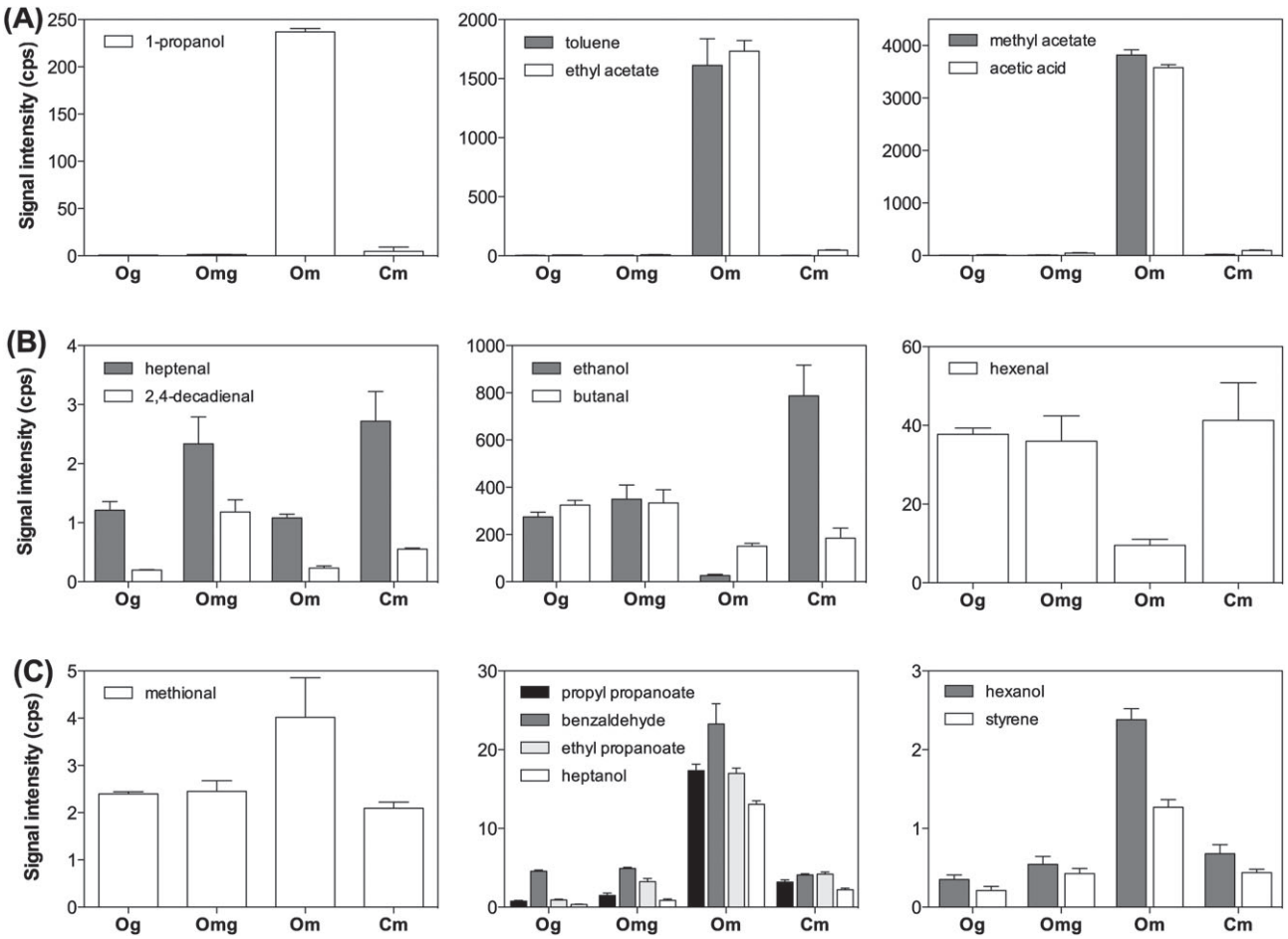


**Figure 3.** Signal intensity of interesting compounds (group 'a') monitored by PTR-TOF-MS: (A) group 'a'; (B) group 'b'; (C) group 'c' (Og, Ogliarola green; Omg, Ogliarola green to mature; Om, Ogliarola mature; Cm, Cellina mature). Means of four replicates and standard deviations are shown; $n = 4$.

analysis allowed us to individuate three groups of compounds with different abundance among sample.

The first group (A) consisted of VOCs which are essentially present in mature fruits of Ogliarola variety. Most of them (e.g. 1-propanol, ethyl acetate, methyl acetate and acetic acid) are undesirable flavours, probably due to over-ripeness of the fruits.[28] The analysis showed also higher level of some compounds which are usually found in mature fruits, such as toluene[24] (Fig. 3A).

The second group (B) consisted of VOCs observed in green and green to mature olives of Ogliarola which maintain high levels during fruit ripening, then decrease in completely mature fruits; some of them have already been observed by other authors; e.g. *cis*-3-hexenal, heptenal and 2,4-decadienal.[24,28,32] Their presence was higher in mature fruits of the Cellina variety than in those of Ogliarola, thus confirming the previous observation on the spatial distribution of groups in the scatter plot in Fig. 1A. In fact, samples from mature fruit of Cellina were closer to green and green to mature fruit of Ogliarola than to samples of Ogliarola with similar ripening stage (Fig. 3B).

The third group (C) consisted of VOCs that increased during the whole ripening process as already reported. Among these, benzaldehyde[24] and 1-hexanol,[29] which confers a banana-like character to the oil, have been used also to discriminate the maturity stages of different cultivars.[27,32,35] Interestingly, the amount of these compounds in mature Ogliarola olives was higher than in Cellina (Fig. 3C).

Mature fruits of Cellina did not produce unique compounds but they showed some VOCs in higher quantity with respect to the other samples, and were thus helpful for variety characterisation. These compounds corresponded to those highlighted with PCA analysis.

## CONCLUSION

In this research, a PTR-TOF-MS instrument, a novel, rapid, high sensitive and non-invasive tool for VOC analysis, was used to study the volatile emission in olive fruits harvested at different degrees of ripeness and belonging to two different Italian varieties.

The PTR-TOF-MS approach has been shown to be able to discriminate the aromatic compounds which are characteristic of each sample, allowing a distinction between different varieties as well as among fruits at different ripening stage.

PCA and HCA applied to PTR-TOF-MS data were both helpful to group together samples; the first analysis was shown to be more sensitive, since clusters of each different olive sample did not overlap each other. Furthermore, PCA analysis, allowing the individuation of the compounds that mainly contribute to the description of variability, was shown to be very efficient also in the characterisation of olive fruit samples, highlighting the typical compounds that have been used for olive oil and fruit characterisation in previous studies.[27,32,35]

Due to the high resolution, large spectra and small fragmentation, the PTR-TOF-MS technology is very useful not only for fingerprinting purposes but also for a deeper study of the physiological processes, such as fruit ripening, thus giving some preliminary information on the final aroma and quality of olive oil.

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