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Short note

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Comparative analysis of volatile compounds (potential aromatic ability) in the fruit of 15 olive Italian cultivars

C. Taiti [1], M. Redwan [1], E. Marone [2 (*)], G. Atzori [1], E. Azzarello [1], S. Mancuso [1]

[1] *Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, Università degli studi di Firenze, Viale delle Idee, 30, 50019 Sesto Fiorentino (FI), Italy.*

[2] *Facoltà di Bioscienze e Tecnologie Agro-Alimentari e Ambientali, Università degli Studi di Teramo, Via R. Balzarini, 1, 64100 Teramo, Italy.*

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Abstract: Virgin olive oils (VOOs) are characterized by peculiar flavors appreciated by the consumers all over the world. Their organoleptic characteristics depend on the aromatic properties of the fruits of the different cultivars, which will originate the final products. VOCs spectra of fifteen certified Italian olive cultivars of the University of Florence Germplasm collection, chosen as their different geographical origin, diffusion, and product purpose, were acquired using a Proton Transfer Reaction Time-of-Flight Mass Spectrometer (PTR-ToF-MS). The VOCs analyses highlighted a great variability among the fifteen cultivars, mostly due to compounds (C6 and C5) deriving from polyunsaturated fatty acids through the LOX pathway. The early identification in the olive fruit of these compounds which are considered among the major contributors to the positive VOOs attributes, would be useful to produce high quality olive oils, and get useful information to individuate the best parents for the genetic improvement.

[(*)] **Corresponding author:**
emarone@unite.it

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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1. Introduction

Virgin olive oil is worldwide considered a "commodity", and until the Second World War, a strategic food (Fiorino *et al.*, 2010). The complex flavor of virgin olive oil is mainly produced by volatile organic compounds (VOCs) whose formation is related to olive fruit cell destruction (Morales *et al.*, 1996; Angerosa, 2004). The total amount and types of VOCs emitted change during the processing steps of olive fruit in the olive mill (Morales *et al.*, 1997). In particular, VOCs emission is linked to the destruction of the cell structure of olive fruits which activates a specific chain of enzymatic reaction (LOX cascade) (Angerosa *et al.*, 2004). The C6 and C5 are the compounds which most affect the aroma of olive oil generating the positive attributes such as fruity and herbaceous notes (Marone *et al.*, 2017). These compounds are usually responsible for typical aromas and flavors and play a dominant role in determining the peculiar aroma and quality of olive oils (Zunin *et al.*, 2005). The synthesis of C5 and C6 compounds is linked to the level of LipOXygenase (LOX) activity to be catabolized through its pathway during the mill process (Garçia-Vico *et al.*, 2017). Different C6 straight chains are produced by the action of hydroperoxide lyase on polyunsaturated C18 fatty acids (linoleic C18:2 and linolenic C18:3). While, C5 seem to be synthesized through another branch of the LOX pathway starting from 13-hydroperoxides derived from linolenic acid (Garçia-Vico *et al.*, 2017).

Currently, the most innovative analytical technique used to detect VOCs emitted by fruits, which provides a high resolution coupled to a rapid screening power of samples and easy to handle without sample manipulation, is the Proton Transfer Reaction-Time of Flight-Mass Spectrometer (PTR-ToF-MS) (Mayr *et al.*, 2002; Masi *et al.*, 2015; Taiti *et al.*, 2017 a).

Currently, a little information is available and at the best of our knowledge one study was carried out to understand the VOCs profile emission from olive fruits related to two cultivars (Masi *et al.*, 2015). The aim of this study was to develop a quick, accurate and relatively simple evaluation method based on the PTR-MS technique, to define the aromatic potentiality of each olive cultivar starting from olive fruits.

This method could potentially help to address the choice of the cultivars in new plantations and to identify parents for future crosses, aimed to improve some organoleptic characteristics of the oils.

2. Materials and Methods

Plant material

Fruits of 15 certified Italian olive cultivars, clonally propagated, different in their geographical origin, distribution and fruit's use were obtained from plants of Olive Germplasm collection deriving from the Italian and World olive Gene bank (Montepaldi experimental farm (43° 40' 39'' North latitude, 11° 08' 46" East longitude, 210 m asl), University of Florence) (Table 1). The rainfed, bush trained, fourteen years old olive plants are ordinarily cultivated, grown on a slight slope of a sedimentarious gipsy-arenaceous soil; the annual rainfall average is 867 mm, the average annual temperature results 13.3°C.

Fruit sampling

For each cultivar, 1 kg of sound and healthy fruits was collected on October 20th from different parts of the trees of each cultivar. The day after, 100 washed fruits from each cultivar were weighted (W) and the color index (CI) was determined (Uceda and Hermoso, 1998). Subsequently, for each cultivar nine whole and cut samples of fruits (~10 g) were submitted to the VOCs analysis.

Statistical analyses

One way analysis of variance (ANOVA) was per-

Table 1 - List of the characteristics of the genetic material

Cultivar	Origin	Product use	Fruit average weight (g)	Color Index (0-7)
Ascolana tenera	Marche	Table	4.80 ± 0.51	2.2
Bianchera	Friuli Venezia Giulia	Olive oil	2.68 ± 0.12	1.5
Carolea	Calabria	Olive oil/Table	3.91 ± 1.17	1.2
Coratina	Apulia	Olive oil	2.05 ± 0.60	1.5
Fasolona	Basilicata	Olive oil/Table	3.80 ± 0.58	3.1
Frantoio	Tuscany	Olive oil	1.96 ± 0.36	1.5
Itrana	Lazio	Olive oil/Table	2.19 ± 0.94	1.4
Leccino	Tuscany	Olive oil	2.02 ± 0.19	2.2
Maiatica di Ferrandina	Basilicata	Olive oil/Table	2.75 ± 0.93	1.2
Moraiolo	Tuscany	Olive oil	1.42 ± 0.28	2.1
Nocellara del Belice	Sicily	Olive oil/Table	3.84 ± 0.76	1.8
Palmarola	Apulia	Olive oil/Table	2.15 ± 0.72	3.1
Sant'Agostino	Apulia	Table	4.36 ± 0.81	0.7
Santa Caterina	Tuscany	Table	5.80 ± 0.48	0.4
San Francesco	Tuscany	Table	2.68 ± 0.22	1.5

formed to compare the considered groups of chemical compounds: C5, C6, other VOCs, and total VOCs. Separation of means was performed by the Fisher's LSD test (p = 0.01). Computations were performed by Statgraphics Centurion XV v. 15.0.04.

A Factor Analysis (FA) was applied to the spectral data of 273 olive oil samples, considering as factors three grouping of compounds: total VOCs, C5, and C6, respectively. Computations were performed by XLSTAT 2014.5.03.

A Principal Component Analysis (PCA, unsupervised method) was applied to the whole spectral data of 273 olive oil samples, submitted to a logarithmic transformation and mean centering as pre-processing. Computations were performed by PLS-Toolbox v. 8.0.2 (Eigenvector Research Inc., West Eaglerock Drive, Wenatchee, WA) for MATLAB_ R2015b (Mathworks Inc., Natick, MA, USA).

Volatile compounds detection

Through a PTR-ToF-8000 (IONICON Analytik, GmbH, Innsbruck, Austria) measurements were performed in a similar way to the one reported by Taiti *et al.* (2017 b). Each analyzed sample consisted of ~10 g of olive fruits intact or cut into 4 parts. Subsequently the sample was inserted into a 3/4 L glass container plugged with a cover in which two Teflon tubes are inserted, connected respectively to a zero-air generator (Peak Scientific) and to the PTR-ToF-MS. The drift tube had the following ionization conditions: pressure of 2.20 mbar, voltage 600V and temperature 110°C. Mass spectrometric data were collected over a mass range of *m/z* 21 to 210 and the acquisition time for each samples was 0.1 ns, for 120 seconds. The instrument internal calibration was based on: *m/z* = 29.997 (NO^+); *m/z* = 59.049 ($C_3H_7O^+$) and *m/z* = 137.132 ($C_{10}H_{17}^+$) and was performed off-line. The raw data were acquired with the TofDaq Software (Tofwerk AG, Switzerland) as cps (count per second), and subsequently were converted in ppbv following the formula described by Lindinger and Jordan (1998) on the basis of the primary ion signal.

3. Results and Discussion

The whole intact olive fruits of the fifteen cultivars showed no detectable quantities of VOCs emission; in fact, the LipOXygenase pathway is activated when the fruit is damaged by the cut, producing a large quantity of volatile compounds, among which different C5 and C6 compounds, which are the main volatiles responsible for the positive aroma of olive oil (Angerosa *et al*., 2004). Meanwhile a great variability exists among the total VOCs emitted by the cut fruits of fifteen different cultivars (Table 2), ranging from 1061.8 ppbv in 'Ascolana Tenera' to 8767.5 ppbv for 'Carolea'. Therefore, the total emission from 'Carolea' was significantly higher compared to 'Ascolana Tenera'. The cultivars can be arbitrarily divided in three main groups according to their total VOCs emission: (a) low VOCs emission, including together with the cv. Ascolana tenera, Fasolona and

Table 2 - Analysis of variance (ANOVA) for the C5, C6, and total VOCs related to the fifteen cultivars

Cultivar	C5 Compounds (ppbv)	C6 Compounds (ppbv)	Total VOCs (ppbv)	VOCs emission (Arbitrary ranking)
Ascolana tenera	8.01±0.61 A	180.73 ± 47.17 A	1061.77 ± 120.13 A	low
Bianchera	35.29±2.15 G	1143.77 ± 148.60 F	5101.34 ± 305.77 G	high
Carolea	53.90±7.83 I	2864.58 ± 543.60 I	8767.54 ± 1024.09 G	high
Coratina	12.02±1.15 D	261.36 ± 34.62 ABC	3177.13 ± 281.13 F	medium
Fasolona	6.64±0.44 AB	229.19 ± 35.91 AB	1845.41 ± 124.20 DE	low
Frantoio	11.86±1.43 D	525.42 ± 96.22 DE	2076.17 ± 149.65 ABC	medium
Itrana	43.63±1.75 H	2497.20 ± 222.50 H	7877.17 ± 491.75 G	high
Leccino	9.07± 0.51 BC	264.10 ± 58.53 ABC	3380.28 ± 148.72 F	medium
Maiatica di Ferrandina	24.49±3.21 F	1581.16 ± 262.15 G	5339.86 ± 472.56 EF	high
Moraiolo	17.41±1.52 E	533.93 ± 67.89 DE	2768.21 ± 150.04 BC	medium
Nocellara del Belice	16.77±1.15 E	544.07 ± 104.33 E	2986.40 ± 128.63 D	medium
Palmarola	9.01±0.57 B	404.83 ± 64.22 CDE	3142.02 ± 218.24 DE	medium
Sant'Agostino	7.65±1.66 AB	148.79 ± 27.75 A	2309.40 ± 141.08 DE	medium
Santa Caterina	8.73±1.61 B	402.58 ± 55.28 CDE	2121.11 ± 148.07 AB	medium
San Francesco	11.54±0.76 CD	368.17 ± 43.71 BCD	1934.07 ± 65.61 C	low

Different upper case letters within a column indicate the difference by the LSD test at the 99% confidence leve (p= 0.01).

San Francesco; (b) medium VOCs emission, including 'Leccino', 'Coratina', 'Palmarora', 'Nocellara del Belice', 'Moraiolo', 'Sant'Agostino', 'Santa Caterina', 'Frantoio' and (c) high VOCs emission, including 'Carolea', 'Itrana', 'Maiatica di Ferrandina', 'Bianchera'. Also C6 and C5 straight chain showed an ample variability; in particular, C6 compounds ranged from 148.79 ppbv for the cv. Sant'Agostino to 2864.58 ppbv for the cv. Carolea, with a fluctuation among the different cultivar of about 1:20. As the high amount of developed C6 compounds it is noteworthy to highlight the cv. Carolea, Coratina, and Itrana. On the other hand, the C5 compounds showed a minimum value of 6.64 ppbv (cv. Fasolona), and a maximum of 53.90 ppbv (cv. Carolea).

A biplot from Factor Analysis (Fig. 1) simultaneously represents the relationship between total VOCs, C6, and C5 compounds, highlighting the relative distances among the fifteen olive cultivars. The first axis explains the 97.25% of the total variability in the spectral data. The samples are clustered in two main groups (Fig. 1), characterized by higher VOCs emission (right quadrants), and lower VOCs emission (left quadrants). Although the different total amount, and despite the quantitatively different responses of the different cultivars, C5 and C6 compounds resulted directly related to the total VOCs emission (0.952 ppbv, and 0.964 ppbv, respectively), as a consequence of a more general phenomenon, the LipOXygenase (LOX) pathway, common to all the examined cultivars. Therefore, by evaluating the total VOCs amount, it is possible to analytically extrapolate the contribution of C5 and C6 in the total volatile compounds emission. Moreover, by the Factor Analysis plot it can be noted that some cultivars are characterized by higher C6 compounds emission (e.g. 'Itrana' and 'Maiatica di Ferrandina'), while other cultivars showed higher C5 emission (e.g. 'Bianchera' and 'Carolea').

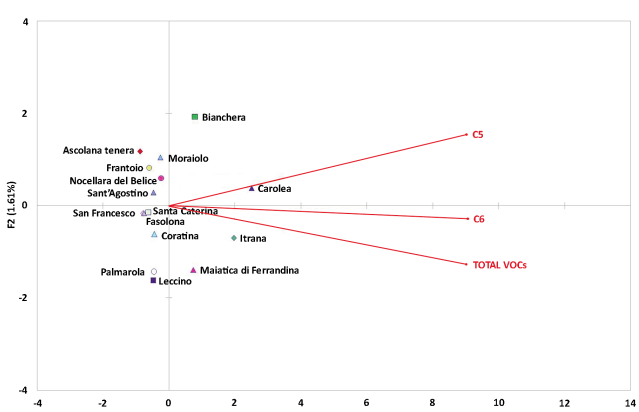
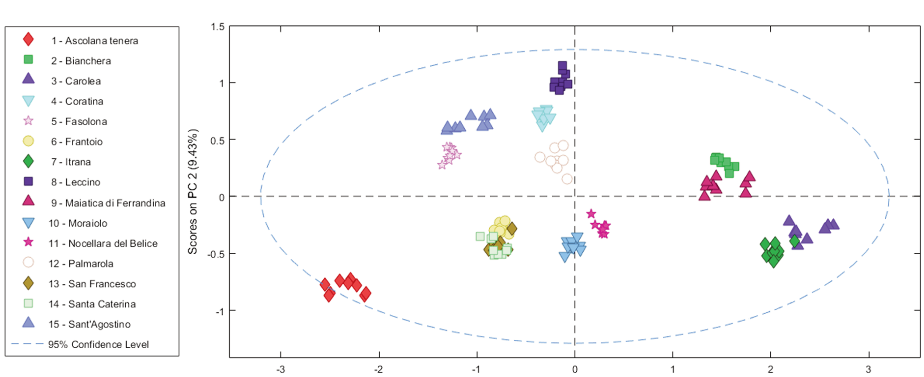


Fig. 1 - Biplot from Factor analysis. Relationships among the fifteen cultivars and the three groups of chemical compounds (C5, C6, total VOCs).

Subsequently a PCA (Fig. 2) was performed on the whole VOCs data set to (1) give a general overview of the cultivars ordination and (2) to detect if the cultivars can be grouped basing on their geographical origin and/or final product use. ANOVA results confirm (Table 2) that a high variability appears in the VOCs emission from the different cultivars, widely distributed without any particular tendency to clustering, excluding any possible link of the different cultivars due to their geographical origin or to the final product use. The first two components justified 58.32% for PC1 and 9.43% for PC2 of the total variability, respectively, indicating that the greatest amount of the total variance is explained by the PC1. The most important PC1 loadings resulted in eight *m/z* as reported in figure 2. In particular, *m/z* = 81.069 [Tentatively Identified (T.I.) as Hexenal fragment] and *m/z* = 99.080 (T.I. as 2,3-Hexenal) directly deriving from linolenic acid and responsible for the freshly cut grass odors, are previously reported as positive attributes in oils by Marone *et al.* (2017) and Taiti and Marone (2017).



Protonated m/z	Chemical formula	PC 1 loadings	Min value	Max value
53.038	$C_4H_5^+$	0.22	10.02	172.29
55.054	$C_4H_7^+$	0.22	52.68	1476.80
59.049	$C_3H_7O^+$	0.22	8.16	173.27
65.038	$C_5H_5^+$	0.21	0.94	23.79
71.086	$C_5H_{11}^+$	0.21	0.00	13.63
79.054	$C_6H_7^+$	0.23	16.20	364.47
81.069	$C_6H_9^+$	0.28	81.83	3641.62
99.080	$C_6H_{11}O^+$	0.24	5.12	116.01

Fig. 2 - Score plot from PCA related to the VOCs dataset (ppbv) for the fifteen cultivars; in the table the main PC1 loadings are reported.

The reported data confirmed the possibility to predict and quickly evaluate during all stages of the fruit ripening the presence of VOCs characterizing the positive flavors of the olive fruits obtained from defined cultivars or new breeding.

4. Conclusions

The data show a great variability in both amount and type of VOCs emitted by olive fruits, simultaneously collected in the same environment from fifteen different Italian cultivars. For example the total emission by 'Carolea' was significantly higher compared to 'Ascolana Tenera'. On the other hand, C5 and C6 compounds which most affect the aroma adding positive attributes to olive oil, were respectively 95% and 96% correlated to the total VOCs. Among the cultivars it is possible to note a different relationship between C5 and C6: the cv. Bianchera showed the highest C5/total VOCs ratio, while the cv. Itrana, and Carolea the highest C6/total VOCs ratio.

The presence of these two groups of compounds (C5 and C6) confirms that the cut of the fruit triggers the same phenomena that verifies at the moment of the olive processing, that is the activation of the LOX cascade, which gives rise to VOCs considered to be positive attributes for the olive oil aroma.

Further studies would be necessary to deepen and to develop the information that can be obtained from the use of the fruits to: (1) understand the behavior of the derived oils, and (2) to widen the genetic platform from which information can be acquired and (3) to start verifying the responses of fruits deriving from predetermined crosses. In conclusion, the possibility to early identify these compounds at the fruit level can allow to individuate the best conditions to produce high quality olive oils, and get useful information to individuate the best parents for the olive genetic improvement.

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