Adv. Hort. Sci., 2018 32(1): 105-111 DOI: 10.13128/ahs-22807

AHS
Advances in Horticultural Science

Volatile compounds from different fruit parts of two cultivars of *Cydonia oblonga*

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Key words: fruit tissues, Proton Transfer Reaction Time-of-Flight Mass Spectrometer, physicochemical fruit parameters, quince, VOCs.

Abstract: Quince is characterized as a fragrant fruit which, unlike other pomes (apple, pear), is not used for fresh consumption due to its astringency and compactness, but only in its processed form (jams, jelly, distillery products, and nutraceutical compounds). As a consequence, there is little knowledge currently available concerning the characteristics of the fruit, and in particular its aromatic and chemotaxonomic patterns. In this work, carpometric, chemometric and spectrophotometric measurements were performed on quince fruits. VOCs emitted by different tissues or parts of the fruit were studied to describe its aromatic profile. The study was carried out on the fruits of an old, well-known cultivar ('Gigante di Wranja', commonly called 'Wranja') and a new Tuscan accession. Intact, halved and solely pulp (cubed) samples were evaluated for each individual fruit. Data obtained from VOC analysis through Proton Transfer Reaction Time-of-Flight Mass Spectrometer (PTR-ToF-MS) were evaluated by multivariate statistical analysis. The spectra obtained from the intact fruit samples showed a higher amount of masses corresponding to terpenes or terpenoid compounds, which fundamentally characterize the aroma of this type of fruit; these substances were found to be much less present in the VOCs emitted by the pulp, where high values of masses linked to the maturation processes were instead found.

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Citation:
TAITI C., GIORDANI E., PALM E., PETRUCCI W.A., BENNATI G., GESTRI G., MARONE E., AZZARELLO E., MANCUSO S., 2018 - *Volatile compounds from different fruit parts of two cultivars of Cydonia oblonga.* - Adv. Hort. Sci., 32(1): 105-111

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 2 December 2017
Accepted for publication 6 March 2018

1. Introduction

Plants are not only a dietary source for both human beings and animals but also serve as medicinal, nutritional, and ornamental purposes

(Ashraf *et al.*, 2016). The potential of plants as medicine is supported by abundant scientific evidence (Lattanzio *et al.*, 2009) and researchers are currently focused on isolating new aromas and active phytochemicals produced by different plant organs. Quince is the fruit of an ancient tree, *Cydonia oblonga* Miller of the Rosaceae family, and has a very wide origin area that ranges from Anatolia to the Caucasus. It was known to the Greeks and Romans, and it seems that the name of the genus *Cydonia* comes from the ancient name of the town of Chania on the island of Crete (Roversi, 1991). It is a climacteric, large-sized (up to 500 g) fruit, namely pome (false fruit), and generally oblong (pyriform types) or rounded (apple-shared types) (Bellini *et al.*, 2007). The harvest is still carried out by hand, although the desire to mechanize this operation with systems similar to those used for the mechanical harvesting of the apple tree is increasing (Fiorino *et al.*, 2010). When ripening, the color of the fruit epidermis turns from greenish-yellow to bright yellow when fully ripe. The pulp is very firm, and not directly edible due to the presence of tannins and pectic substances (Bellini *et al.*, 2007). Ripe fruits are commonly used as a source of pectin, and to produce jam or jelly, liquors, and distilled products with aromatic notes typical of quince (Silva *et al.*, 2004). The fruits can also be beneficial to human health thanks to their high phytochemical content (Ashraf *et al.*, 2016). Many studies have reported *Cydonia oblonga* as an excellent natural source of phenolic and flavonoid compounds which are considered potent antioxidants (Silva *et al.*, 2004; Oliveira *et al.*, 2007). Quince fruit is considered an important dietary source of health-promoting compounds due to its antioxidant, antimicrobial and antiulcerative properties (Magalhães *et al.*, 2009).

Although the aroma is one its most important fruit-derived parameters, only a few studies have focused on the volatile emission by *Cydonia* (Tateo and Bononi, 2010), and in particular, by different tissues of the fruit. Indeed, the intense and pleasant aroma released by *Cydonia* may represent an important starting point in the genetic improvement of this species.

Recently, Proton Transfer Reaction-Time of Flight-Mass Spectrometry (PTR-ToF-MS) was proposed as an innovative analytical technology for volatile organic compound (VOC) detection and quantification on fruit matrices due to its capacity to rapidly provide a comprehensive mass spectrum with high-time resolution and without sample treatment (Mayr *et al.*, 2003; Taiti *et al.*, 2017 a). The aim of the current study was to analyze the aroma profile of different fruit parts of two *Cydonia oblonga* genotypes, both grown in Italy, to increase the chemotaxonomic knowledge, fruit management and quince product trade.

2. Materials and Methods

Plant materials

Quince fruits belonging to an old, well-known cultivar ('Gigante di Wranja', commonly called 'Wranja') and a new Tuscan accession were harvested in Tuscany (Italy) in the last week of September 2017. For each cultivar, 12 homogeneous and healthy fruits were selected, gently brushed to eliminate the hair cover and washed in deionized water. Fruits were stored in a climatic chamber (14±1°C, 85-90% relative humidity) for three days prior to laboratory analysis. For all the fruits, color, weight, maximum diameter and height were determined. Six fruits were used for the spectrophotometric tests by PTR-ToF-MS and the remaining six for the physicochemical measurements. For chemical analyses (Lugol starch test and refractometric grade), fruits were divided in half so that one half of each fruit was used for the starch test and the other to evaluate refractometric grade.

Color and physicochemical fruit parameters

Peel color. The identification of the different peel color of quince fruits was assessed using a Minolta CR-200 Chroma-Meter (Minolta, Ramsey, NJ, USA) according to the Hunter scale, as previously described by Taiti *et al.* (2017 b).

Fruits dimensions and firmness. For each cultivar, the weight (g), maximum diameter (cm), and height (cm) of twelve samples were measured. The firmness of each fruit (expressed as kgf) was measured as the force needed to reduce fruit diameter by 2 mm using a penetrometer (Model 53205D, Turoni, Italy).

Total soluble solids (TSS). To evaluate TSS concentration, six quince halved fruits for each genotype were squeezed to collect a few drops of juice; then TSS levels were measured with an N1 Atago refractometer (Atago Co., Japan) and expressed as °Brix.

Starch Iodine Test. Starch-iodine test (Lugol solution) was performed by visual evaluation on halved fruits and by scoring samples on a 'Golden Delicious' standardized 1-9 scale (Smith *et al.*, 1979).

Volatile compounds detection

VOCs analyses were carried out with a high-reso-

lution PTR-ToF-MS (IONICON Analytik GmbH, Austria) using H_3O^+ as ion donor. For each genotype, six fruits were used; the same fruit was first analyzed as intact whole, then divided into two parts, analyzing the halves together; finally, two cubes were got from each one of the halved fruit respectively, without peel (5 × 5 × 5 cm, about 10 g each one); all the measurements were performed in triplicates on the same sample. All whole and halved samples were weighed, and the VOCs data were based on 100 g samples for whole and halved samples to allow for comparisons between the obtained results.

The drift tube of PTR-ToF-MS instrument was set to 80°C and operated with a drift pressure of 2.30 mbar and a voltage of 550 V. These settings lead to an E/N ratio (E, electric field strength in the drift tube; N, buffer gas number density in the drift tube) equal to 135 Townsends (1 Td = 1017 Vcm^2). Mass/charge ratio of peaks detected at *m/z* 21 (signal for H_3O^+) and *m/z* 37 [signal for water clusters $(H_2O)H_3O^+$] were monitored during all measurements to check the instrument's stability and cluster ion formation. To reach a good mass accuracy (up to 0.001 Th), internal calibration was based on three points and was performed off-line. Acquisition and post-processing data were performed as reported by Marone *et al.* (2017).

Statistical analyses

One-way analyses of variance (ANOVA) were performed (1) to compare the physicochemical parameters of two quince genotypes, and (2) to compare the VOC emission profiles whole, halved, and cubed fruit samples. Separation of means was performed by the Fisher's LSD test (p= 0.05). Computations were performed by Statgraphics Centurion XV v. 15.0.04. A Principal Component Analysis (PCA) was applied to the whole spectral data of 36 quince samples, previously 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). A Correspondent Analysis (CA) was applied to the spectral data of the 36 quince samples to build up a simultaneous ordination of quince samples and protonated *m/z*, thus facilitating the evaluation of their reciprocal relationships. The analysis preserves the X^2-distances between the data matrix rows and columns. Data were weighted using the symmetric option (module HIERCLUS, SYN-TAX 2000 program package).

3. Results and Discussion

Physicochemical analyses

The results of the physicochemical analyses on the different cultivars are reported in Table 1, and are largely in agreement with a previous study on different cultivars (Leonel *et al.*, 2016). Among the physicochemical parameters analyzed, some significant differences were observed between the two *Cydonia* accessions, with regard to firmness, °Brix values and starch contents. In particular, the °Brix content was lower for 'Wranja' (12.2±0.9) compared to Tuscan accession (17.2±0.9); vice versa the starch content was higher for 'Wranja'. The starch and sugar contents measured for the Tuscan accession indicate that these fruits may have been further along in the ripening process than the 'Wranja' fruits.

VOCs headspace analysis

Samples (whole, halved and cubed) belonging to two different *Cydonia* genotypes were analyzed by PTR-ToF-MS detecting a total of 67 peak signals (Fig. 1). These signals represent different groups of volatile compounds including hydrocarbons, esters, alcohols, terpenoids, aldehydes, ketones and lactones. These results show that, even if no different volatile compounds were identified, different emission intensities were found between the two genotypes (Fig. 1). On the other hand, among fruit tissues, both differences in number and emission intensity of each detected compound were instead observed. Figure 1 shows that for both cultivars, the intensity and number of compounds emitted were higher in

Table 1 - One-way analysis of variance (ANOVA) related to the quince fruits physicochemical parameters (average ± sd)

Cydonia accessions	Weight (g)	Ø (mm)	High (mm)	Firmness (kgf)	°Brix	Starch	Overcolor			Background color		
							L*	A*	B*	L*	A*	B*
Wranja	268.9	82.8	77.8	6.1	12.2	4.25	77.7	-12.5	62.6	72.6	-15.9	58.4
	±54.5	±7.0	±4.5	±0.9 a	±0.9 a	±0.5 a	±2.4	±2.4	±3.3	±4.1	±2.3	±3.1
Tuscan	320	86.5	85.4	7.6	17.2	2.75	76.9	-13.1	57.6	76.6	-12.8	60
	±92.6	±9.2	±9.7	±0.4 b	±0.9 b	±0.5 b	±4.7	±2.3	±5.6	±2.6	±2.2	±2.9

Different lowercase letters within a column indicate differences by the LSD test at the 95% confidence level (p= 0.05).

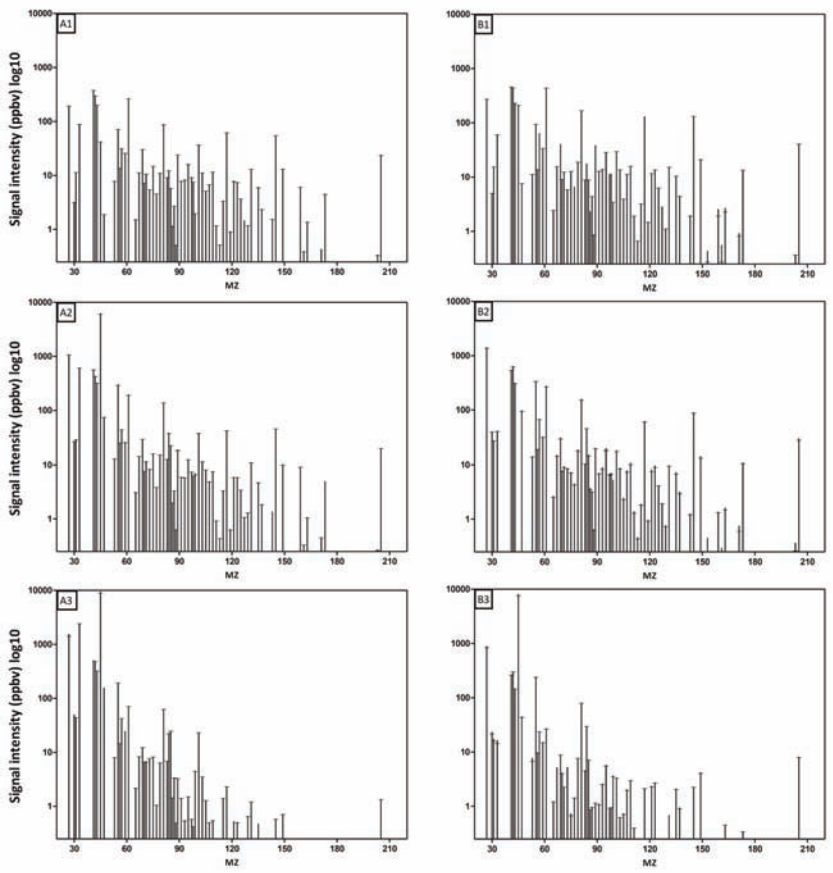

Fig. 1 - Typical PTR-MS mass spectrum (average, n=6) obtained by different fruit parts of two cultivars of *Cydonia*. (A) Tuscan accession, (B) 'Wranja'; (1) whole fruit, (2) halve fruit, (3) cube fruit.

the whole fruit followed by halved fruit, and lowest in cubed fruit. These results are in agreement with a previous study of Imayoshi *et al.* (1995) on Nashi pear, where differences in the total number of VOCs among pulp, peel, and whole fruit were found. Indeed, as reported by Paillard (1981) for many fruits species, VOC production changes among different fruit tissues, being highest in the skin and nearby tissues. It is noteworthy that fruit's aroma depends on the combination of VOCs produced, and on the concentration and odor threshold of each in the blend. A further investigation of the spectral data was performed by a multivariate ordination. The PCA (Fig. 2) approach applied to the whole dataset (ppbv) of 67 detected protonated masses (data not shown) give a general view of the quince sample ordination, based on the analysis of defined fruits parts (intact, halved, and cubed fruits) related to the two chosen accessions (Tuscan accession and 'Wranja'). The first three components accounted for 96.4% of the total variability (66.1%, 17.7%, 12.7%, respectively). A strong effect related to the type of the sample used rather than the cultivar is evident. The whole fruit samples of both cultivars, joined in the lower right quadrant of the diagram, strongly differ from those obtained from the head space of the pulp only, both correlated with the negative part of the x-axis (PC1), but clustered based on the cultivar, and respectively related to the positive part (Tuscan accession) and to the negative part (Wranja) of the y-axis (PC2). The halved fruit group is placed in the upper right quadrant, with the two cultivars partially overlapped, indi-

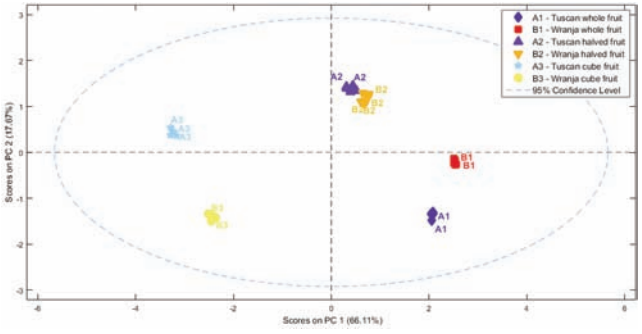

Fig. 2 - PCA scores of the quince samples based on the full spectra distribution of the 'Tuscan accession' and 'Wranja'. (A1) = 'Tuscan accession' whole fruits; (A2) = 'Tuscan accession' halved fruits; (A3) = 'Tuscan accession' cube fruits; (B1) = 'Wranja' whole fruits; (B2) = 'Wranja' halved fruits, (B3) = 'Wranja' cube fruits.

cating a greater similarity of masses with the intact whole fruit, even if positively correlated with PC2. To better evaluate the effect of specific chemical family compounds, the 20 masses with statistically significant (p= 0.05) discriminating power among the different parts of the fruit were selected by ANOVA within the whole spectrum (Table 2), upon which a Correspondence Analysis was subsequently applied.

The join plot from CA (Fig. 3) simultaneously displays quince different sample and protonated *m/z* ordinations based on the results of the variable selection. The intact fruits of both cultivars are characterized by a group of masses in which terpenes and terpenoids are well represented. In particular, the highest presence of monoterpene (*m/z* 135, *m/z* 137), terpenoid (*m/z* 153) and sesquiterpene (*m/z* 205) compounds distinguished the whole fruit from the other two types of cut samples. According to Taiti *et al.* (2017 b), the higher emission intensity and number of VOCs detected in whole fruits was associated to the presence of the peel. It is well known that a great diversity of VOCs linked to the pericarp tissue of the peel are emitted, such as esters, alcohols, aldehydes and terpenes (Chervin *et al.*, 2000; Rodriguez *et al.*, 2013).

The VOCs linked to cutting, such as the green leaf volatiles (GLVs) (i.e., hexenal, hexanal), are highest in halved and cubed fruits, compared to intact fruits where the intensities of these signals were very low (Table 2), and confirmed by the fact that these compounds were well represented in pulp tissue.

Moreover, halved fruits occupy an intermediate position, while cubed fruit samples are shifted to the right in the diagram (Fig. 3), with a differentiation along the axis of the y (axis 2) determined by the masses *m/z* = 45.033, *m/z* = 47.049 and *m/z* 33.033 (only for the Tuscan accession). As reported by Taiti *et al.* (2015) the methanol emission in fruits increase

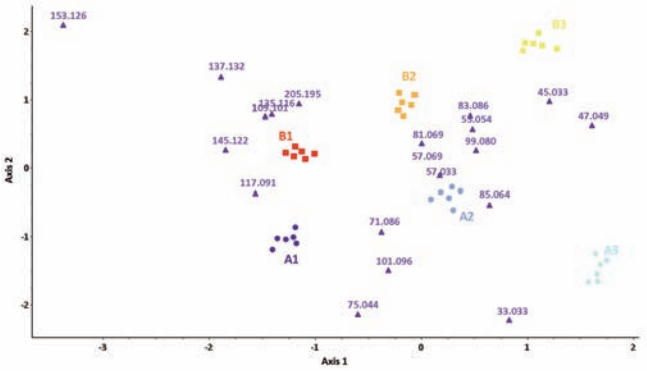

Fig. 3 - Join plot from Correspondence Analysis. (A1) = 'Tuscan accession' whole fruits; (A2) = 'Tuscan accession' halved fruits; (A3) = 'Tuscan accession' cube fruits; (B1) = 'Wranja' whole fruits; (B2) = 'Wranja' halved fruits, (B3) = 'Wranja' cube fruits. Numbers correspond to the protonated m/z.

Table 2 - Protonated selected masses discriminating whole, halved and cubed fruit samples, identified via PTR-ToF-MS: Mass/charge (m/z) ratios, chemical formula, tentative identifications, minimum and maximum values detected (ppbv) and VOC, as previously reported in the literature (Cydonia*; PTR-MS#)

No.	Protonated *m/z*	Chemical formula	Tentative identification	Min detected value (ppbv)	Max detected value (ppbv)	References
1	33.033	CH_5O^+	Methanol	11.7	2724.1	Khoubnasabjafari and Jouyban (2011)*
2	45.033	$C_2H_5O^+$	Acetaldehyde	35	16416.9	Umano *et al.* (1986)*
3	47.049	$C_2H_7O^+$	Ethanol	1.5	199.8	Umano *et al.* (1986)
4	55.054	$C_4H_7^+$	C4 aldehydes fragment	65.3	433.1	Taiti *et al.* (2017 b)#
5	57.033	$C_3H_5O^+$	Alkyl fragment (hexanal/hexyl acetate)	8.5	25.4	Taiti *et al.* (2017 b)#
6	57.069	$C_4H_9^+$	Alkyl fragment (Hexanol/valeric acid)	18.1	75.2	Taiti *et al.* (2017 b)#
8	75.044	$C_4H_{11}O^+$	2-butanol/2-methyl-1-propanol	0.5	19.7	Umano *et al.* (1986)*
9	81.069	$C_6H_9^+$	Terpenes and C6 fragments	49.8	203.5	Maleknia *et al.* (2007) #
10	83.085	$C_6H_{11}^+$	C6 fragments /hexenol fragment	8.8	50.7	Maleknia *et al.* (2007)#
11	85.064	$C_5H_9O^+$	2-Methyl-2-butenal/1-Penten-3-one	4.6	26.2	Khoubnasabjafari and Jouyban (2011)*
12	99.080	$C_6H_{11}O^+$	(E)-2-hexenal	1.6	8.9	Umano *et al.* (1986)*
13	101.096	$C_6H_{13}O^+$	Hexanal/(Z)-3-hexen-1-ol	1.7	44.8	Tateo and Bonomi (2010)*
14	109.101	$C_8H_{13}^+$	Terpene fragments	0.4	23.6	Maleknia *et al.* (2007) #
15	117.091	$C_6H_{13}O_2^+$	Isobutyl acetate/Ethyl butyrate/butyl acetate	1.5	176.7	Tateo and Bonomi (2010)*
16	135.116	$C_{10}H_{15}^+$	p-Cymene	0.4	11.9	Tateo and Bonomi (2010)*
17	137.132	$C_{10}H_{17}^+$	Monoterpenes (e.g. limonene, g-terpinene)	0	5.1	Tateo and Bonomi (2010)*
18	145.122	$C_8H_{17}O_2^+$	Ethyl hexanoate	0.5	153.9	Tateo and Bonomi (2010)*
19	153.127	$C_{10}H_{17}O^+$	Oxygenated Terpenes (e.g. geranial)	0	0.4	Tsuneya *et al.* (1983)*
20	205.195	$C_{15}H_{25}^+$	Sesquiterpenes (e.g. bergamotene)	1.1	49.8	Tateo and Bonomi (2010)*

steadily throughout the ripening process. On the other hand, ethanol is the product of anaerobic metabolism and, together with acetaldehyde, accumulates in pome fruit under imposed hypoxia and poor gas exchange in the pulp tissue (Pinto *et al.* 2001). Pome fruit contains ethanol and acetaldehyde as part of their aroma (Ritenour *et al.* 1997) and as reported by Rapparini and Predieri (2003) these compounds increase together with the ripening process and their emission increased at a faster rate with the onset of fruit senescence.

4. Conclusions

The typical quince fruit aroma depends on a mix of volatile compounds which originate by different parts of the pome. By the analyses of different fruit tissues, differences in VOCs number and in their emission intensity were observed, while little difference was observed between the two varieties analyzed. Moreover, it has been shown that the intensity and number of compounds emitted was highest in the whole fruit, followed by halved fruit and cubed fruit. Through multivariate analysis, some masses have been highlighted that determine the differentiation of the spectral composition between the VOCs emitted by the pulp (halved and cubed fruit samples) and those obtained from intact fruit that could be specifically responsible for the particular aroma of quince fruit. The four masses referring to terpenes and terpenoids are prevalent in the peel, and are moderately related to the cultivar. Further work should be done to increase the knowledge concerning the chemotaxonomic profiles of the different cultivars, and to understand how through this tool, innovations can be developed and moved to quince genetically improved and to the food industry.

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