Morpho-chemical and aroma investigations on autochthonous and highly-prized sweet cherry varieties grown in Tuscany

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Key words: fruit aroma, morpho-chemical parameters, *Prunus avium* L., PTR-ToF-MS, volatile compounds.

Abstract: The morpho-chemical and aromatic characteristics of four sweet cherry cultivated varieties (*Prunus avium* L.) grown in the area of Lari (Pisa, Central Italy) were evaluated with the aim to investigate their properties, mainly concerning volatile organic compounds (VOCs). Of these, three cultivars ('Di Giardino', 'Di Nello', and 'Marchiana') represent ancient sweet cherries recovered through a private cultivation program (belonging to the group of the so-called 'Ciliegia di Lari'); their evaluation was compared with the commercial cultivar Ferrovia, highly-prized variety marketed in Italy and abroad. Morpho-chemical analyses highlighted statistical differences among the cultivars under study, mainly on total soluble solids (TSS) and tritatable acidity (TA). Aroma investigation was performed with PTR-ToF-MS (proton transfer reaction - time of flight - mass spectrometer) approach, employed here for the first time in cherry fruits. About 50 VOCs were detected; among them, those belonging to the chemical classes of aldehydes and alcohols were the most represented although with different intensities between samples. Tentative identification of some key VOCs for cherry fruit was also performed and preliminary conclusions on the characterization of ancient and wide spread Italian cultivars were given.

1. Introduction

Sweet cherry fruit (*Prunus avium* L.) is one of the most appreciated spring-summer fruit in temperate areas of Europe, especially in Mediterranean basin (Landi *et al*., 2014). Its economic importance is due to the nutritional, technological and commercial value of fruits. Fruits are rich in many antioxidants and nutrients (Ballistreri *et al*., 2013), such as phenolics, flavonoids, anthocyanins and carotenoids, and are characterized by sensory qualities highly appreciated by consumers.

In Tuscany region (Central Italy) its cultivation has a long tradition, and the hilly area in the South-South East of Pisa province, especially the area of Lari, is one of the most important and famous districts for the sweet cherry production in Tuscany (Gargani *et al*., 2013).

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Received for publication 30 May 2017
Accepted for publication 19 June 2017

As with other fruit species, the introduction and diffusion of new varieties of sweet cherry in specialized crop systems has marginalized the local ones to the point that some have disappeared, while others are still present as single plants or in mixed orchards.

In those situations, autochthonous and ancient varieties have formed plant populations, resulted from the selective pressure exerted by both natural environment and human cultural practices; importantly, these populations act as a natural reservoir of genetic variability and source of useful genes for the selection of new varieties, for the improvement of the existing ones, with the global objective to guarantee the levels of sustainability and stability of production systems (Di Matteo *et al*., 2016). Concurrently, autochthonous and ancient varieties could represent source of new agro-economic systems, based on the use of crop residuals, or on an alternative use of the products.

Globally, during the last decades, worldwide biodiversity has been lost at an unprecedented rate in all the ecosystems, including agro-ecosystems. Accordingly, a number of instruments and tools that contribute to a sustainable development while addressing objectives and priorities related to biodiversity have been established at different levels, from global to national ones (FAO, 2010); in Italy, for example, since 2015, the law n. 194/2015 has been published with the aim to safeguard and enhance the protection of biological resources relevant to food and agriculture from the risks of extinction and genetic erosion.

In this context, many studies have been performed to explore plant biodiversity, especially through the investigation of plant and fruit metabolomics. Indeed, plants produce a wide range of metabolites, the main part being involved in secondary metabolic pathways; these are the result of different plants responses, through the course of evolution, to specific needs and stimuli. Among such metabolites, volatile organic compounds (VOCs) play a dominant role (Dicke and Loreto, 2010), being released by quite any kind of plant tissues (Peñuela and Llusia, 2001; Dudareva *et al*., 2006) as green leaf volatiles, nitrogen-containing compounds and aromatic compounds. Plants VOCs can be emitted constitutively (Holopainen and Gershenzon, 2010; Holopainen *et al*., 2010), or as a consequence of the interactions of plants with biotic and abiotic factors (Spinelli *et al*., 2011).

Therefore, VOCs can be considered as important metabolites for the characterization of biodiversity. Accordingly, the study of VOCs emitted by fruits, represents a strategic tool for discriminating varieties of the same species growing in the same environment.

To this end, the aim of the present study was to investigate the properties, mainly concerning VOCs, in fruits of a collection of autochthonous and ancient sweet cherry varieties, and to compare them with those of a commercial variety.

2. Materials and Methods

Plant Material

Fruits of four sweet cherry cultivated varieties for fresh consumption (namely: 'Di Giardino', 'Di Nello', 'Marchiana' and 'Ferrovia') used in this study were collected at fully ripe grade from ten years old *Prunus avium* L. trees grown in an experimental farm in Lari (Pisa province, Italy), in late May-early June 2017. The genotypes were planted with a spacing of 6.0×4.0 m, trained as spindle-bushes, and managed according to standard cultural practices. Yield per tree was comparable among cultivars (around 35-40 tons per hectare).

The cherry orchards were located about 130 m above sea level (lat. 43° 33' 08'' N, long. 10° 35' 21'' E). Three of these cultivars ('Di Giardino', 'Di Nello', 'Marchiana') represent ancient native sweet cherry cultivars recovered through a private cultivation program (belonging to the group of the so-called 'Ciliegia di Lari'); instead 'Ferrovia', highly-prized variety marketed in Italy and abroad, has been used as commercial cultivar in order to compare the morpho-chemical and the aromatic characteristics with the native ones.

For each cultivar, 1 kg of homogeneous and healthy fruits were harvested randomly from multiple trees at a commercial ripening stage based on color change and fruit firmness. Samples were transported to the laboratory in isothermal plastic bags within 2 h from harvesting and stored at 4°C until the analysis were performed (at least 24 h after the sampling). Subsequently, the measurements were made in the following order, according to the degree of destruction: fruit skin color, morphological parameters, volatile compounds and chemical parameters. Before the measurements, fruit samples were washed in deionized water.

Morpho-chemical parameters

Fifteen fruits from each sweet cherry cultivar were used to assess the morphological parameters and the skin color. The total weight (fruit and seed) was determined using a digital balance (Sartorius TE1502, USA) while their three linear dimensions (length, width and thickness) and the stalk length were measured using a electronic digital caliper (Stainless Hardened, sensitivity of 0.01 mm). The ripening stage of sweet cherries is characterized by fruit color changing from green to red (Diaz-Mula *et al*., 2009). The color characteristics were analyzed on the fruit surface (skin) using a Minolta CR-200 chromatometer (Minolta, Ramsey, NJ) and *L* (lightness), *a* (green to red) and *b* (blue to yellow) values were measured. Subsequently, *a* and *b* values were used to calculate the color index (*a*/*b*) since this value shows a continuous increase during sweet cherry un grirororrorirorо ripening (Díaz-Mula *et al*., 2009). Furthermore, Hue angle ($\tan^{-1} b/a$) and the Chroma index ($\sqrt{a^2 + b^2}$) were assessed as two main parameters used to describe visual color appearance (Little, 1975; McLellan *et al*., 1995). Results are reported as mean ± deviation standard.

The chemical parameters were determined in triplicate thus each sample was represented by the pulp of three fruits. Few drops of fruit juice were used to determine the total soluble solids (TSS) with a refractometer (N1 Atago Co., Japan) and expressed as °Brix. Subsequently, the fruit pulp was shredded and blended with 150 ml of deionized water. The obtained solution was filtered and used to measure pH with a digital pH meter (Basic20, Crison Instruments) and titratable acidity (TA) by tritation with 0.1 N NaOH up to pH 8.1 and expressed as percentage of malic acid. Furthermore, TSS/TA ratio has been assessed since this value is linked to the fruit flavor and is one of the main indicator of the fruit quality (Alonso and Alique, 2006). Data are reported as mean ± deviation standard.

PTR-ToF-MS profiling

The volatile profile of four different sweet cherry samples and the tentative identification of each detected compound was evaluated by PTR-ToF-MS (model 8000, Ionicon GmbH, Innsbruck, Austria) which guarantees high sensitivity with a very high-time resolution (Taiti *et al*., 2017).

A further description of PTR-ToF-MS is given by Lindinger *et al*. (1998). The analysis method and instrumental settings were carried out following the procedure previously used by Taiti *et al*. (2016). Briefly, each sample consisted of four freshly cut fruits (including the seed). For the analysis, each fruit was cut in 2 parts, inserted in a clear glass jar (3/4 l at 22°C, with a dynamic headspace flushing flow rate of 0.75 l per meter, lpm) equipped with two Teflon inlet and outlet tubes on opposite side, connected respectively to a zero-air generator (Peak Scientific) and the PTR-ToF-MS. Before each analysis, the jar was cleaned for 1 minute with free VOCs air and subsequently was incubated for 80 s. Blank measurements were carried out between samples to monitor background air. The analyses were performed in independent triplicates and an averaged mass spectrum per sample was calculated after background and transmission correction (N=10). The mass spectral data (m/z = 20-210) of four sweet cherry cultivars was assessed after the removal from the dataset of masses m/z = 32 (O_2^+) and m/z = 37 (water cluster ion), other interfering ions and their isotopologues. The instrument was operated at E/N value of 133 Townsend (1 Td = 10^{-17} cm^2 V^{-1} s^{-1}). The chamber ionization conditions were kept as follows: drift temperature 60°C, drift voltage 580 V and drift pressure 3.80 mbar. Each sample measurement was performed with an acquisition rate of 1 spectrum/s for 80 s.

The raw data were acquired by the TOFDAQ Viewer® software (Tofwerk AG, Thun, Switzerland) and the count losses due to the ion detector dead time were corrected off-line following the methodology based on Poisson correction as previously reported by Titzmann *et al*. (2010). Moreover, to reach a good mass accuracy (up to 0.001 Th), the instrumental calibration was based on m/z = 29.997 (NO^+), m/z = 59.049 ($C_3H_7O^+$) and m/z = 137.137 ($C_{10}H_{17}^+$) and was performed off-line. Finally, the VOCs identification was based on models of fragmentation available in the literature (Buhr *et al*., 2002; Lee *et al*., 2006; Aprea *et al*., 2007; Maleknia *et al*., 2007; Kim *et al*., 2009; Jardine *et al*., 2010; Tani, 2013) and compared with published VOCs emitted from sweet cherry fruits (Table 1).

Statistical analysis

Analysis of mean values and deviation standard, multivariate analysis of variance (ANOVA, $p \leq 0.05$), and mean separation by Tuckey's test ($p \leq 0.05$) were performed using the statistical package GraphPad Prism 5.0 software, IL, USA).

3. Results and Discussion

As known, cherry fruit quality (expressed as concentration of nutritive and bioactive compounds) is mainly affected by genotype, environment and orchard management (Predieri *et al*., 2004; Gonçalves *et al*., 2006). In this work all cherry cultivars were obtained from the same farm and all trees were grafted onto the same rootstock, therefore, the differences observed should be attributed almost exclusively to genetic characteristics.

Morpho-chemical parameters

Fruit color and size are the main parameters employed to visually evaluate the sweet cherries (Romano *et al.*, 2006). The morpho-chemical attributes of the four cherry cultivars are shown in Table 2.

Among comparative cultivars the highest average fruit weight was measured in 'Ferrovia' (7.24±0.82 g), while all other autochthonous accession showed lower values; furthermore, 'Di Giardino' (5.46±0.65 g) and 'Di Nello' (4.62±0.62 g) were also statistically different compared to 'Ferrovia' and 'Marchiana'.

As far as concerning other morphological parameters, cv. Ferrovia confirmed to be the biggest (21.54±1.24, 24.33±0.84, 21.07±0.97 mm for length, width and thickness, respectively), while cv. Marchiana was the smallest (15.59±0.99, 18.76±0.82, 15.12±0.73 mm for length, width and thickness respectively). Fruits belonging to 'Di Giardino' and 'Di Nello' showed intermediate shape. Interestingly, the stalk length was highest for 'Di Nello' which belongs to the category of varieties with 'medium stalk length', while the others (less than 39 mm) are culti-

Table 1 - List of VOCs detected in the four sweet cherry samples. Chemical classes and references, where available, are indicated

m/z	Protonated chemical formula	Chemical class (tentative identification)	References
27.022	$C_2H_3^+$	alkyl fragment	
30.046	$C_2H_6^+$	alkene	
31.018	CH_3O^+	aldehyde	
31.054	$C_2H_7^+$	alkyl fragment	
33.033	CH_5O^+	alcohol	
39.022	$C_3H_3^+$	unknown fragment	
41.038	$C_3H_5^+$	alcohol fragment/ester fragment	
43.018	$C_2H_3O^+$	ester fragment	
43.054	$C_3H_7^+$	alcohol fragment	
45.033	$C_2H_5O^+$	aldehyde	1
47.049	$C_2H_7O^+$	alcohol	2
51.038	$CH_7O_2^+$	alcohol	
53.040	$C_4H_5^+$	alkyl fragment	
55.055	$C_4H_7^+$	aldehyde fragment	
57.033	$C_3H_5O^+$	aldehyde	
59.059	$C_3H_7O^+$	aldehyde/ketone	3
61.028	$C_2H_5O^+$	acid	4
65.038	$C_5H_5^+$	alkyl fragment	
67.054	$C_5H_7^+$	alkyl fragment	
69.033	$C_4H_5O^+$	heterocyclic aromatic compound	5
69.069	$C_5H_9^+$	terpene	1
71.049	$C_4H_7O^+$	aldehyde	
71.085	$C_5H_{11}^+$	alcohol	
73.065	$C_4H_9O^+$	aldehyde/ketone	6
75.044	$C_3H_7O_2^+$	ester	
77.040	$C_6H_5^+$	alkyl fragment	
79.049	$C_6H_7^+$	alkene	
81.069	$C_6H_9^+$	terpene fragment	
83.086	$C_6H_{11}^+$	alcohol fragment	
85.065	$C_5H_9O^+$	ald	
87.044	$C_4H_7O_2^+$	ketone	
89.059	$C_4H_9O_2^+$	ester	
91.054	$C_7H_7^+$	unknown fragment	
93.069	$C_7H_9^+$	alkyl	6
95.086	$C_7H_{11}^+$	unknown fragment	
97.064	$C_6H_9O^+$	aldehyde	1
99.080	$C_6H_{11}O^+$	aldehyde	1
101.096	$C_6H_{13}O^+$	aldehyde	2
103.075	$C_5H_{11}O_2^+$	acid	4
107.049	$C_7H_7O^+$	aldehyde	2
109.101	$C_8H_{13}^+$	unknown fragment	
115.075	$C_6H_{11}O^+$	acid	4
117.091	$C_6H_{13}O_2^+$	acid	2, 7
119.101	$C_9H_{11}^+$	alkyl fragment	
121.065	$C_8H_9O^+$	aldehyde	6
127.111	$C_8H_{15}O^+$	ketone	7
137.137	$C_{10}H_{17}^+$	terpene	1

Refereces legend for data available on cherry fruit: (1) Vavoura *et al*., 2015; (2) Serradilla *et al*., 2012; (3) Mattheis *et al*., 1992; (4) Wen *et al*., 2014; (5) Zhang *et al*., 2007; (6) Bernalte *et al*., 1999; (7) Sun *et al*., 2010. Where legend is missing, the VOCs identification was based on fragmentation models available in literature (Buhr *et al*., 2002; Lee *et al*., 2006; Aprea *et al*., 2007; Maleknia *et al*., 2007; Kim *et al*., 2009; Jardine *et al*., 2010; Tani, 2013).

vars with 'short stalk length' (Roselli and Mariotti, 1999; Fajt *et al*., 2005).

Color parameters, especially *a/b*, Hue angle and Chroma indices, were comparable to those reported by Díaz-Mula *et al*. (2009) for ripe cherry fruits. In this work, based on the Hue angle values reported by Crisosto *et al*. (2002), the analyzed varieties showed fruits with color tending to: full light red for 'Marchiana' (25.29±1.22), between full light red and 50% bright red for 'Ferrovia' (22.72±1.49), between 50% bright red and full dark red for 'Di Nello' and 'Di Giardino' (Table 2; see also pictures inserted in figure 1, representing cherry samples for each cultivar under study).

The chemical parameters of sweet cherries as total soluble solids (TSS) and titratable acidity (TA), as well as indicators of the degree of ripening, are also important quality indexes for cherry cultivars evaluation (Crisosto *et al*., 2003).

The results of the chemical parameters are presented in Table 2. Significant differences were found in TSS values especially with regard to cv. Di Nello (12.94±1.08 °Brix) which showed values significantly lower than the other cultivars under study. Moreover, the highest values were detected in 'Ferrovia' (22.77±0.58 °Brix) while 'Marchiana' and 'Di Giardino' showed very similar values to each other (Table 2). Thus, excluding the cv. Di Nello, all cultivars analyzed were above the limit of 14-16°Brix, considered acceptable for marketing cherries as suggested by Crisosto *et al*. (2003).

Some differences ($p \leq 0.05$) were also found in TA among the sweet cherry cultivars (Table 2). The highest average values were found in 'Ferrovia' (0.90±0.05 as percentage of malic acid per fresh weight) followed by 'Di Giardino' and 'Marchiana' (0.58±0.05 and 0.64±0.02, respectively), and finally by 'Di Nello' cultivars that showed the lowest values (0.37±0.05). Accordingly, 'Ferrovia' fruits showed the lowest pH value, and 'Di Nello' the highest one ($p \leq 0.05$).

Moreover, since the TSS/TA ratio has being related to the consumer acceptance, giving that the sugar concentration increases while acidity remains relatively constant during the maturation or ripening process (Spayd *et al*., 1986), such index can be used as well as a quality parameter in sweet cherry (Alonso and Alique, 2006). Indeed, as it has been observed elsewhere for sweet cherry fruits (Crisosto *et al*., 2003; Garcia-Montiel *et al*., 2010) the increase in TSS/TA ratio during ripening process is due to the higher increase in TSS than the increase in TA.

Table 2 - Morpho-chemical proprieties of sweet cherry samples. Data represent mean ± deviation standard

	'Di Giardino'	'Di Nello'	'Marchiana'	'Ferrovia'
Morphological proprieties				
Fruit weight (g)	5.46±0.65 b	4.62±0.62 c	6.68±0.64 a	7.24±0.82 a
Fruit length (mm)	22.47±0.94 a	20.33±0.92 b	15.59±0.99 c	21.54±1.24 a
Fruit width (mm)	19.24±0.75 bc	19.83±0.92 b	18.76±0.82 c	24.33±0.84 a
Fruit thickness (mm)	20.16±1.01 a	18.60±1.21 b	15.12±0.73 c	21.07±0.97 a
Stalk length (mm)	30.34±3.05 b	41.26±3.92 a	30.79±4.04 b	32.12±3.82 b
Skin color				
L	31.26±1.64 b	30.68±1.36 b	33.67±1.53 a	31.09±1.34 b
a	13.66±2.85 b	13.36±2.71 b	19.72±2.06 a	13.50±2.99 b
b	4.35±2.06 b	4.70±1.79 b	9.36±1.41 a	5.73±1.59 b
a/b	2.94±0.51 a	2.69±0.45 ab	2.12±0.11 c	2.40±0.17 bc
Hue angle (deg)	18.74±2.72 c	20.10±4.22 bc	25.29±1.22 a	22.72 ±1.49 ab
Chroma	13.13±3.95 b	14.23±2.95 b	21.83±2.45 a	14.67±3.37 b
Chemical proprieties				
pH	3.68±0.10 b	4.03±0.10 a	3.47±0.01c	3.52±0.03 c
TSS (° brix)	16.34±2.57 b	12.94±1.08 c	16.10±0.65 bc	22.77±0.58 a
TA	0.58±0.05 b	0.37±0.05 c	0.64±0.02 b	0.90±0.05 a
TSS/TA ratio	29.39±1.99 ab	32.80±3.47 a	25.22±1.81 b	25.26±1.39 b

The letters after the values indicate the significant differences within the same row according to Tuckey's test (p≤0.05).

Specifically, this ratio was highest in 'Di Nello' (32.80±3.47) and lowest in 'Marchiana' and 'Ferrovia' (25.22±1.81 and 25.26±1.39, p≤0.05) (Table 2). This data suggest that also cv. Di Nello, that had reported the lowest TSS (12.94±1.08 °Brix), given the low acidity (0.37±0.05) could be considered as qualitatively comparable to the other cultivars, from the point of view of the fruit maturity.

PTR-ToF-MS profiling

Beside fruit sweetness and skin color, aroma is perhaps the most appreciated fruit characteristics (Romano *et al.*, 2006). The typical aroma composition of each fruit is affected by their chemical composition (including phytonutrients) as: fatty acids, amino acids, carotenoids, phenols and terpenoids (Sun *et al.*, 2010). Thus, the fruits aroma is often for-

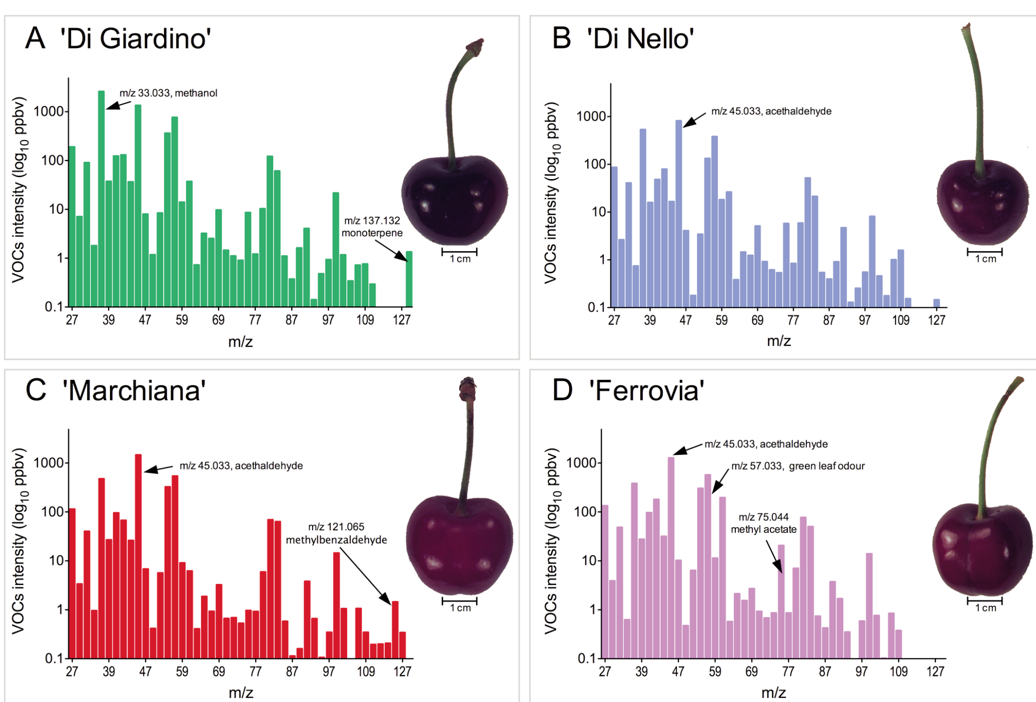

Fig. 1 - VOCs profile of the four sweet cherry cultivars under study. Representative pictures of each cultivar are also shown.

mulated by a complex mixture of VOCs, whose composition is species-specific and sometimes is variety-specific (El Hadi *et al*., 2013). It follows that, it is important to identify at first the entire volatile imprint for all cherry types and subsequently investigate which compounds contribute to create the unique aroma/flavor of each sweet cherry, as it represents a fundamental quality parameter used by the consumer.

By the PTR-ToF-MS analysis 47 peak signals were identified (Table 1); among them, a minimum of 40 peaks for ‘Ferrovia’ to a maximum of 45 peaks for Marchiana cultivar were detected (Fig. 1). As a general overview, cultivars under study showed highest intensities for different VOCs (Fig. 1). In particular, among all samples, the biggest peaks detected, corresponding to VOCs with highest intensity, were the following m/z: 27.022, 31.018, 33.033, 41.038, 43.018, 45.033, 57.033, 61.028, 75.044, 81.069, 83.086 (each present in percentages >1 on the total). In respect to each variety, cv. Di Giardino showed the main peak at m/z = 33.033 (2592 ppbv, on average over replicates), tentatively identified (TI) as methanol, linked to mild alcoholic odour; cv. Marchiana and Di Nello were rich in m/z = 45.033, TI as acetaldehyde (respectively 1459 ppbv and 816 ppbv, on average over replicates), linked to pungent/fruity odour (this volatile compound was well represented in all samples); cv. Ferrovia had the biggest signal at m/z = 33.033, followed by m/z = 57.033 (568 ppbv, on average over replicates), TI as fragment of hexanal and/or hexyl acetate, linked to green leaf odour (Fig. 1).

In addition, ‘Di Giardino’ showed high signal intensities for terpene compounds compared to the other varieties used in study (Fig. 1A). Being monoterpenes (C10) and sesquiterpenes (C15) the compounds that most affect the aromatic profile in some fruits, their presence in ‘Di Giardino’ determines probably a characteristic aroma. The different terpenes composition in sweet cherry varieties has been also observed by Vavoura *et al*. (2015) that showed, in five varieties, a different composition in types and amount of terpenes compounds. Interestingly, peak detected at m/z = 121, TI as methylbenzaldehyde, referred as cherry-like scent (Bernalte *et al*., 1999), was observed only in ‘Marchiana’ (Fig. 1C). Instead, ‘Ferrovia’ showed an interesting peak at m/z = 75.044, TI as methylacetate linked to ether sweet fruity odour (Fig. 1D). Concerning the cultivar Di Nello, it is worth noting that intensities of VOCs related to green leaf odour (m/z = 57, 81, 99 and 101) were inferior in respect to the other sample varieties (Fig. 1B).

As reported by Sun *et al*. (2010), the aroma compounds in sweet cherry is determined by a great number of organic components, especially aldehydes, alcohols, esters, acids and terpenes. Hence, each peak detected and tentatively identified was then clustered according to the chemical class (Table 1). This allowed to drawing some further interesting consideration and to better appreciate the differences between aroma profiles of sweet cherry fruits belonging to different cultivars (Fig. 2).

Aldehydes are the most abundant class of VOCs (in percentage) in all cherry samples (73%, 66%, and 63% respectively in ‘Marchiana’, ‘Ferrovia’ and ‘Di Nello’, Fig. 2B,C,D), excluding ‘Di Giardino’ where alcohols were the biggest class (47%, Fig. 2A). Among aldehydes, the peaks detected at m/z = 45, 57, 59, 99, 101 are linked to compounds known to be among the most important aroma compounds of sweet cherry fruit (Mattheis *et al*., 1992; Wen *et al*., 2014; Vavoura *et al*., 2015). In contrast to the results of Vavoura *et al*. (2015) but according to Serradilla *et al*. (2012), 2-propanone (m/z = 59) was not the most abundant compound identified, although it has been detected in all cherry cultivars under study. On the contrary, the compounds known as ‘green leaf volatiles’ such as 2-hexenal (m/z = 99.080), hexanal (m/z = 101.096) and their main fragment (m/z = 57.033) were found. All of them, even in very small quantities as hexanal, give a strong odour due to their low perception threshold (Matsui, 2006).

Besides aldehydes, alcohols revealed to be abundant in all cherry samples (Fig. 2), and in fact they represent important compounds for the aroma in sweet cherry (Sun *et al*., 2010). In this study, methanol (m/z = 33), ethanol (m/z = 47) and propanol (m/z = 61) were highlighted. Such alcohol compounds are precursors of natural aroma and appear as a result of anaerobic respiration in fruits and are linked to the normal maturation process (Taiti *et al*., 2015). In addition, both autochthonous and commercial cherries analyzed were rich in ethanol, which most probably derives from carbohydrates metabolism (glycolysis) (Mattheis *et al*., 1992). These data also suggest that the intensity of ethanol emission is at least partially associated to TSS content; in fact, the lowest intensity of ethanol was found in ‘Di Nello’ (4.05±1.40 ppbv), the cultivar with lowest TSS (Table 2), while the highest was found in ‘Ferrovia’ (10.2±1.60 ppbv), the cultivar richest in TSS (Table 2); similarly, the other two varieties, ‘Di

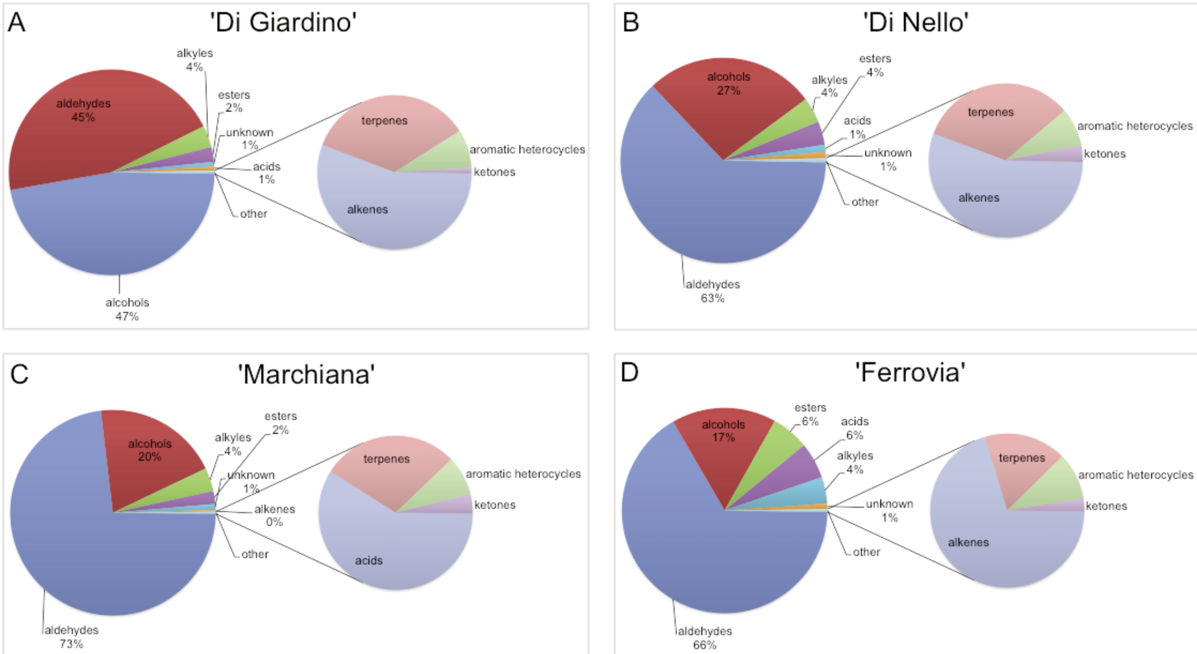

Fig. 2 - Pie charts of the VOCs emitted by the four sweet cherry cultivars under study, expressed as chemical classes according to Table 1.

Giardino' and 'Marchiana', had similar ethanol content, at intermediate intensity in respect to 'Di Nello' and 'Ferrovia', according to TSS data (Table 2).

As for the determination of acids, a total of four acids were detected, acetic (m/z = 59), 3-methylbutanoic (m/z = 103), hexenoic (m/z = 115) and (E)-2-hexanoic acid (m/z = 117), confirming results reported by Sun *et al*. (2010). In our cherry samples, 'Ferrovia' showed the highest percentage (6%) of this class of compounds (Fig. 2D).

Finally, esters were found in all cherry cultivars (Fig. 2) but in low concentrations (especially for 'Marchiana') (Fig. 2C). For example ethyl acetate (m/z 89.059), that is identified as impact volatile in sweet cherry fruits (Zhang *et al*., 2007) and is associated to pineapple aroma, showed the highest emission level in 'Ferrovia' (3.73±0.83 ppbv), followed by 'Di Giardino' (1.67±0.77 ppbv), 'Di Nello' (0.91±0.15 ppbv) and finally by 'Marchiana' (0.16±0.08) with values below 1 ppbv.

4. Conclusions

In this study, fruit properties of a collection of autochthonous and ancient sweet cherry varieties (*Prunus avium* L.) were investigated and compared to the properties of a commercial variety.

Morpho-chemical analyses showed statistical differences among the cultivars, the main ones found on fruit total soluble solids (TSS) and acidity. Concerning TSS, two of the authoctonous cultivars investigated, as well as the commercial one 'Ferrovia', resulted acceptable for the market (TSS above the limit of 14-16 °Brix, Crisosto *et al*., 2003). Fruits from the cultivar Di Nello, on the other hand, despite they turned out to be not so rich in sugars, showed the biggest TSS/TA ratio, another important index related to fruit quality and acceptance (Alonso and Alique, 2006).

Concerning the analysis of aromatic compounds, the use of PTR-ToF-MS (the first in our knowledge) allowed to distinguish each cultivar's peculiarities. Among autochthonous cultivars, 'Di Nello' was the weakest in emitting green leaf related odours; 'Di Giardino' showed to be rich in the chemical classes of terpenes, which most affect the aromatic profile in some fruits; methylbenzaldehyde (cherry scent) was detected only in 'Marchiana'. Such results would suggest potentiality interest for all these autochthonous varieties, not only for commercial purposes, but also for breeding ones.

In conclusion, this study on sweet cherry fruits provides preliminary information that can be useful tools for the enhancement and utilization of native cultivars.

Acknowledgements

This study was supported by funds of the Regione Toscana ''PRAF 2012–2015 MISURA 1.2 e)'' program (call ‘Agrifood’, project VOLATOSCA). We gratefully acknowledge the ‘Azienda Agricola Mastrociliegia’ (Lari, Pisa, Italy) in the person of Dr. Leonardo Puccioni for his technical support and as provider of cherry samples.

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