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Volatiles Profile of Red Apple from Marche Region (Italy)

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Abstract: The volatile composition of different parts (whole fruit, peel and flesh) of a rare wild red apple named "Mela Rossa Val Metauro" was established by SPME (Solid Phase Micro-Extraction) sampling, followed by GC-MS analysis. The most prominent constituent in all the three parts was the sesquiterpene *(E,E)*-α-farnesene. The presence of the ester ethyl hexanoate particularly in the flesh (39.8%) gives a special exotic flavor to the fruit.

Keywords: Apple; *Malus x domestica* Borkh; volatiles profile; *(E,E)*-α-farnesene; ethyl hexanoate.

1. Introduction

The genus *Malus* is native to the temperate zones of the northern hemisphere, Europe, Asia, and North America and it comprises about 30-35 species of small deciduous trees or shrubs in the Rosaceae family [1].

The domesticated table apple, *Malus x domestica* Borkh. is considered to be a complex interspecific hybrid. The main ancestor is thought to be *Malus sieversii* M. Roem [2,3] along with other ancestors, those being *Malus sylvestris* (L.) Mill., *Malus pumila* Mill. and *Malus dasyphylla* Borkh [4]. The ancestors are generally known as "wild apples", name derived from their typically small and tart fruits [5,6]. Among the ancestors, *Malus pumila* Mill. produces fruits that show red coloration in both the skin and flesh. It is tart, relatively non juicy and it oxidizes easily [7].

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Aroma volatile compounds are of the utmost importance on the establishment of fruit quality criteria and especially on determining consumer acceptance. Apple aroma profiles are complex since they are constituted by a large number of volatile compounds that contribute to the overall sensory quality. These compounds include carboxylic esters, alcohols, aldehydes, ketones, free acids and ethers, but just about 20 of these chemicals are really crucial to characterize the apples aroma such as ethylacetate, ethyl butyrate and methyl anthranilate [8]. Volatile esters such as 2-methylbutyl acetate, hexyl acetate, butyl acetate and others have been found in different apple cultivars. Among them, 2-methyl butyl acetate and butyl acetate are considered as the most important volatile esters contributing to the characteristic apple aroma [9].

Some chemicals are emitted at very low concentration, such as ethyl 2-methyl butyrate, but they are extremely important in the overall definition of the apple flavour. Other compounds, such as *(E)*-2- hexenal, contribute to the aroma intensity, while the presence of alcohols regulates the quality of the flavouring [10].

The final aroma profile of a fruit is the result of a balance between all the emitted volatile compounds, and any modification in this fine balance would result in changes of the fruit flavour [11].

Among the most frequently consumed apples, Fuji is considered one of the most aromatic ones, while the green apple cultivar Granny Smith is considered to be one of the least aromatic [12, 13-15]. In this work, for the first time, we report the composition of the volatile boquet of a rare wild italian apple named “Mela Rossa Val Metauro” [16] a volunteer *Malus domestica*, similar to the fruit of *Malus pumila* Mill.,with red skin and flesh but covered in bloom, juicy, and fragrant. The fruits are edible, having a pleasant taste, being not tart, and medium sized comparable to the known table apples. Analysis of the volatile compounds was conducted by SPME (Solid Phase Micro-Extraction), a solventless sampling technique that has been applied to the analysis of volatile and non volatile compounds present in several complex matrixes and also for the analysis of volatiles in a large variety of fruits [17-21].

2. Materials and Methods

2.1. Plant Material

The apple samples used in this study were harvested at maturity from few scattered plants during august 2010 found and classified by Prof. Giovanna Giomaro University of Urbino “Carlo Bo” in Metauro valley (PU), Marche region, Italy. (Patent registered). The fruits were stored at room temperature until analysis.

2.2 Gas Chromatography-Mass Spectrometry

Supelco SPME devices coated with polydimethylsiloxane (PDMS, 100 µm) were used to sample the headspace of two whole fruits inserted into a 300 ml glass container sealed with aluminium foil and allowed to equilibrate for 60 min. The peel and the flesh of the fruits were then treated in the same way.. After the equilibration time, the fiber was exposed to the headspace for 25 min at room temperature. Once sampling was finished, the fiber was withdrawn into the needle and transferred to the injection port of the GC–MS system. GC– EIMS analyses were performed with a Varian CP 3800 gas-chromatograph equipped with a DB-5 capillary column (30 m x 0.25 mm x 0.25 µm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions were as follows: injector and transfer line temperatures were 250 and 240 °C, respectively; oven temperature was programmed from 60 to 240°C at 3°C/min; carrier gas was helium at 1 mL/min; splitless injection. The identification of the constituents was based on the comparison of their retention times with those of authentic samples, comparing their linear retention indices (LRI) relative to a series of *n*-hydrocarbons, and on computer matching against commercial (NIST 98 and Adams) and homemade library mass spectra, and MS literature data [22-25]. Moreover, the molecular weights of all the identified substances were confirmed by GC–CIMS, using methanol as ionizing gas, using the following parameters: CI storage level 19 *m/z*, ejection amplitude: 15 V, background mass 55 *m/z*, max ionization time 2000 µsec, max reaction time 40 msec. All the analyses were performed in triplicate.

3. Results and Discussion

Comparative analysis of the volatiles of the whole apple, peel and flesh of mature apples "Mela rossa Val Metauro" is reported in Table 1. Altogether, 30 compounds were identified, accounting from 98.1% to 98.6% of the whole volatiles. The main volatiles were terpenes in the case of whole fruits and peel (90.5% and 79.5% , respectively) and aliphatic esters for the flesh (57.8%). However, the most prominent constituent in all the three samples was the sesquiterpene *(E,E)*-α-farnesene: 90.0% in the whole fruit, 78.0% in the peel and 30.5% in the flesh. Ferreira et al. (2009) [8], found α-farnesene to be the most abundant substance in three varieties of apples. Paliyath et al. (1997) [26] detected the same compound as the major volatile in other apples varieties, with the highest levels produced by Red Delicious apples, followed by McInthosh, Gala and Empire varieties. It also appears that an increased biosynthesis of α-farnesene and its subsequent oxidation to conjugated trienes, causes a disease in apple and pear named "scald", characterized by damages on the surface of the fruit [26]. Its olfactory properties remind the characteristic smell of green apple and, together with *(E)*-β-farnesene, detected in smaller amounts (0.2%, 0.1% and traces, in whole apple, peel and flesh, respectively) represents an attraction cue for some parasites of apples, such as *Cydia pomonella* L. and *Rhagoletis pomonella* Walsh [27,28].

About other volatiles, Rapparini et al. (2001) [29] reported that the monoterpenes δ^3-carene and α-phellandrene were characteristic of apple and cherry, but these compounds were not detected in our samples. Several authors report that the developmental stages and the phenological status of the plant can play a significant role on volatile production in fruits [30,31].

Besides farnesene, other terpenes were present in very small amounts in all the samples.

Only small amounts of typical esters of apple fruits such as ethyl hexanoate (3.5%), hexyl hexanoate (1.7%) and butyl hexanoate (1.4%) were detected in the whole fruit, while their concentration significantly increased in the peel and flesh, in particular ethyl hexanoate (39.8% and 12.0% in flesh and peel, respectively) contribute to the typical aroma (pineapple) of this apple fruit. Butyl hexanoate was also found by Zhang et al. (1999) [28], together with butyl butanoate and pentyl hexanoate in five varieties of apples, while hexyl hexanoate plus butyl hexanoate were found as the two main volatiles characterizing Royal Gala apples, a known red-stripper cultivar [32]. In Renetta Canada apples, twelve of the fourteen identified volatiles were esters, among which six were butanoic acid ones [33]. In our red apple, the ester butyl butanoate (1.4% in the flesh) could strengthen the aroma of pineapple due to the presence of ethyl hexanoate (39.8%) in the flesh. Worthy of note was the presence in the flesh of alcohols and other C-6 derivatives namely 1-hexanol and *(E)*-2-hexenal: (4.3% and 1.6%, respectively), responsible for the singular herbaceous notes: in particular *(E)*-2-hexenal was detected only in the flesh, while 1-hexanol was also present in the peel (0.8%).

Table1. Chemical composition of the volatile compounds of red apple "Mela rossa Val Metauro"

Constituents	**L.R.I**	**Whole Fruit %**	**Peel%**	**Flesh%**	**Odor notes**
ethyl 2- methylbutanoate	853	0.1	0.1	-	apple (m)(*)
(E)-2-hexenal	855	-	-	1.6	green, leafy (s)
1-hexanol	870	0.2	0.8	4.3	green, floral (m)
n-nonane	900	t	0.2	1.0	petrol (m)
methyl hexanoate	919	t	0.1	0.4	fruity, sweet (m)
6-methyl-5-hepten-2-one	984	t	-	-	citrus, green, apple (m)
butyl butanoate	992	0.2	0.3	1.4	banana, pineapple (m)
ethyl hexanoate	997	3.5	12.0	39.8	fruity, pineapple (s)
Limonene	1032	-	-	t	citrus, lemon (m)
1-octanol	1071	-	-	t	burnt, mushro-oms (m)
pentyl butanoate	1092	t	0.2	0.6	banana, tropical (m)
ethyl heptanoate	1099	t	0.1	1.2	fruity, pineapple (m)
nonanal	1102	-	-	t	citrus, green (s)
methyl octanoate	1126	t	t	-	orange (m)
hexyl isobutanoate	1151	t	t	-	fruity, apple, pear (m)
3-decanol	1184	0.5	0.4	2.6	floral (m)
butyl hexanoate	1190	1.4	2.6	11.2	fruit, apple, wine (m)
ethyl octanoate	1196	0.4	0.8	0.5	fruit, fat (m)
decanal	1205	-	-	t	citrus, fat (s)
hexyl 3-methylbutanoate	1242	-	0.2	0.4	green fruits, apple peel (m)
pentyl hexanoate	1286	0.1	0.1	0.3	fruity (m)
hexyl hexanoate	1386	1.7	1.2	2.0	apple skin, cut grass (s)
(E)-geranylacetone	1455	-	-	t	magnolia, green (m)
(E)-β-farnesene	1458	0.2	0.1	t	wood, citrus, sweet (m)
β-selinene	1487	-	tr	-	grass (m)
(Z,E)-α-farnesene	1491	-	0.1	-	green (m)
bicyclogermacrene	1495	-	1.0	0.3	green, wood (m)
(E,E)-α-farnesene	1506	90.0	78.0	30.5	green apple (m)
germacrene B	1556	0.1	0.1	t	wood, earth, spicy (m)
dendrolasin	1565	0.2	0.2	t	lemongrass (m)
Aliphatic esters		**7.4**	**17.7**	**57.8**	
Terpenes		**90.5**	**79.5**	**30.8**	
Others		**0.7**	**1.4**	**9.5**	
Total identified		**98.6**	**98.6**	**98.1**	

t = trace (< 0.1) (*) w = weak m = medium s = strong L.R.I. Linear Retention Indices

On the contrary, compounds such as the esters ethyl butanoate, hexyl acetate, 2-methyl butyl acetate, butyl acetate, ethyl 2-methylbutanoate [12, 34, 35], together with *(E)*-β-damascenone, a strong flavour compound in many foods [36], and aldehydes such as *(Z)*-3-hexenal [32], that play a very important role in the definition of the quality of known commercial apples, were not detected in our variety "Mela rossa Val Metauro" except for small amounts of ethyl 2-methylbutanoate, 0.1 % both in whole apple and peel. However it is known that the relative contribution of these volatiles to aroma of apple is also dependent on the apple cultivar, its maturity, and the methods used for volatile extraction [32]. In conclusion the very high amounts of *(E,E)*-α-farnesene in all the fruit parts of our red apple, together with relatively high amounts of ethyl hexanoate, especially in the flesh, could make this variety interesting as a table apple, characterized by a particular exotic flavour. Furthermore, it could be used to develop new cultivars.

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