Characterization of nutrients, polyphenols and volatile components of the ancient apple cultivar 'Mela Rosa dei Monti Sibillini' from Marche region, central Italy

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Abstract

In the present work we reported for the first time a comprehensive study on the phytonutrients, i.e. natural compounds able to provide benefits to health, found in an old apple variety cultivated in orchards of the Sibillini Mountains, central Italy, known as Mela Rosa dei Monti Sibillini. This fruit has recently been promoted by authorities and local institutions as a typical food of the Marche Region. For the purpose, analysis of its nutrients, phenolics, triterpenes and volatile components as well as a morpho-anatomy study, was carried out in order to give an added value for its consumption and promotion at regional and national level. ICP-MS, HPLC-MS^n and GC-MS analyses were useful techniques for giving a typical fingerprint to this apple, given by a high content of K and B, quercetin derivatives as the main phenolic compounds and carboxylic esters, aldehydes, alcohols and (*E*,*E*)-α-farnesene as the main key odorants.

Keywords: Mela Rosa dei Monti Sibillini; phytonutrients; phenolic compounds; volatile components; HPLC-DAD-MS^n; SPME-GC-MS; histochemistry.

1. Introduction

The 'Mela Rosa dei Monti Sibillini' is an ancient apple variety resulting from *Malus communis* Borkh. that has been cultivated in the Sibillini mountains, Marche, central Italy, since the Roman age (Fig. 1). The hallmarks of this old variety are the small size, the flat shape, the green peel with pink-reddish shades, an intense aroma and a sour and sweet taste. Notably, the pre-Apennine zone of Sibillini Mountains, mainly on the hills between 400 and 900 m of altitude, has been recognised as the traditional area for cultivation of the 'Mela Rosa' in the Marche region, central Italy (Fig. 1) (D'Abrosca, Pacifico, Cefarelli, Mastellone & Fiorentino, 2007).

For a long period, the cultivation of this fruit has been overlooked and almost abandoned, but regional authorities and local institutions, in the last decade, tried to recover and preserve the germplasm of this old apple variety. Currently, this apple is cultivated in a dozen of ha sited in the territory of Monti Sibillini National Park, Marche, central Italy (Fig. 1). Numerous initiatives (e.g. recognition as Slow Food) are in progress in this area to improve the cultivated surface of this ancient apple cultivar and increase the local economy, especially after the 2016 central Italy earthquakes.

This renewal of attention on the 'Mela Rosa' is due to its distinctive characteristics such as particular shape, taste, aroma, and long storability though its physical aspect (e.g., small size) makes it less attractive on a commercial perspective (Fig. 1).

Despite the old age and longstanding use of this apple in central Italy, there are no data in the literature about its chemical and nutritional profile. Many studies have highlighted that apple is one of the richest sources of antioxidants (Escarpa & Gonzalez, 1998; Vrhovsek, Rigo, Tonon & Mattivi, 2004; Todea et al., 2014; D'Abrosca et al., 2007). Among them, flavan-3-ols, flavonols, anthocyanins, procyanidins, hydroxycinnamic acids and dihydrochalcones are

63 recognized as the most important secondary metabolites of apples (Escarpa et al., 1998; Tsao,
64 Yang, Young & Zhu, 2003; Vrhovsek et al., 2004). The aroma of apple, which frequently
65 influences the consumer's preference, is due to aliphatic compounds, mainly esters,
66 terpenoids, aldehydes and alcohols (Lee, Jang, Jeong, Yoo & Ha, 2017; Giannetti, Mariani,
67 Mannino & Marini, 2017; Reis, Rocha, Barros, Delgadillo & Coimbra, 2009; Ferreira,
68 Perestrelo, Caldeira & Câmara, 2009).
69 The popular saying 'an apple a day keeps the doctor way' is due to the health promoting
70 effects displayed by its phytonutrients. In fact, their intake has been associated to the
71 prevention of cardiovascular diseases, lung dysfunctions and some types of cancer (Tsao et al,
72 2003). Notably, apple procyanidins showed antitumor, anti-inflammatory and antioxidant
73 effects (Jia, Ren, Nie & Yang, 2017; Hyson, 2011). Quercetin, one of the most abundant apple
74 flavonols, has shown effectiveness in the treatment of obesity and hypertension (Larson,
75 Symons & Jalili, 2012; Porras et al, 2017). Phloretin, has reported as effective in the therapy
76 and prevention of diabetes and cancer (Singh, Barden, Mori & Beilin, 2001; Yang et al.,
77 2009). Phloridzin, a glycosylated derivative of phloretin, is exploited in cosmetics due to its
78 inhibitory properties on the melanogenesis and skin protective effects against UV radiation
79 (Gaudout, Megard, Inisan, Esteve & Lejard, 2006).
80 Chlorogenic acid, the main apple hydroxycinnamic acid, is endowed with ameliorating effects
81 against cancer, oxidative stress, cardiovascular diseases, diabetes and obesity (Cho et al.,
82 2010).
83 Besides secondary metabolites, apple is also an important source of minerals with high
84 relative nutritional value such as K (Todea et al., 2014). This mineral significantly reduces
85 pain in women with rheumatoid arthritis (Kianifard and Chopra, 2018). Some studies also
86 showed a reverse association between the potassium intake and metabolic syndrome in adults
87 (Shin, Joh, Kim & Park, 2013).

In order to valorise the healthy properties of the Mela Rosa dei Monti Sibillini, improve its local cultivation and promote its reputation in the regional and national territory, in the present work we furnished a comprehensive phytochemical analysis of this fruit by studying its phytonutrients, including macro- and micro-nutrients, aroma components, phenolic compounds and triterpenes. For the purpose, the different classes of phytonutrients of this fruit were analysed by HPLC-DAD-MS^n, GC-MS and ICP-MS. Results of this work provide the fingerprint of this variety useful to consumer for distinguishing it from the commercial ones and to identify possible health benefits derived from its consumption. Moreover, we studied the morphoanatomy and histochemistry of the fruit by means of light microscopy, since literature does not provide data about this issue. This additional information may contribute to retaining and increasing the biological values of this apple variety.

2. Materials and methods

2.1. Sampling

The fruits were picked at ripening in different organic apple orchards sited in Montedinove (GPS coordinates: N 42°57′44”; E 13°36′15”), Monte San Martino (N 43°01’58”; E 13°26’22”), Montefalcone Appennino (N 42°59’17”; E 13°27’32”), and Montottone (N 43°03’43”; E 13°35’04”), Marche region, central Italy, in October-November 2017. Samples were kindly furnished by 10 farmers and differed from each other for graft and altitude (Table 1). Intact, defect-free apples were collected from the central part of the crown of randomly chosen trees. Storage was practiced at ambient temperature (~ 15-20°C) according to the traditional use. Fruits devoted to nutritional and volatile analyses were used as fresh, whereas the pulp and peel of the remaining ones were separately dried using a Biosec De Luxe B12 dryer (Albrigi luigi, Verona, Italy) at 40°C for 18 h, then grinded into 1 mm-size particles using an IKA-WERK MFC DCFH 48 (Staufen, Germany). Once powdered, samples were

stored in 50 mL Falcon tubes at room temperature and protected from light until further analyses. While collecting the fruits and preparing the samples for the morphological investigation, special attention was addressed to avoid touching the surface area to use it for analysis and to limit degrading the external cuticle. Data including fresh weight (g), water content (%), height (cm) and width (cm) were randomly gathered by measuring 15 different apples for each sample. The average values are reported in Table 1.

2.2. Nutritional analysis and mineral content

Five different whole apple samples (1-5) were analyzed for chemical composition (moisture, total carbohydrates, fat, protein, dietary fiber, and ash) using the AOAC procedures (1995) and data are reported in Table 2. The moisture content was calculated by oven drying the sample to a constant weight (24 h, 133°C). The protein content was estimated by the Kjeldahl method; the crude fat was determined by extracting a known weight of powdered sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at 600 ± 15 °C. Dietary fiber content was determined by a gravimetric method after acidic hydrolysis of the samples. Total carbohydrates were calculated by difference. Total energy was calculated according to the following equations (Manzi, Marconi, Aguzzi, & Pizzoferrato, 2004): Energy (kcal) = 4 X (g protein + g carbohydrate) + 9 X (g lipid). Glucose, fructose, sucrose and sorbitol were analyzed by HPLC using a refractive index (RI) detector (Kubola, Siriamornpun, & Meeso, 2011).

Different edible parts of apple, i.e. whole apple, pulp and peel (Table 1SM), were analyzed for mineral content in five different apple samples. The determination was performed by mineralization of the weighed dried samples with nitric acid (Suprapur, Merck) according to the methodology described elsewhere (Nasuti, Ferraro, Giovannetti, Piangerelli, & Gabbianelli, 2016) that uses inductively-coupled plasma mass spectrometry (ICP-MS, Agilent Technologies, Santa Clara, CA, USA)

138 *2.3. Analysis of polyphenols and triterpenes in apple peel and pulp*

139 Methanol, acetonitrile, formic acid, rutin, phloridzin, catechin and chlorogenic acid were
140 obtained from Sigma-Aldrich (St. Louis, MO, USA). Annurcoic acid was purified as reported
141 in Sut, Poloniato, Malagoli & Dall'Acqua (2018). Five hundred mg of dried pulp and peel
142 samples (1-10) were transferred in a flask with 15 mL of methanol:water (50%) solution and
143 sonicated at room temperature for 15 min. Liquid was decanted and filtered and further 5 mL
144 of solvent was added to solid material. Further 15 min of sonication were applied. Liquid was
145 collected and volume adjusted to 25 mL with the same solvent. Samples were then filtered
146 through 0.45 μm membrane filters and used for analysis.

147 *2.3.1. HPLC-DAD-(ESI)-MS*

148 Quali-quantitative analysis of phenolic derivatives was carried out by HPLC-DAD-MS^n. The
149 measurements were performed with an Agilent 1260 chromatograph (Santa Clara, CA, USA)
150 equipped with 1260 diode array (DAD) and Varian MS-500 ion trap mass spectrometer.
151 Separation was achieved using an Agilent Eclipse XDB C-18 (3.0 × 150 mm) 3.5 μm as
152 stationary phase. The mobile phases were acetonitrile (A) and water 0.1% formic acid (B),
153 flow rate was 500 μL/min. The elution gradient started at (95:5) A:B then (85:15) A:B at 15
154 min, (15:85) A:B at 35 min, (0:100) A:B at 48 min and then 5 min for re-equilibration time.
155 At the end of the column a T connector splitted the flow rate to DAD and MS. The DAD
156 detector was used to quantify phenolic compounds using rutin, chlorogenic acid, phloridzin
157 and catechin as reference compounds. The chromatograms were monitored at 280, 330 and
158 350 nm and UV-Vis spectra were acquired in the range of 200-650 nm in order to assign
159 different peaks to each class of constituents (Fig. 1 Supplementary Material). The sample
160 injection volume was 10 μL. MS spectra were recorded in negative ion mode in 50–2000 Da
161 range, using ESI ion source. Fragmentation of the main ionic species was obtained by the
162 turbo data depending scanning (TDDS) function. Identification of compounds was obtained

matching the fragmentation spectra as well as by comparison with the literature and injection of available reference compounds. Quantification of phenolic constituents was obtained using the method of calibration curve; rutin, chlorogenic acid, catechin and phloridzin were used as external standards in the concentration range of 5-100 µg/mL for quantification of flavonoid, caffeoylquinic acid derivatives, procyanidins and chalcone derivatives, respectively. Four-point calibration curves were as follows: rutin y = 27,788x + 330,7 (r^2 = 0,9981); chlorogenic acid y = 47,359x + 439,99 (r^2 = 0,9951); catechin y = 20,525x + 3,2962 (r^2 = 0.999) ; phloridzin y = 87,029x - 1,832 (r^2 = 0.999).

2.3.2. HPLC-(APCI)-MS analysis

For triterpene analysis an Agilent Eclipse XDB C-18 (3.0 × 150 mm) 3.5 µm was used as stationary phase. Methanol (A) and H_2O 0.05 % formic acid(B) were the mobile phases. The analysis revealed the presence of annurcoic acid (Sut et al., 2018) that was quantified using the four-point calibration curve of the purified compound in the concentration range of 5-50 µg/mL considering the ion species at *m/z* 485. The calibration curve was as follow, y = 12549x + 136925; r^2 = 0.9962.

2.4. Headspace Solid-Phase Microextraction (HS-SPME) sampling

Pulp and peel samples from ten farmers (samples 1-10 of Table 1) were separately homogenized using liquid nitrogen in a mortar until a powder was obtained. Then 80 and 400 mg of grounded peel and pulp, respectively, were inserted in a 4 ml headspace glass vial (Supelco, Bellefonte, PA, USA) covered with a polypropylene cap and sealed with PTFE-silicon septum (Supelco). The vial was then dipped into a thermostatic water bath (RCT basic IKAMAG ® safety control, IKA®-Werke GmbH & Co.KG, Staufen, Germany) with magnetic stirring bar at 40˚C. After 10 min of equilibration, a 100 µm polydimethylsiloxane (PDMS) (Supelco) fibre, 1 cm long, was inserted into the vial by manually penetrating the septum. The selection of this fibre was made by comparison with divinylbenzene-carboxen™-

polydimethylsiloxane (DVB-CAR-PDMS, 50/30 μm, Supelco) fibre. Notably, the use of PDMS fibre resulted in higher and larger peaks of the marker compounds of apple aroma (Fig. 2 Supplementary Material). The good response of PDMS fibre towards apple marker volatiles was consistent with results of Matich, Rowan & Banks (1996) and Song, Gardner, Holland & Beaudry (1997). The coating of the PDMS fibre was exposed to the apple headspace for 20 min. Afterwards, the fibre was withdrawn and introduced into the GC injection port and thermally desorbed for 3 min at 250°C in splitless mode. Furthermore, a non-destructive analysis of the whole fruit was performed as well. For the purpose, the fruit was inserted into a glass jar of 1700 mL volume and exposed to the PDMS and DVB-CAR-PDMS fibres at room temperature for 40 min. A mixture of *n*-alkanes (C_6-C_{22}, Sigma-Aldrich, St. Louis, USA) was also prepared in *n*-hexane (Carlo Erba, Milan, Italy) and inserted into the 4 mL vial. After 5 min of equilibration at 40°C, this mixture was exposed to the PDMS and fibre for 20 min, subsequently desorbed into the GC-MS system to calculate the linear retention indices (RIs) of apple volatiles. All the measurements were made with the same fibre which was conditioned in the injector of the GC system before analyses. All experiments were carried out in duplicate and the standard deviation values were calculated.

2.5. GC-MS Analysis

Apple volatiles were analysed on an Agilent 6890N gas chromatograph (GC) equipped with a single quadrupole mass spectrometer detector 5973N (MS). Separation was achieved on a HP-5-MS (30 m x 0.25 mm i.d., 0.1 μm film thickness, Agilent, Folsom, CA, USA) capillary column which was exposed to the following oven temperature program: 45˚C held for 6 min, raised up to 130˚C at 10˚C/min, then to 180˚C at 3˚C/min, finally to 300˚C at 20˚C/min held for 3 min. The GC injector port was equipped with a SPME inlet liner (0.75 mm i.d, Supelco) operating in splitless mode. Carrier gas was helium with a flow rate of 1.0 mL/min Injector and transfer line temperatures were set up to 250°C and to 280°C, respectively. The

acquisition of detector was in the 29-400 *m/z* range. Chromatograms were analysed using the MSD ChemStation software (Agilent, Version G1701DA D.01.00) and the NIST Mass Spectral Search Program for the NIST/EPA/NIH Mass Spectral Library v. 2.0. Mass spectra (MS) of the peaks were studied using the following libraries: ADAMS, NIST 17 and WILEY275. In addition, the temperature-programmed retention indices. The relative abundance of components, expressed in percentages, were extrapolated from the peak areas without using correction factors.

2.6. Principal Component Analysis (PCA)

This analysis was carried out to understand the relationships among different apple samples based on phenolic and volatile compositions and to determine the main constituents influencing the chemical variability. A covariance data matrix composed of 20 apple samples and 19 variables for phenolic compounds (380 data), and 21 apple samples (including the intact fruit) and 22 variables for volatile fraction (462 data), was prepared and subjected to Principal Component Analysis (PCA) using STATISTICA 7.1 (Stat Soft Italia S.r.l., Vigonza, Italy). For SPME analysis, only constituents occurring in percentages $\geq$ 1% in at least one sample were included in the data matrix. Eigenvalues were calculated and score- and loading plots including both apple samples and volatile and non-volatile constituents were generated.

2.7 Morphological and histochemical analysis

Samples 1-10 were studied for the morpho-anatomical and the histochemical features of the fruits by means of light microscopy. For consistency, samplings were carried out from the blushed side of the fruit (the pink sun-exposed), and not from the green-shaded side, for all the investigated samples. Both fresh and fixed material was analysed. After harvest and storage, vibratome-cut ca 25 μm-thick cross-sections from fresh small fragments (ca. 9 mm^2) of two fruits per sample were made. Samples were also fixed in FAA for a minimum of 2 days, then they were dehydrated in a graded ethanol series and embedded in Technovit 7100

(Kulzer). Cross sections ca. 3 µm-thick of these samples were then prepared using a Reichert Om U3 Automatic Microtome. Observations were made with a Leitz DM-RB Fluo optic microscope equipped with a digital camera. The following morphometric parameters were assessed in at least 10 slides per samples: cuticle thickness, cell layer number and thickness of the epidermis, cell layer number and thickness of the hypodermis, overall thickness of the peel. The following histochemical procedures were employed: Toluidine Blue as a general staining, Sudan III/IV for total lipids, Nadi reagent for terpenes, PAS-reaction for total polysaccharides, Lugol's solution for starch, and Ferric trichloride for total phenolics (Giuliani, Tani, Maleci Bini, Fico, Colombo & Martinelli, 2018). Standard control procedures were carried out simultaneously for each histochemical dye. Primary fluorescence was also evaluated under UV and Blue lights.

3. Results and discussion

3.1. Nutritional analysis and mineral content

The proximate analysis and mineral content of five samples of Mela Rosa dei Monti Sibillini (whole fruit) are reported in the Table 2. The moisture content ranged from 77.8% in sample 1 to 86.0 % in sample 3. Total carbohydrates were found in high levels in sample 4 (13.9%) and sample 1 (12.4%). Their levels were similar to those reported by Aprea et al. (2017) on different apple cultivars. Among sugars, the most abundant one was fructose with an average level of 6.5% (range 5.7-7.5%), followed by glucose with an average level of 2.8% (range 1.9-3.5%) and finally by sucrose and sorbitol with an average level of 2.2% and 0.8%, respectively.

Fructose is an important ketohexose with a lower calorific value and a higher sweetening power compared to sucrose, usually known as 'table sugar', a white, odorless, crystalline powder with a sweet taste, usually taken as reference for the calculation of sweetener power

of carbohydrates. Sorbitol and glucose, which are produced by photosynthesis in leaves, translocated through the phloem to reach fruit tissue, where they are converted, depending on the developmental stage, into fructose, glucose, malic acid, or starch (Aprea et al., 2017). Glucose is preferentially incorporated into starch while the predilection site of fructose is the vacuoles of apple cells. As a result, fructose is always higher than glucose in fruit tissue as confirmed by our and previous data (Aprea et al., 2017). Sorbitol is another important apple sugar as it influences the perceived sweetness in apple (Aprea et al., 2017). Thus, the five apple samples analyzed might display little difference in sweetness according to the different level of sorbitol, being sample 1 and 4 the sweetest one.

Dietary fiber, an important component with beneficial effects to organism, ranged from 1.9 % of sample 5 to 2.4% in sample 4 with an average content of 2.2%. Protein was found in low levels in different samples ranging from 1% (sample 1) to 1.7% (sample 3). Fat ranged from 0.2% in sample 1 to 0.4% in samples 3 and 4. Ash content varied between 1.2% in sample 5 to 1.6% in sample 2 (mean values of 1.3%). On the basis of the proximate analysis, it was calculated that the whole apple samples provided from 53.0 (sample 3) to 70.0 kcal (sample 4). Our results on nutritional analysis are in accordance with those reported by Feliciano et al. (2010) that performed the nutritional characterization of traditional and exotic apple varieties from Portugal. In the nine apple varieties analyzed, they found similar moisture content (range 78.1- 83.5%) and total sugars (range 9.99-13.25%) but lower protein content (range 0.07-0.1%) with respect to our samples. According to the specific sugars analyzed, the levels of fructose and glucose found in our samples are slightly higher (around 1%) with respect to those reported by these authors (Feliciano et al., 2010); on the other side, the reported amounts of sucrose are comparable to our findings.

The analysis of minerals was carried out on both the entire fruit (Table 2) and separate peel and pulp of apple samples (Table 1 Supplementary Material). For the purpose, 31 different

minerals including 5 major elements such as Mg, P, S, K and Ca, and 26 minor elements such as Li, B, Na, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Mo, Ag, Cd, Sn, Sb, Cs, Ba, Pb and U, were quantified in apple samples 1-5, including 5 entire fruits, 5 peels and 5 pulps. Data are expressed on a dry weight basis (w/w). The mineral analysis showed K as the most abundant mineral with an average concentration of 6.67 g/kg in the whole apple samples, being sample 5 the richest one (8.0 g/kg). Potassium plays an important role in the human body, especially in the transmission process of nerve signals, but also in fluid balance and proper function of heart, muscles, kidney and hormones (Todea et al., 2014). The concentration of K found in our samples is noteworthy, as it is at least 6-fold higher with respect to those reported by Feliciano et al. (2010) in various apple samples grown in Portugal (i.e. traditional apples with an average content of 1.0 g/kg and exotic apples with an average content of 0.99 g/kg). On the other side, this value is almost five times lower with respect to those obtained by Todea et al. (2014), that found an average level of 31 g/kg in different commercial apple varieties with the 'Golden Delicious' as the richest one. Concerning the other four major elements, the average concentration of P, Mg, Ca, and S in the whole pink apple samples were 0.48, 0.28, 0.25 and 0.18 g/kg, respectively. Feliciano et al. (2010) found lower levels of these minerals with respect to our data (i.e. 0.084, 0.039, 0.025 and 0.031 g/kg, and 0.11, 0.035, 0.032 and 0.028 g/kg in traditional and exotic apples, respectively). The values reported by Todea et al (2014) were once again higher with respect to our findings. The high content of the four major elements found in the analysed apple samples is noteworthy, as calcium and magnesium play an essential role in muscle function, nerve transmission, bone and teeth formation and hormone secretion; furthermore, magnesium is required for processing adenosine triphosphate (ATP) (Todea et al., 2014). Phosphorus plays an important role in the formation of bones and teeth, in the formation of deposits of energy in the form of ATP, in the absorption and transport of nutrients and in the constitution of

313 enzymes, proteins, phospholipids, nucleotides and nucleic acids; while sulfur is essential for
314 the synthesis of collagen and many mucopolysaccharides (Todea et al., 2014).
315 Among the minor minerals, B was the most abundant one with an average concentration of
316 21.7 mg/kg in the five whole apple samples analysed, followed by Na (18.40 mg/kg), Fe
317 (10.57 mg/kg), Rb (6.27 mg/kg), Mn (3.25 mg/kg), Zn (2.50 mg/kg) and Cu (2.35 mg/kg)
318 among the most abundant ones. Contrarily, Todea et al. (2014) found lower levels of Na (9.62
319 mg/kg), Fe (3.68 mg/kg), Mn (1.81 mg/kg) and Zn (1.93 mg/kg), and higher levels of Cu
320 (3.57 mg/kg) with respect to our findings. Feliciano et al. (2010) reported lower amounts of B
321 (4.2 and 3.7 mg/kg for traditional and exotic varieties, respectively), Na (8.2 and 9.4 mg/kg,
322 for traditional and exotic varieties, respectively), Mn (0.4 mg/kg for traditional and exotic
323 varieties), Zn (0.3 and 0.4 mg/kg for traditional and exotic varieties, respectively) and Cu (0.6
324 and 0.4 mg/kg for traditional and exotic varieties, respectively) with respect to our results.
325 Concerning minor elements, the apple variety under study can be considered a very good
326 source of boron, sodium and iron. Boron seems to improve the ability of the body to absorb
327 calcium and magnesium and can manage many dangerous conditions such as arthritis and
328 osteoporosis (Nielsen, 1997). Sodium plays a key role in muscle and nerve function and work
329 with potassium to coregulate ATP and fluids. It contributes to the maintenance of osmotic
330 balance, and numerous acid-base equilibria, affects the permeability of the cell membrane and
331 participates to the conduction of the nerve impulse. Iron is necessary to produce hemoglobin
332 and myoglobin, proteins that carry oxygen in the human body (Todea et al., 2014). In Table 1
333 Supplementary Material the mineral content in separate peel and pulp apple samples is
334 reported. From these results it is clear that peel results richer in all minerals than pulp except
335 for Na and Rb. Concerning the major elements, K was the most abundant major mineral
336 (average content of 8.3 g/kg in peel and 7.2 g/kg in pulp), followed by P (average content of
337 1.3 g/kg in peel and 0.3 g/kg in pulp), being samples 5 the richest one in both elements (9.2

g/kg for K and 1.6 g/kg for P). Concerning the minor elements, most of them are more concentrated in peel with B (27.9 mg/kg), Fe (19.5 mg/kg), Zn (6.8 mg/kg) and Mn (7.1 mg/kg) as the most abundant ones. On the other side, pulp contained higher levels of Na (26.0 mg/kg) and Rb (8.9 mg/kg). Notably, peel sample 1 was the richest in B (42.3 mg/kg), Mn (10.0 mg/kg) and Rb (13.7 mg/kg), whereas sample 5 showed the highest amounts of Fe (25.3 mg/kg) and Cu (10.3 mg/kg). Pulp sample 4 was the richest in Na (51.6 mg/kg).

3.2. HPLC-DAD-MS^n Analysis

The HLPC-DAD-MS^n analysis allowed to identify nineteen different constituents in the peel and pulp samples of the Mela Rosa dei Monti Sibilini. Their list is reported in Table 3.

Five different classes of constituents, namely flavan-3-ols, flavonols, dihydrochalcones, hydroxycinnamic acids and triterpenes, were identified in pulp and peel of the ten apple samples. Overall, peel was richer in these constituents than pulp (average values 8.04 vs 3.93 mg/g, respectively), though a significant variability was observed in the various samples from both apple parts. Procyanidins and catechin average concentrations were similar in the two different tissues, showing concentrations ranges of 1.18-3.33 and 1.23-2.36 mg/g, respectively. Flavonoid concentrations were definitely higher in peels (0.78-6.97 mg/g) than pulps (0.04-0.10). Average values of dihydrochalcones ranged from 0.18 to 0.85 mg/g and 0.14 to 0.40 mg/g in peels and pulps, respectively. Amounts of chlorogenic acid derivatives showed high variation in both peel and pulp samples, with ranges of 0.03-3.09 and 0.35-5.10 mg/g, respectively.

Different apple varieties present significant variations in phenolics (Kalinowska, Bielawska, Lewandowska-Siwkiewicz, Priebe & Lewandowski, 2014), thus the measured values are in the ranges previously reported. It is notable that there is a large variation (nearly ten-fold rate) of the flavonoid amounts in peels as well as that of chlorogenic acid (fifteen -old rate) in pulps. It is notable that for the ten different samples the most abundant class of compounds is

363 that of flavonoids in peels except for samples 9 and 10 that contained proanthocyanidins as
364 the most abundant constituents. Alike, it is worth noting that samples 2,4 5 and 6 showed the
365 higher contents of chlorogenic acid derivatives in both peel and pulp. We assume that
366 pedoclimatic conditions, ripening stage and graft type of the different samples analyzed may
367 contribute to the highlighted variance of the samples.
368 A PCA analysis considering the various amounts of the secondary metabolites in peel and
369 pulp samples is depicted in Fig. 2 a,b where score and loading plots representing 93.78% of
370 data variability are included. The components with the highest variance were quercetin-3-O-
371 galactoside (values of eigenvectors: -1.4; -0.3) in the first principal component and
372 chlorogenic acid (values of eigenvectors: -0.2; 1.3) in the second principal component.
373 Looking at the score plot (Fig. 2 a) it is interesting to observe that peel samples were
374 correlated with high levels of flavonols, mostly quercetin-3-O-galactoside and, to a minor
375 extent, rutin and quercetin-3-O-arabinoside, whereas pulp samples were mostly influenced by
376 chlorogenic acid and, to a minor extent, catechin.
377 PCA was also restricted to the composition of peels in order to observe specific compositions
378 of the various samples. As reported in Fig. 2a Supplementary Material, samples 3, 9 and 10
379 formed a cluster characterized by similar levels of annurcoic acid, procyanidins and
380 rhamnetin-3-O-glucoside as discriminant constituents. Samples 1,5,6,7 and 8 were clusterized
381 by procyanidins, phloretin-2-O-glucoside, quercetin-3-O-xyloside and rhamnetin-3-O-
382 glucoside. A further cluster was represented by samples 2 and 4 that were characterized by
383 chlorogenic acid, rutin and quercetin-3-O-galactoside.
384 Two different clusterizations were observed for pulp samples (Fig. 2b Supplementary
385 Material). Samples 2, 4, 5 and 6 contained higher levels of chlorogenic acid and catechin
386 whereas all the remaining samples showed similar amounts of the identified compounds.
387 Considering both the tissues, apple samples 2, 4, 5 and 6 showed a similar clusterization. With

388 regard to pulp samples, sample 2 showed the highest concentrations of phloretin and
389 flavonoid derivatives (0.397 and 0.118 mg/g, respectively), sample 7 those of procyanidins
390 (2.601 mg/g) and sample 4 those of chlorogenic acid derivatives (5.05 mg/g). In peels, the
391 highest content of phloretin derivatives was observed in sample 5 (0.85 mg/g), with sample 4
392 showing the highest amount of procyanidins (3.34 mg/g) and sample 2 being the richest in
393 chlorogenic acid (3.09 mg/g) and flavonoids (6.97 mg/g).
394 In conclusion, the phenolic profile of this central Italy ancient variety is very interesting and
395 deserves further investigation since the total level of constituents detected in the peels (8
396 mg/g) appeared to be higher than that found in Annurca (4 mg/g) which is one of the most
397 important cultivars of southern Italy (Mari et al., 2010).
398 *3.3. Volatile composition*
399 In order to characterize the aroma profile of the Mela Rosa dei Monti Sibillini, a HS-SPME
400 coupled to GC-MS analysis was performed. For the purpose, we separately analysed fresh
401 peel and pulp of 10 apple samples furnished by local farmers. In addition, a comparison with
402 an intact fruit was made. Actually, the fruit aroma perceived is due to the interaction of all
403 volatile components released to the air.
404 A total of 134 volatile components were identified in different samples of intact fruit, peel and
405 pulp, accounting for 96.5-98.5% of the total compositions. The richest part was the peel, with
406 112 identified compounds, followed by pulp (72) and intact fruit (52) (Table 4). Carboxylic
407 esters (55.8%) and terpenoids (36.0%) were the characteristic compounds in the headspace of
408 the intact fruit, with butyl hexanoate, hexyl butanoate (sum of both = 33.6%) and hexyl
409 hexanoate (9.6%), and (*E*,*E*)-α-farnesene (35.2%) as the most characteristic compounds,
410 respectively. It is important to note that butyl hexanoate and hexyl butanoate co-eluted in the
411 column; their coelution was also reported by other authors (Song et al., 1997). Other flavour
412 compounds occurring in appreciable percentages (>1%) were hexyl 2-methyl butanoate

413 (3.7%), hexanol (2.2%), phenoxyethanol (1.3%), pentyl hexanoate (1.5%) and butyl
414 heptanoate (1.5%). Notably, the terpene fraction was mostly represented by (*E*,*E*)-α-
415 farnesene, whereas limonene, a marker compound of other apple varieties such as Fuji, was
416 missing. Terpenoids are involved in the plant defence against herbivores and parasites (Paré
417 and Tumlinson, 1999). (*E*,*E*)-α-farnesene is an acyclic sesquiterpene occurring in the coating
418 of several pomoidea fruits where it contributes to the green apple-like scent and sweet-wood
419 odor (Maggi, Bilek, Cristalli, Papa, Sagratini & Vittori, 2009). This compound is used as a
420 flavouring agent in confectionery and perfumery.
421 Esters are marker compounds of apple aroma having a low odor threshold. They are
422 responsible for the sweet-fruit flavour, thus their presence is considered as an indicator of
423 apple quality. They are characteristics of fruit ripening where degradative reactions involving
424 lipids (for straight chain esters) and amino acids (for branched chain esters) give rise to these
425 volatiles, notably from esterification of even carboxylic acids with ethyl, butyl, and hexyl
426 alcohols (Bartley, Stoker, Martin, Hatfield & Knee, 1985). In the apple variety under study
427 butyl and hexyl esters were the most abundant impact odorants with 11 and 7 contributing
428 compounds, respectively. Notably, butyl hexanoate and hexyl butanoate are odor descriptors
429 for honey and pumpkin, respectively (Aprea et al., 2012). In addition, butanoate esters are
430 characterized by grapefruit and lemon attributes and are typical of apples with red coloration
431 (Lo Scalzo, Testoni & Genna, 2001). On the other hand, acetate esters which are described as
432 the main contributors to the typical apple odor are poor in the apple under study. Alcohols and
433 aldehydes, which are responsible for the herbaceous note of fruits and vegetables, were
434 present in low amounts. They were mostly represented by hexanol (2.2%) and nonanal
435 (0.6%), respectively. Overall, the hallmark of Mela Rosa dei Monti Sibillini aroma was the
436 abundance of straight chain esters and green apple-like farnesene. Interestingly, the wealth of

esters makes this old variety comparable to apples with red peel (Young, Chu, Lu, & Zhu, 2004).

The volatile profile detected in the apple variety under investigation showed some differences with those found in commercial varieties such as 'Royal Gala', 'Ambrosia' and 'Golden Delicious', where the major impact odorants were butyl acetate, hexyl acetate and 2-methylbutyl acetate (Aprea et al., 2012; Lo Scalzo et al., 2001). These compounds were missing or at trace levels in the headspace of the apple under investigation. Important differences in the aroma components were also observed when we compared our samples with those belonging to 'Annurca', a Southern Italy apple cultivar (Lo Scalzo et al., 2001). The latter was characterized by high amounts of esters (79-84% of headspace) such as *n*-pentanol, along with the absence of (*E*,*E*)-α-farnesene.

When we analysed separately apple peel samples we found out a quite similar chemical profile with respect to the intact fruit (Table 4). Peel samples showed esters (27.4-65.4%) and terpenoids (18.4-55.9%) as the most important aroma contributors, whereas aldehydes (3.5-12.6%) and alcohols (2.2-6.3%), though more abundant than those of intact apple, gave a lower contribution. Also in this case, butyl hexanoate + hexyl butanoate (18.8-32.0%), hexyl 2-methyl butanoate (3.1-24.8%) and hexyl hexanoate (3.8-10.9%) were the most important carboxylic esters of the peel fruit, whereas the apple-like (*E*,*E*)-α-farnesene (18.2-54.4%) was the principal terpenoid. Hexanal (2.3-7.0%) and (*E*)-2-hexenal (0.9-5.1%), and hexanol (0.5-1.8%) and 1,3-octadeniol (0.9-3.5%) were the most representative compounds among aldehydes and alcohols, respectively. Notably, (*E*)-2-hexenal, hexanal and hexanol are responsible for the green apple-like, grass-minty and herbaceous odors, respectively. In combination with esters they contribute to the so-called 'moscato grape' odor (Amaro, Beaulieu, Grimm, Stein & Almeida, 2012). Aldehydes and alcohols with six carbon atoms are biosynthetically formed from unsaturated fatty acids through a β-oxidation followed by

462 lipoxygenase action leading to hydroperoxides that are in turn converted into aldehydes then
463 to alcohols (Bartley et al., 1985).
464 The pulp samples displayed a quantitatively different volatile profile (Table 4), with
465 terpenoids (26.9-45.8%), alcohols (10.5-32.3%) and aldehydes (5.7-30.6%) as the most
466 abundant chemical groups. These were represented by (*E*,*E*)-α-farnesene (26.3-45.3%),
467 hexanol (4.1-11.7%) and 1,3-octanediol (2.6-14.2%), and hexanal (4.1-22.8%) and (*E*)-2-
468 hexenal (1.4-8.4%), respectively. However, in samples 2 and 6 esters were found abundant
469 (33.7 and 42.5%, respectively). They were mostly represented by butyl hexanoate + hexyl
470 butanoate (18.0 and 25.6%, respectively). These differences may be due to the advanced
471 ripening stages of these sample.
472 In conclusion, the pulp of Mela Rosa dei Monti Sibillini is characterized by green apple-like,
473 grass-minty and herbaceous notes.
474 PCA of peel, pulp and intact fruit samples in reported in Fig. 2 c,d where score and loading
475 plots summarize 91.62% of data variability. The main contribution was provided by the first
476 principal component which explained 74.54% of variance, with the second principal
477 component accounting for 17.08%. In this regard, butyl hexanoate + hexyl butanoate (values
478 of eigenvectors: 12.8; 1.2) and hexanal (values of eigenvectors: -7.6; -2.5) were the
479 components influencing most the variability in the first principal component, whereas (*E*,*E*)-
480 α-farnesene (values of eigenvectors: -4.6; 7.6) was the main contributor to the variance in the
481 second principal component (17.08%). As a result, peel and pulp samples clustered in two
482 separate groups, with the former characterized by esters such as butyl hexanoate, hexyl
483 butanoate, hexyl 2-methyl butanoate and hexyl hexanoate, and the latter more influenced by
484 aldehydes and alcohols such as hexanal, 1,3-octanediol, hexanol and 2-methyl-1-butanol. In
485 this respect, the intact fruit sample took place in the same part of peel samples (Fig. 2c), being

characterized by butyl hexanoate, hexyl butanoate and hexyl hexanoate. The green apple-like (*E*,*E*)-α-farnesene influenced both pulp and peel samples with a higher impact on the former.

3.3 Morphological and histochemical analysis

The overall anatomical and histochemical features of the fruits proved to be homogeneous across all of the investigated samples. Fig. 4 Supplementary Material shows the median cross-section of the peel and pulp of the sample 1. The peel was composed of:

(cu) an external cuticle covering the epidermis. It is formed by cutin and cuticular waxes, staining deep orange-red with Sudan III/IV and displaying primary fluorescence under UV and Blue lights. The cuticle often occcurs not only on the outer wall of the epidermal cells, but is also deposited within the anticlinal wall and occasionally on the inner tangential walls (Fig. 4a Supplementary Material). The cuticle layer may externally exhibit several microcracks which however do not reach the epidermal tissue.

(ep) a single- or double-layered epidermis composed by polygonal to rounded cells with a small lumen. The cytoplasm of this cells displayed an intense positive response to both the test ferric trichloride and the Nadi reagent, indicating the presence of phenolics and terpenes, respectively (Fig. 4f,g Supplementary Material).

(hy) a hypodermis composed of several (four to eight) layers of flattened tetragonal to hexagonal collenchymatous cells with a variable-sized diameter and containing numerous amyloplasts (Fig. 4h Supplementary Material). The cells are thick-walled and close-packed.

The fruit pulp consists of parenchymatous tissue containing large, rounded, thin-walled cells, each with a thin lining cytoplasm and a large central vacuole. The intercellular spaces are generally large.

The morphometric analysis on the fruit peel proved the existence of a high level of variability among the samples for all the examined parameters (Table 2 Supplementary Material). Cuticle thickness ranged from 16.8±1.8 μm in the sample 4 to 28.1±3.9 μm in the sample 3.

With regards to the epidermis, in most of the samples it was single-layered with the exception of the samples 1, 7, 8 and 9; its thickness was variable, ranging from 10.0±0.9 μm (sample 5) up to 24.3±0.9 μm (sample 1). Concerning the hypodermis, the cell layer number was not homogeneous and its thickness varied from 76.9±5.7 μm (samples 7) to 180.2±5.6 μm (sample 8). The overall thickness of the peel was in the range 122.9±3.9 (sample 10) n - 216.7±6.7 μm (sample 9).

The morphometric observations showed varying thickness of the diverse parts of the peel, namely cuticle, epidermis and hypodermis, as well as a diverse structure. Besides the genetic backgrounds, climatic factors and microenvironmental conditions at the growing sites may affect all these parameters. A thicker cuticle on the blushed side of the fruit may allow a major defence for the fruit interior towards the severe damages of UV radiation. On the other hand, a thicker hypodermis increases the resistance of the peel against compression and deformation, thus protecting the fruit interior.

4. Conclusions

The present work represents the first investigation carried out on the so-called 'phytonutrients' of the Mela Rosa dei Monti Sibillini, an old apple variety cultivated for thousands of years in Central Italy and worthy of preservation and economic valorization in this area. Analysis of nutrients and polyphenols evidenced the quality of this fruit, with the peel as the richest part containing healthy promoting compounds such as K, P, B, Na and Fe among micronutrients and quercetin derivatives among polyphenols. The high phenolic content found in this apple is worthy of further investigations from a nutraceutical perspective. The fruit aroma was characteristic when compared with other apple varieties; it was mainly given by carboxylic esters, mainly butyl and hexyl esters, as the main contributors of the peel, and aldehydes and alcohols as key odorants of the pulp, with the presence in both

parts of the green apple-like farnesene. In addition, we correlated this information with a morphological survey which allowed to elucidate the presence of phenolic and terpenic components in the epidermis of the peel. These evidences are consistent with the results of the phytochemical investigation. In conclusion, this old apple variety showed nutritional, phenolic and volatile profiles that give an added value for its consumption allowing to improve its production at both regional and national levels.

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Conflict of interest

Authors declare they have no conflict of interest.

References

Amaro, A. L., Beaulieu, J. C., Grimm, C.C., Stein, R.E., & Almeida, D.P. (2012). Effect of oxygen on aroma volatiles and quality of fresh-cut cantaloupe and honeydew melons. *Food Chemistry*, *130*, 49-57.

AOAC (1995). Official methods of analysis (16th ed.). Arlington VA, USA: Association of Official Analytical Chemists.

Aprea, E., Charles, M., Endrizzi, I., Corollaro M. L., Betta E., Biasioli, F., & Gasperi, F. (2017). Sweet taste in apple: the role of sorbitol, individual sugars, organic acids and volatile compounds. *Scientific Reports*, *7*, 44950.

560 Aprea, E., Corollaro, M. L., Betta, E., Endrizzi, I., Demattè, M. L., Biasioli, F., & Gasperi, F.
561 (2012). Sensory and instrumental profiling of 18 apple cultivars to investigate the
562 relation between perceived quality and odour and flavour. *Food Research*
563 *International*, *49*, 677–686.
564 Bartley, I.M., Stoker, P.G., Martin, A.D.E., Hatfield, S.G.S., & Knee, M. (1985). Synthesis of
565 aroma compounds by apples supplied with alcohols and methyl esters of fatty acids.
566 *Journal of the Science of Food and Agriculture*, *36*, 567–574.
567 Cho, A.S., Jeon, S.M., Kim, M.J., Yeo, J., Seo, K.I., Choi, M.S., & Lee, M.K. (2010).
568 Chlorogenic acid exhibits anti-obesity property and improves lipid metabolism in
569 high-fat diet-induced-obese mice. *Food and Chemical Toxicology*, *48*, 937-943.
570 D'Abrosca, B., Pacifico, S., Cefarelli, G., Mastellone, C., & Fiorentino, A. (2007).
571 'Limoncella'apple, an Italian apple cultivar: Phenolic and flavonoid contents and
572 antioxidant activity. *Food Chemistry*, *104*, 1333-1337.
573 Escarpa, A., & Gonzalez, M.C. (1998). High-performance liquid chromatography with diode-
574 array detection for the determination of phenolic compounds in peel and pulp from
575 different apple varieties. *Journal of Chromatography A*, *823*, 331-337.
576 Feliciano R.P., Antunes C., Ramos A., Serra A.T., Figueira M.E., Duarte C.M.M., de
577 Carvalho A., & Bronze, M.R. (2010). Characterization of traditional and exotic apple
578 varieties from Portugal. Part 1 – Nutritional, phytochemical and sensory Evaluation.
579 *Journal of Functional Foods*, *2*, 34-45.
580 Ferreira, L., Perestrelo, R., Caldeira, M., & Câmara, J.S. (2009). Characterization of volatile
581 substances in apples from Rosaceae family by headspace solid-phase microextraction
582 followed by GC-qMS. *Journal of Separation Science*, *32*, 1875-1888.
583 Gaudout, D., Megard, D., Inisan, C., Esteve, C., & Lejard, F. (2006). *U.S. Patent No.*
584 *7,041,322*. Washington, DC: U.S. Patent and Trademark Office.

585 Giannetti, V., Mariani, M.B., Mannino, P., & Marini, F. (2017). Volatile fraction analysis by
586 HS-SPME/GC-MS and chemometric modeling for traceability of apples cultivated in
587 the Northeast Italy. *Food Control*, *78*, 215-221.
588 Giuliani, C., Tani, C., Maleci Bini, L., Fico, G., Colombo, R., & Martinelli, T. (2018).
589 Localization of phenolic compounds in the fruits of *Silybum marianum* characterized
590 by different silymarin chemotype and altered colour. *Fitoterapia*, *130*, 210-218.
591 Hyson, D.A. (2011). A comprehensive review of apples and apple components and their
592 relationship to human health. *Advances in Nutrition*, *2*, 408-420.
593 Jia, M., Ren, D., Nie, Y., & Yang, X. (2017). Beneficial effects of apple peel polyphenols on
594 vascular endothelial dysfunction and liver injury in high choline-fed mice. *Food &*
595 *Function*, *8*, 1282-1292.
596 Kalinowska, M., Bielawska, A., Lewandowska-Siwkiewicz, H., Priebe, W., & Lewandowski,
597 W. (2014). Apples: Content of phenolic compounds vs. variety, part of apple and
598 cultivation model, extraction of phenolic compounds, biological properties. *Plant*
599 *Physiology and Biochemistry*, *84*, 169-188.
600 Kianifard, T., & Chopra, A. (2018). A therapeutic role for potassium (K) to reduce pain and
601 complications related to the cardiovascular system and bone in rheumatoid arthritis
602 (RA): A clinical research perspective. *Rheumatology Research*, *3*, 1-12.
603 Kubola, J., Siriamornpun, S., & Meeso, N. (2011). Phytochemicals, vitamin C and sugar
604 content of Thai wild fruits. *Food Chemistry*, 126, 972–981.
605 Larson, A. J., Symons, J. D., & Jalili, T. (2012). Therapeutic potential of quercetin to decrease
606 blood pressure: review of efficacy and mechanisms. *Advances in Nutrition*, *3*, 39-46.
607 Lee, J., Jang, H.W., Jeong, M.C., Yoo, S., & Ha, J. (2017). Analysis of volatile compounds as
608 quality indicators for Fuji apples after cold storage. *Journal of Food Biochemistry*,
609 *41*(6), e12410.

610 Lo Scalzo, R., Testoni, A., & Genna, A. (2001). 'Annurca' apple fruit, a southern Italy apple
611 cultivar: textural properties and aroma composition. *Food Chemistry*, *73*, 333-343.
612 Maggi, F., Bilek, T., Cristalli, G., Papa, F., Sagratini, G., & Vittori, S. (2009). Comparison of
613 the characterization of the fruit-like aroma of *Teucrium flavum* L. subsp *flavum* by
614 hydrodistillation and solid-phase micro-extraction. *Journal of the Science of Food and*
615 *Agriculture, 89*, 2505-2518.
616 Manzi, P., Marconi, S., Aguzzi, A., & Pizzoferrato, L. (2004). Commercial mushrooms:
617 Nutritional quality and effect of cooking. *Food Chemistry*, *84*, 201–206.
618 Mari, A., Tedesco, I., Nappo, A., Russo, G. L., Malorni, A., & Carbone, V. (2010). Phenolic
619 compound characterisation and antiproliferative activity of "Annurca" apple, a
620 southern Italian cultivar. *Food Chemistry*, *123*, 157-164.
621 Matich, A.J., Rowan, D.D., & Banks, N.H. (1996). Solid phase microextraction for
622 quantitative headspace sampling of apple volatiles. *Analytical Chemistry*, *68*, 4114-
623 4118.
624 Nasuti, C., Ferraro, S., Giovannetti, R., Piangerelli, M., & Gabbianelli, R. (2016). Metal and
625 microelement biomarkers of neurodegeneration in early life permethrin-treated rats.
626 *Toxics*, *4*, 3.
627 Nielsen, F.H. (1997). Boron in human and animal nutrition. *Plant and Soil*, *193*, 199-208.
628 Paré, P.W., & Tumlinson, J.H. (1999). Plant volatiles as a defense against insect herbivores.
629 *Plant Physiology*, *121*, 325-331.
630 Porras, D., Nistal, E., Martínez-Flórez, S., Pisonero-Vaquero, S., Olcoz, J.L., Jover, R.,
631 González-Galego, J., Garcia-Mediavilla, M.V., & Sánchez-Campos, S. (2017).
632 Protective effect of quercetin on high-fat diet-induced non-alcoholic fatty liver disease
633 in mice is mediated by modulating intestinal microbiota imbalance and related gut-
634 liver axis activation. *Free Radical Biology and Medicine*, *102*, 188-202.

635 Reis, S.F., Rocha, S.M., Barros, A.S., Delgadillo, I., & Coimbra, M.A. (2009). Establishment
636 of the volatile profile of 'Bravo de Esmolfe' apple variety and identification of varietal
637 markers. *Food Chemistry*, *113*, 513-521.
638 Shin, D., Joh, H.K., Kim, K.H., & Park, S.M. (2013). Benefits of potassium intake on
639 metabolic syndrome: The fourth Korean National Health and Nutrition Examination
640 Survey (KNHANES IV). *Atherosclerosis*, *230*, 80-85.
641 Singh, R., Barden, A., Mori, T., & Beilin, L. (2001). Advanced glycation end-products: a
642 review. *Diabetologia*, *44*, 129-146.
643 Song, J., Gardner, B.D., Holland, J.F., & Beaudry, R.M. (1997). Rapid analysis of volatile
644 flavor compounds in apple fruit using SPME and GC/Time-of-Flight Mass
645 Spectrometry. *Journal of Agricultural and Food Chemistry*, *45*, 1801-1807.
646 Sut, S., Poloniato, G., Malagoli, M., & Dall'Acqua, S. (2018). Fragmentation of the main
647 triterpene acids of apple by LC-APCI-MS^n. *Journal of Mass Spectrometry*, *53*, 882-
648 892.
649 Todea, D. A., Cadar, O., Simedru, D., Roman, C., Tanaselia, C., Suatean, I., & Naghiu, A.
650 (2014). Determination of major-to-trace minerals and polyphenols in different apple
651 cultivars. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *42*, 523-529.
652 Tsao, R., Yang, R., Young, J. C., & Zhu, H. (2003). Polyphenolic profiles in eight apple
653 cultivars using high-performance liquid chromatography (HPLC). *Journal of*
654 *Agricultural and Food Chemistry*, *51*, 6347-6353.
655 Vrhovsek, U., Rigo, A., Tonon, D., & Mattivi, F. (2004). Quantitation of polyphenols in
656 different apple varieties. *Journal of Agricultural and Food Chemistry*, *52*(21), 6532-
657 6538.
658 Yang, K.C., Tsai, C.Y., Wang, Y.J., Wei, P.L., Lee, C.H., Chen, J.H., Wu, C.H., & Ho, Y.S.
659 (2009). Apple polyphenol phloretin potentiates the anticancer actions of paclitaxel

660 through induction of apoptosis in human hep G2 cells. *Molecular Carcinogenesis*, *48*,
661 420-431.
662 Young, J.C., Chu, C.L.G., Lu, X., & Zhu, H. (2004). Ester variability in apple varieties as
663 determined by solid-phase micro extraction and gas chromatography–mass
664 spectrometry. *Journal of Agricultural and Food Chemistry*, *52*, 8086–8093.

Table 1

Table 1. Main information about the samples of Mela Rosa dei Monti Sibillini analysed.

Sample No	Farmer	Graft	Altitude (m)	Fresh weight (g)	Height (cm)	Width (cm)	Water content (%)
1	Orsolini	M111/106	480	111.1±22.4	3.5±0.3	5.5±0.5	77.8±0.7
2	Botticelli	M111	300	101.9±23.2	3.5±0.3	5.0±0.6	81.0±0.9
3	Galli	M26/111	250	86.9±20.3	3.1±0.4	4.8±0.6	86.0±0.1
4	Geminiani	M26	400	125.1±19.3	3.7±0.3	5.6±0.4	81.9±0.4
5	Traini	M111	480	77.1±16.1	3.0±0.4	4.5±0.5	84.8±0.6
6	Peretti	M26	550	129.9±13.0	4.1±0.3	5.9±0.3	86.2±0.4
7	Marini	M111	700	81.5±17.2	3.1±0.4	4.5±0.4	81.9±0.4
8	Siliquini	M111	400	81.1±16.2	3.0±0.3	4.6±0.5	79.7±1.7
9	Mazzoni	M9	250	125.6±17.6	3.7±0.4	5.7±0.4	84.3±2.4
10	Gravucci	M111	500	122.6±24.9	3.5±0.4	5.7±0.6	81.5±0.4
		Average	**430**	**104.3±19.0**	**3.4±0.4**	**5.2±0.5**	**82.5±1.1**

Table 2

Table 2**.** Proximate analysis and mineral content of five entire fruits of Mela Rosa dei Monti Sibillini.

	Samples					Mean	SD
	1	**2**	**3**	**4**	**5**		
Moisture (%)	77.8	81.0	86.0	81.9	84.8	**82.3**	3.24
Macronutrients (% fw[a])							
Total Carbohydrate	12.4	10.6	9.6	13.9	10.7	**11.4**	1.70
Sugars							
Glucose	2.4	1.9	2.5	3.5	3.5	**2.8**	0.71
Fructose	7.0	6.3	5.7	7.5	5.9	**6.5**	0.76
Sucrose	3.0	2.4	1.4	2.9	1.3	**2.2**	0.81
Sorbitol	1.0	0.6	0.5	1.0	0.7	**0.8**	0.23
Total dietary fibre	2.2	2.3	2.0	2.4	1.9	**2.2**	0.21
Fats	0.2	0.3	0.4	0.4	0.3	**0.3**	0.08
Proteins	1.0	1.2	1.7	1.2	1.6	**1.3**	0.30
Ashes	1.3	1.6	1.3	1.3	1.2	**1.3**	0.15
Energy (KCal/100 g)	60.0	55.0	53.0	70.0	56.0	**58.8**	6.76
Energy (KJ/100 g)	253.0	229.0	223.0	290.0	235.0	**246.0**	27.04
Major minerals (g/kg dw[b])							
Mg / 24	0.4	0.2	0.3	0.2	0.3	**0.3**	0.08
P / 31	0.5	0.4	0.4	0.4	0.7	**0.5**	0.13
S / 34	0.2	0.1	0.2	0.1	0.3	**0.2**	0.07
K / 39	6.8	7.4	6.1	5.1	8.0	**6.7**	1.12

Ca / 44	0.4	0.2	0.3	0.2	0.2	**0.3**	0.07
Minor minerals (mg/kg dw[b])							
Li / 7	0.0[c]	0.0	0.0	0.0	0.0	**0.0**	0.00
B / 11	30.0	15.3	22.8	21.0	19.4	**21.7**	5.40
Na / 23	11.4	19.0	20.0	23.6	18.0	**18.4**	4.44
Ti / 47	0.4	0.2	0.2	0.3	0.4	**0.3**	0.11
V / 51	0.0	0.0	0.0	0.0	0.0	**0.0**	0.00
Cr / 52	0.3	0.1	0.7	0.2	0.3	**0.3**	0.20
Mn / 55	4.8	1.1	4.5	3.0	2.9	**3.3**	1.48
Fe / 56	9.4	5.5	8.4	19.4	10.1	**10.6**	5.22
Co / 59	0.0	0.0	0.0	0.0	0.0	**0.0**	0.01
Ni / 60	0.4	0.2	0.7	0.2	0.1	**0.3**	0.23
Cu / 63	2.2	1.1	1.9	1.1	5.3	**2.4**	1.74
Zn / 66	4.1	1.7	1.9	1.1	3.7	**2.5**	1.36
Ga / 69	0.2	0.3	0.2	0.3	0.2	**0.3**	0.03
As / 75	0.0	0.0	0.0	0.0	0.0	**0.0**	0.00
Se / 77	0.0	0.0	0.1	0.1	0.1	**0.1**	0.03
Rb / 85	17.5	6.7	5.0	1.0	1.1	**6.3**	6.76
Sr / 88	0.8	0.7	0.7	0.6	0.6	**0.7**	0.08
Mo / 95	0.1	0.1	0.1	0.1	0.1	**0.1**	0.02
Ag / 107	0.0	0.0	0.0	0.0	0.0	**0.0**	0.01
Cd / 111	0.0	0.0	0.0	0.0	0.0	**0.0**	0.00
Sn / 118	0.0	0.0	0.0	0.0	0.0	**0.0**	0.00
Sb / 121	0.0	0.0	0.0	0.0	0.0	**0.0**	0.00
Cs / 133	0.0	0.0	0.0	0.0	0.0	**0.0**	0.02

Ba / 137	1.4	1.6	1.4	1.6	1.2	**1.5**	0.16
Pb / 208	0.0	0.0	0.0	0.0	0.0	**0.0**	0.01
U / 238	0.0	0.0	0.0	0.0	0.0	**0.0**	0.00

[a]fw, fresh weight

[b]dw, dry weight

[c]the value 0.0 correspond to the element whose concentration is under the level of detection.

Table 3

Table 3. Phenolic compounds and triterpene acids in peel and pulp of the Mela Rosa dei Monti Sibillini.

Compound (mg/g)	$[M-H]^-$	MS^2	MS^3	Peel samples										Mean	SD	Pulp samples										Mean	SD
				1	2	3	4	5	6	7	8	9	10			1	2	3	4	5	6	7	8	9	10		
Flavan-3-ols																											
catechin	289	245-205-179		0.15	0.99	0.13	0.32	0.37	0.26	0.34	0.38	0.23	0.11	**0.33**	0.24	0.15	1.15	0.17	0.41	0.46	0.79	0.47	0.39	0.21	0.09	**0.43**	0.33
procyanidin B1	577	451-425-407	407-289	0.68	0.48	0.31	0.92	1.07	0.36	0.84	0.84	0.28	0.34	**0.61**	0.28	0.44	0.23	0.31	0.64	0.56	0.19	0.67	0.44	0.30	0.69	**0.45**	0.19
procyanidin dimer B	577	451-425-407	407-289	0.54	0.40	0.29	0.65	0.62	0.28	0.58	0.61	0.25	0.25	**0.45**	0.16	0.40	0.19	0.32	0.46	0.49	0.15	0.46	0.46	0.32	0.37	**0.36**	0.12
procyanidin trimer B	865	739-695-577-451-425		0.26	0.23	0.13	0.41	0.36	0.16	0.55	0.27	0.12	0.17	**0.27**	0.13	0.15	0.10	0.12	0.21	0.18	0.09	0.42	0.16	0.12	0.26	**0.18**	0.10
procyanidin tetramer B	1153	865-577		0.22	0.27	0.09	0.23	0.23	0.20	0.22	0.08	0.07	0.12	**0.17**	0.07	0.10	0.08	0.08	0.10	0.10	0.08	0.14	0.10	0.07	0.16	**0.10**	0.03
procyanidin pentamer B	1441	1151-865-577		0.15	0.14	0.09	0.28	0.26	0.12	0.26	0.16	0.09	0.12	**0.17**	0.07	0.09	0.08	0.08	0.13	0.11	0.08	0.16	0.10	0.08	0.19	**0.11**	0.04
procyanidin polimer*	1153*	865-577		0.12	0.13	0.08	0.14	0.14	0.10	0.14	0.11	0.07	0.09	**0.11**	0.02	0.08	0.08	0.07	0.08	0.09	0.07	0.11	0.08	0.07	0.11	**0.08**	0.01
procyanidin polymer*	1008*	865-577		0.20	0.20	0.14	0.39	0.12	0.17	0.19	0.30	0.08	0.14	**0.19**	0.09	0.09	0.07	0.06	0.09	0.11	0.08	0.17	0.11	0.07	0.12	**0.10**	0.03
Flavonols																											
rutin	609	301	271-255-179	0.90	1.93	0.42	1.85	0.45	1.23	0.51	1.47	0.22	0.21	**0.92**	0.63	0.01	0.00	0.01	0.02	0.01	0.03	0.03	0.01	0.01	0.01	**0.01**	0.01
quercetin-3-O-rhamnoside	447	301	271-255-179	0.05	0.15	0.02	0.14	0.05	0.07	0.04	0.07	0.02	0.00	**0.06**	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	0.00
quercetin-3-O-arabinoside	433	301	271-255-179	0.55	1.37	0.09	0.42	0.67	1.01	0.16	0.65	0.12	0.09	**0.51**	0.41	0.01	0.03	0.02	0.01	0.02	0.02	0.03	0.01	0.02	0.03	**0.02**	0.01
quercetin-3-O-xyloside	433	301	271-255-179	0.18	0.64	0.04	0.31	0.23	0.28	0.16	0.21	0.04	0.02	**0.21**	0.17	0.01	0.02	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	**0.01**	0.01
quercetin-3-O-galactoside	463	301	271-255-179	3.69	2.85	0.89	3.29	3.24	2.23	1.59	2.92	0.55	0.44	**2.17**	1.16	0.01	0.05	0.02	0.04	0.01	0.04	0.01	0.02	0.02	0.02	**0.02**	0.02
rhamnetin-3-O-glucoside	477	315-285-274		0.10	0.04	0.04	0.09	0.09	0.22	0.03	0.11	0.03	0.03	**0.08**	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	**0.00**	0.00
Dihydrochalcones																											
phloretin-2-O-glucoside	435	273	167-123	0.18	0.49	0.07	0.42	0.49	0.18	0.27	0.33	0.07	0.12	**0.26**	0.15	0.10	0.23	0.06	0.16	0.18	0.13	0.12	0.14	0.06	0.07	**0.13**	0.06
phloretin-2-O-xyloglucoside	567	273	167	0.03	0.32	0.13	0.37	0.37	0.15	0.42	0.47	0.11	0.21	**0.26**	0.14	0.11	0.17	0.09	0.17	0.15	0.08	0.21	0.16	0.08	0.15	**0.14**	0.04
Hydroxycinnamic acids																											
chlorogenic acid	353	191-179	173-171-127-85	0.18	3.06	0.16	2.91	1.35	2.18	0.17	0.07	0.02	0.38	**1.05**	1.17	0.66	3.29	0.51	4.80	2.54	1.97	0.97	0.89	0.28	0.53	**1.64**	1.49
caffeic acid derivative	387	341-179-161-143		0.02	0.03	0.02	0.10	0.11	0.02	0.06	0.01	0.01	0.04	**0.04**	0.04	0.09	0.05	0.09	0.29	0.26	0.05	0.23	0.08	0.07	0.16	**0.14**	0.09
Triterpenes																											
annurcoic acid	485	467.5-423.5-405.6-393.5		0.23	0.21	0.17	0.18	0.18	0.16	0.23	0.10	0.23	0.17	**0.19**	0.04	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd		
Total				**8.43**	**13.93**	**3.30**	**13.41**	**10.40**	**9.37**	**6.76**	**9.17**	**2.61**	**3.03**	**8.04**	**3.89**	**2.50**	**5.83**	**2.05**	**7.61**	**5.28**	**3.87**	**4.21**	**3.16**	**1.80**	**2.97**	**3.93**	1.85

Nd. not detected; * double charged ions $[M-2H]^{2-}$

Table 4

Table X. Volatile components in intact fruit, peel and pulp of mela rosa dei Monti Sibillini.

No	Component[a]	RI calc.[b]	RI lit.[c]	Intact fruit (%)[d]	Peel (%)[d]										Pulp (%)[d]									
					1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
1	methyl butanoate	719	721													0.3								
2	butyl methanoate	729	724		0.7																			
3	2-methyl-1-butanol	731	732			1.0	0.7	0.7	1.2	1.7	1.0	0.9	0.8	0.3	3.8	2.6	10.1	7.9	5.7	3.2	9.6	6.6	11.4	2.2
4	pentanol	762	762							0.2		0.2										1.3		
5	hexanal	800	801		3.1	2.3	4.0	3.1	7.0	3.2	4.1	3.4	3.8	6.8	22.4	16.6	21.2	10.6	22.8	4.1	21.5	15.8	19.6	20.1
6	(*E*)-2-hexenal	844	846		1.1	1.2	2.4	1.4	5.1	1.9	2.5	0.9	1.7	1.8	6.1	4.9	5.0	4.2	8.4	1.4	7.6	3.6	4.4	3.1
7	hexanol	863	863	2.2	0.6	1.1	0.9	0.5	1.3	1.8	0.9	1.1	0.7	1.6	5.9	5.2	7.7	7.3	8.3	4.1	8.3	8.2	7.4	11.7
8	2-methylbutyl acetate	876	875			0.1																		
9	propyl butanoate	902	898		0.1	0.4		0.1		0.6	tr[e]	0.1	tr			1.1	0.1	0.2		0.6			0.2	
10	heptanal	904	901						tr				tr											
11	butyl propanoate	913	910	0.1	0.1	tr		0.1			0.1	tr	0.1											
12	methyl hexanoate	933	930													0.1								
13	propyl 2-methyl butanoate	954	944		tr	0.1		tr		0.2	tr	tr	tr			0.3				0.1			0.1	
14	butyl isobutanoate	962	955		tr	tr	tr	tr		tr	tr	tr	tr			tr				tr				
15	isobutyl butanoate	964	955		tr	0.2	tr	tr	tr	0.3	tr	tr	tr			0.3				0.3				
16	heptanol	952	959	tr																				
17	2-methylbutyl propanoate	979	975		tr	tr		0.1			0.1	tr	tr											
18	1-octen-3-one	984	972				tr	tr	tr															
19	3-methylmercapto propanol	987	983																	tr		tr		0.1
20	octan-3-one	991	990					tr																
21	6-methyl-5-hepten-2-one	992	990	0.3	0.1	0.1	0.1	tr	0.2	0.2	0.1	tr	tr	0.2	0.1	0.1	0.1	0.1	0.1	tr	0.1		0.1	
22	6-methyl-5-hepten-2-ol	996	997	0.1	tr	0.1	0.1	0.1	0.1	0.3	0.1	0.1	tr	0.1	0.3	0.4	0.2	1.0	0.4	0.5	0.4	0.6	0.3	0.5
23	octan-3-ol	998	998														0.3					0.3		
24	butyl butanoate	998	998	1.0	3.0	2.4	1.1	1.7	1.0	4.2	2.5	2.4	1.9	0.5	0.5	2.5	0.5	2.9	0.7	4.2	1.1	0.8	1.2	0.2
25	ethyl hexanoate	1002	1002			0.2										0.7								
26	octanal	1004	998						tr								tr							
27	isobutyl 2-methyl butanoate	1005	1004							0.2										0.2				
28	hexyl acetate	1019	1018	tr		0.1		tr	tr	tr														
29	*p*-cymene	1023	1023													0.1		0.1						
30	limonene	1027	1024					tr								0.2								
31	isopropyl hexanoate	1044	1034			tr				tr						tr				tr				

32	butyl 2-methyl butanoate	1046	1044	0.3	0.8	1.3	0.3	1.1	0.2	2.1	1.0	0.9	1.6	tr	0.1	0.9	0.8	2.5	0.4	1.4	0.8	0.8	2.1	tr
33	2-(2-hydroxypropoxy)-1-propanol	1055	1046	tr																				
34	butyl pentanoate	1059	1063			tr																		
35	isopentyl butanoate	1063	1060	0.2	0.5	0.6	0.4	0.6	0.3	1.2	1.0	0.7	0.5	tr	tr	0.4	0.1	0.3	0.1	0.7	0.2	0.1	0.2	
36	*cis*-linalol oxide	1073	1067			tr			tr	tr				tr	tr	0.1		tr	0.1	tr		tr		tr
37	octanol	1076	1076	tr	tr	tr		tr	tr	tr	tr	tr	tr	tr	tr	tr	0.1	tr	0.1	tr	tr	tr	0.1	0.1
38	*trans*-linalool oxide	1088	1084						tr	tr									tr					
39	pentyl butanoate	1095	1089	0.4	0.8	1.0	0.3	0.8	0.3	1.1	0.5	1.0	0.7	0.1		0.3				0.5				
40	propyl hexanoate	1095	1096	0.5	0.8	1.0		0.8	0.3	1.1	0.5		0.7			0.6				0.2				
41	*n*-undecane	1099	1100	0.1																				
42	nonanal	1104	1100	0.6					0.2					0.1	0.3	0.4	0.4		0.5		0.7			0.3
43	2-methylbutyl 2-methyl butanoate	1104	1104		0.1	0.3	0.3	0.4		0.6	0.4	0.5	0.5					0.9		0.4		0.4	0.6	
44	hexyl propanoate	1107	1108	0.6	0.2	0.2	0.2	0.5	0.2	0.1	0.4	0.3	0.2	tr		tr								
45	*trans*-rose oxide	1110	1115													tr						tr		
46	benzeneethanol	1112	1111						tr					tr		tr	tr			tr	0.1	tr	tr	0.1
47	butyl tiglate	1136	1136		tr	tr	tr	tr		tr	tr	tr	tr				tr	tr		tr	tr	tr	tr	
48	pentyl 2-methyl butanoate	1139	1142	0.1	0.1	0.2	0.1	0.4	0.1	0.3	0.1	0.5	0.4	tr		0.1	tr	0.2	tr	0.2	tr	0.1	0.1	
49	hexyl isobutanoate	1149	1147	tr	tr	0.1	0.2	0.1	tr	0.1	0.1	0.1	0.1	tr		0.1				tr	tr	tr		
50	menthone	1150	1150														tr							
51	hexyl 2-methyl propaonate	1150	1150															tr						
52	isobutyl hexanoate	1152	1149	tr	0.1	0.2	0.1	0.1	tr	0.1	0.1	tr	0.1	tr		0.2				0.1				
53	2-methylbutyl pentanoate	1156	-		tr	tr	tr	0.1	tr	tr	tr	0.1	0.1											
54	(*E*)-2-nonenal	1158	1160		tr	tr	tr	tr	0.1	tr	tr		tr	tr	0.1	0.1	0.1	0.1	0.2	tr	0.1	tr	0.1	0.1
55	2,6,6-trimethyl-decane	1168	1165	tr																				
56	nonanol	1171	1165	0.1		tr		tr	tr				tr											
57	(*Z*)-5-hexenyl butanoate	1181	1183	0.1	0.1	tr	0.1	0.1	tr	0.1	0.1	0.1	0.1	0.1		0.1				0.1				
58	(*Z*)-3-hexenyl butanoate	1183	1185	tr		tr	tr		tr	0.1	tr	0.1		tr		tr				0.1				
59	butyl hexanoate + hexyl butanoate	1190		33.6	31.8	21.0	23.7	25.4	18.8	24.6	32.0	30.9	26.4	20.6	0.3	18.0	0.6	0.5	0.5	25.6	1.0	0.6	0.9	0.9
60	methyl chaavicol	1193	1193												tr		tr	0.1			tr	0.1		0.1
61	dodecane	1195	1200	0.4																				
62	decanal	1200	1201	0.5	tr	tr	tr	tr	0.1	tr	tr	tr	tr		0.3	0.2	0.3	0.2	0.3	0.1	0.7	0.2	0.5	0.5
63	*sec*-butyl 2-methyl hexanoate	1216	-									tr												
64	phenoxyethanol	1216	1221	1.3		tr				tr				tr			tr				tr	tr	tr	tr
65	(*Z*)-3-hexenyl isovalerate	1223	1223		tr	0.1	0.1	0.2	tr	0.1	0.1	0.2	0.1	tr		tr		tr		tr				

66	(*Z*)-3-hexenyl-α-methylbutanoate	1228	1229		tr	tr	tr	tr	tr	tr	tr	tr	tr							tr				
67	citronellol	1229	1229											tr										
68	hexyl 2-methylbutanoate	1233	1233	3.7	5.4	8.4	14.8	12.3	3.1	8.2	10.3	24.8	17.6	0.7	0.3	3.6	0.4	3.2	0.8	3.8	1.4	1.4	0.8	0.2
69	2-methylbutyl hexanoate	1248	1246	0.7	1.1	0.9	0.6	2.1	0.5	0.7	2.6	0.9	1.7	0.1		0.4				0.4	0.1		0.2	0.1
70	1,3-octanediol	1262	1275	0.2	1.6	0.9	2.0	1.1	3.5	2.1	2.3	2.0	1.0	3.4	10.6	5.3	11.1	9.5	10.3	2.6	13.9	12.2	10.9	14.2
71	nonanoic acid	1271	1271						0.1															
72	pentyl hexanoate	1283	1282	1.5	1.5	1.1	0.8	2.1	0.5	0.8	1.2	1.3	1.4	0.2		0.5				0.5				
73	butyl heptanoate	1283	1289	1.5	1.5	1.1		2.1	0.5	0.1			1.4											
74	propyl octanoate	1286	1290	0.1	0.1	0.1	tr	0.1	tr		0.1		0.1											
75	*n*-tridecane	1292	1300	0.2																				
76	undecanal	1300	1307	0.1									tr		0.1				0.1			0.1		0.1
77	theaspirane	1308	1305								tr	tr		tr										
78	hexyl tiglate	1323	1326	tr	tr	0.1	0.2	0.1		0.2	0.1	0.2	0.1	tr		0.2	tr	0.1		0.1	0.1			
79	heptyl 2-metyhylbutanoate	1326	1332		tr	tr	tr	tr			tr	tr	tr											
80	2-methylpropyl octanoate	1339	1343	tr	tr	tr	tr	tr	0.2		tr	tr	tr	tr										
81	3-methylbutyl heptanoate	1342	1334	0.1	0.1	tr	0.1	0.2	0.1		0.1	0.1	0.1											
82	gamma-nonalactone	1354	1348			tr			tr															
83	ethyl decanoate	1358	1373						0.1															
84	decanoic acid	1364	1364						0.1															
85	(*Z*)-5-hexenyl hexanoate	1369	-			0.1	0.1	0.3		0.1	0.1	0.1	0.1	tr		tr				tr				
86	(*Z*)-3- hexenyl hexanoate	1369	1375	0.1																				
87	3-octenoic acid. butyl ester	1372	1391			0.1																		
88	butyl octanoate	1379	1387														0.1	tr	0.1		0.1	0.1	0.1	0.1
89	hexyl hexanoate	1380	1382	9.6	10.4	5.8	6.8	10.7	7.9	3.8	10.9	5.3	8.2	4.9	0.1	2.9	0.3	0.1	0.2	3.0	0.4	0.2	0.4	0.3
90	*n*-tetradecane	1391	1400	0.1																				
91	dodecanal	1402	1408	0.1		tr	tr	tr	tr	tr	tr	tr	tr	tr		0.1	tr	0.1	0.1		0.1	tr	0.1	0.1
92	(*E*)-caryophyllene	1411	1417										tr											
93	β-copaene	1421	1430										tr											
94	butyl phenylacetate	1424	1433																					tr
95	6-methyl-4-heptenyl pentanoate	1424	1438									tr	tr											
96	octyl 2-methyl butanoate	1426	1430			tr		tr			tr	tr	tr											
97	2-phenyletyl butanoate	1432	1440							tr						tr				tr				
98	2-methylbutyl octanoate	1441	1444	0.3	0.4	0.1	0.2	0.6	0.2	0.1	0.8	0.2	0.3	tr		tr	0.1	tr	tr	tr	0.1	tr	0.1	tr
99	geranyl acetone	1444	1446	0.6	0.1	0.1	tr	tr	0.3	0.1	0.2		tr	0.1	0.1	tr	0.1	tr	0.1	tr	0.1	tr	0.1	0.1

100	(*E*)-β-farnesene	1448	1450		tr	tr	tr	tr		0.1	tr	tr	tr	0.1										
101	dodecanol	1449	1458						0.1															
102	germacrene D	1472	1475								tr	tr	0.2											
103	phenylethyl isovalerate	1479	1482													tr								
104	isopentyl phenylacetate	1484	1490													tr		tr	tr		0.1		0.1	
105	(*Z*,*E*)-α-farnesene	1490	1491	0.2	0.2	0.4	0.4	0.3	0.4	0.3	0.2	0.2	0.2	0.4	0.4	0.2	0.4	0.3	0.3	0.4	0.2	1.7	0.3	0.3
106	*n*-pentadecane	1493	1500	tr																				
107	(*E*,*E*)-α-farnesene	1509	1505	35.2	30.2	43.7	36.1	27.2	40.1	33.5	19.8	18.2	25.1	54.4	45.3	26.3	36.7	44.8	36.1	37.9	28.2	41.2	34.4	40.9
108	(Z)-γ-bisabolene	1520	1519				0.1			0.1			0.1	0.2										
109	sesquirosefuran	1544	1557		0.1	0.1	0.1	tr	0.1	0.1		0.1	tr	0.1	0.2	tr	0.1	0.4	0.1	0.1	0.1	0.6	0.4	0.2
110	dodecanoic acid	1552	1565						0.1															
111	(*Z*)-nerolidol	1554	1557		tr																			
112	butyl 9-decenoate	1559	-	0.2	0.1			0.1	0.2		tr													
113	dendrolasin	1563	1570		0.1		tr	tr	0.1	tr	0.1	tr	tr		tr				tr					
114	hexyl benzoate	1566	1579		tr				0.1		tr													
115	hexyl octanoate	1572	1581	0.4	0.4	0.1	0.2	0.3	0.5	0.1	0.3	0.1	0.2	0.1										
116	butyl decanoate	1577	1588		0.1			0.1																
117	β-copaen-4-a-ol	1577	1590				0.1							0.1	tr					tr				
118	tetradecanal	1597	1606						tr		tr	tr	tr											
119	phenetyl hexanoate	1631	1639			tr																		
120	2-methylbutyl decanoate	1638	1647		tr	tr	tr	tr		tr	tr	tr	tr	tr										
121	(*Z*)-methyl dihydrojasmonate	1640	1654	0.1					tr															
122	(*E*)-methyl dihydrojasmonate	1644	1682						tr															
123	octyl ether	1651	1657						0.1															
124	hexyl nonaoate	1673	1684		tr																			
125	2,3-dihydrofarnesol	1682	1688							tr	tr													
126	pentadecanal	1705	1706						tr															
127	hexadecylene oxide	1707	1708						tr															
128	(*E*,*Z*)-farnesol	1714	1714		0.4	0.2	tr	0.1	0.7	0.2	1.3	tr	0.1	0.5										
129	(*E*,*E*)-farnesal	1734	1740		tr	tr			0.1	tr	tr	tr	tr	tr										
130	tetradecanoic acid	1762	1762						0.4															
131	hexyl decanoate	1776	1783		tr				tr				tr											
132	butyl dodecanoate	1782	1786		tr				tr			tr	tr											
133	isopropopyl tetradecanoate	1823	1828	0.8																			0.1	

134	2-methylbutyl laurate	1842	1851								tr	tr									tr	
	Total identified (%)			97.8	97.8	98.3	97.7	98.1	96.7	97.2	98.2	98.0	98.5	97.7	97.3	96.5	96.9	97.6	96.7	97.2	97.0	97.0	97.1	96.6
	Grouped compounds (%)																							
	Aliphatics																							
	Esters			55.8	60.1	47.1	50.7	63.4	35.0	51.2	65.4	70.9	64.5	27.4	1.3	33.7	3.1	11.0	2.7	42.5	5.4	4.4	7.1	1.8
	Alcohols			2.6	2.2	3.1	3.7	2.4	6.3	6.1	4.3	4.1	2.5	5.4	20.6	13.4	29.4	25.8	24.8	10.5	32.3	29.3	30.1	28.8
	Aldehydes			1.2	4.3	3.5	6.4	4.5	12.6	5.2	6.6	4.4	5.6	8.7	29.2	22.3	27.1	15.0	32.4	5.7	30.6	19.7	24.7	24.3
	Ketons			0.3	0.1	0.1	0.1	0.1	0.2	0.2	0.1	0.1	tr	0.2	0.1	0.1	0.1	0.1	0.1	tr	0.1		0.1	
	Terpenoids			36.0	31.0	44.3	36.7	27.6	41.6	34.3	21.6	18.4	25.8	55.9	45.8	26.9	37.2	45.3	36.5	38.3	28.4	43.0	34.7	41.3
	Others			2.0	0.1	0.1	0.2	0.1	1.0	0.2	0.1	0.1	0.1	0.1	0.3	0.1	0.2	0.5	0.2	0.1	0.3	0.7	0.5	0.5

[a] Component are ordered according to their elution from a HP-5MS (30 m x 0.25 mm, 0.1 mm) column. [b] Arithmetic index calculated. [c] Retention index taken from Adams and NIST 17 libraries. [d] Relative abundance (%). [e] Traces, % < 0.1%.