

# Musk strawberries: the flavour of a formerly famous fruit reassessed<sup>†</sup>

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**ABSTRACT:** Fruits of wildy growing and naturally ripened musk strawberries (*Fragaria moschata*) from two highland sites were studied during two seasons. The fruits were characterized on site by two flavourists and a full profile of the volatile compounds was extracted immediately after collection using solid phase extraction and analysed by gas chromatography–mass spectrometry (GC-MS) and gas chromatography–olfactometry (GC-O). The aroma of whole fruits was characterized with dynamic headspace coupled to GC-MS. Single fruits evaluated on site were characterized by green, spicy, seedy and sweet exotic notes, while the fruit bunches showed a complex tropical smell. More than 100 distinctive volatile compounds were detected by GC-MS. Some of them are reported for the first time in the *Fragaria* species, most notably the abundant coniferyl alcohol. GC-O revealed that mesifuran, eugenol, methyl butyrate, furaneol and 3-mercaptohexyl acetate were the key components of the highland musk strawberry flavour. A comparison of the volatile pattern with the much more known woodland strawberry (*F. vesca*) from a nearby location contrasted the differences in the volatile composition of the musk strawberry. Repeated analysis of fruits harvested 2 years after the first vintage confirmed these data. Copyright © 2012 John Wiley & Sons, Ltd.

**Keywords:** *Fragaria moschata*; analysis; volatile; aroma

## Introduction

Strawberries belong to one of the most valued fruits all around the globe. While for consumers the aroma of strawberries is the most important quality indicator,<sup>[1]</sup> factors such as colour and size of the fruits, texture, shelf life and yield of the crops have been until recently in the centre of the breeding aims. Not surprisingly, certain criticism of consumers on sensory quality of commercial garden strawberries is pervasive.<sup>[2]</sup>

The most commonly planted variety of strawberry, *Fragaria x ananassa*, is a result of an accidental hybridization between *F. chiloensis* from South America and *F. virginiana* from North America.<sup>[3]</sup> From its parents the garden strawberry obtained several positive properties, such as large fruits (from the Chilean strawberry), red colour (from the Virginian strawberry) and a pleasant flavour from both. Nevertheless, its flavour is usually less appreciated when compared to the more fragrant wild strawberries.

The genus *Fragaria* (Rosaceae) comprises about 22 wild species,<sup>[4]</sup> while around 1000 cultivars are preserved in germplasm collections worldwide.<sup>[5]</sup> The most common of these, the woodland strawberry (*F. vesca*), is a diploid, and it is also the only species found in both Eurasia and America.<sup>[4]</sup>

The wild Musk strawberry (*F. moschata*) is the only hexaploid ( $2n=6x=42$ ) from all strawberry species. Until the boom of the garden strawberry in the 19th century, it was the musk strawberry, together with woodland strawberry, which was mostly planted.<sup>[6]</sup> The Musk strawberry is believed to be the first strawberry with a given cultivar name 'Gallobelgis des Chapirons'.<sup>[6,7]</sup> It was also the preferred strawberry variety of Jane Austen and F. D. Roosevelt, who encouraged strawberry breeders to experiment with musk varieties.<sup>[8]</sup>

The Musk strawberry is most likely a result of natural cross between the diploid species *F. vesca* and *F. viridis*, which sometimes co-occur.<sup>[4]</sup> It is native to highland areas from Great Britain and France through to Siberia. Its berries tend to remain uneven in colour as they ripen and its specific strong flavour gave this strawberry its name.

The flavour of musk strawberry was analysed for the first time by Drawert *et al.*<sup>[9]</sup> The musk strawberry, together with other wild *Fragaria* species, was found to contain more volatile components than the garden strawberry. In a recent study, Ulrich *et al.*<sup>[10]</sup> confirmed these findings, with the wild growing musk strawberry 'Cotta' containing the largest number of volatiles of all tested species. Two clones of musk strawberry ('Capron Royal' and 'Profumata di Tortona') were analysed, among others, by Urruty *et al.*,<sup>[11]</sup> finding that the *F. moschata* clones were again among the most fragrant strawberry types of all.

In this work, we strived to characterize the volatile composition of musk strawberries from its most characteristic location (highlands) immediately after harvesting of the naturally ripened fruits. For doing this, we used various isolation and separation techniques to obtain a full profile of *F. moschata* volatiles. Moreover, an established gas chromatography–olfactometry (GC-O) method<sup>[12,13]</sup> was used to compile a list of its key aroma compounds.

## Materials and Methods

### Botanic Material and Sensory Characterization

The fruits of *F. moschata* were collected in highlands (650–800 m above sea level) near the villages Budiná and Kremnické Bane in

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Slovakia in two vintages/harvests (2009 and 2011). In 2011, in the Budiná location, we found the fruits of *F. vesca* too. Fruits were picked in all cases at full ripeness by the end of July. Berries were left on the stems until solid phase extraction (SPE). The physiology of the botanical material matched the description of *Fragaria moschata* and *Fragaria vesca* given by Darrow;<sup>[6]</sup> the identity was additionally confirmed in the Research Institute of Fruit and Decorative Trees (Bojnice, Slovakia).

A sensory characterization (smelling and tasting) was performed on site by two experienced flavourists first on single fruits and then on the headspace of a bunch of collected strawberries.

### Chemicals

All chemicals were of GR for Analysis (<http://www.surechem.co.uk/pdf/chemicalCompweb14509.pdf>) grade, purchased from Merck (Darmstadt, Germany). Double-distilled water was used for all necessary tasks.

### Extraction of Volatiles

Volatile organic compounds were collected using SPE, which is especially suitable for quantification of major strawberry flavour constituents: furaneol (4-hydroxy-2,5-dimethyl-3(2*H*)-furanone) and mesifuran (4-methoxy-2,5-dimethyl-3(2*H*)-furanone).<sup>[14,15]</sup> Twenty grams of berries free of sepals were gently crushed and mixed with 40 ml of water. The slurry was immediately filtered through a pre-washed Whatman paper and 30 ml of the filtrate was passed at 5 ml/min through a cartridge packed with 200 mg of Lichrolut EN resins (Merck, Darmstadt, Germany). The sorbents were preconditioned with 4 ml of dichloromethane, 4 ml of methanol and 4 ml of water. Two replicate extractions were made from each strawberry specie. The extraction was performed on a 12-place SPE station (Biotage, Uppsala, Sweden) equipped with a vacuum pump Laboport N820 FT.18 (KNF Neuberger, Freiburg, Germany). After sample loading, the charged sorbents were washed with 10 ml of water and were further dried by using a weak vacuum for 30 min. LiChrolut EN is very efficient at trapping headspace volatiles too,<sup>[16]</sup> hence, two additional LiChrolut cartridges were put on the top of the main cartridge to prevent contamination from the ambient. Dry cartridges were placed into a tight glass vessel, free of odour and the next day transported to the laboratory, where they were extracted with 1.5 ml of dichloromethane and analysed. Representativeness of the extract was confirmed using paper blotters.

### Gas Chromatography–Mass Spectrometry

An Agilent 7890 (Agilent Technologies, Palo Alto, CA, USA) gas chromatograph equipped with a non-polar fused silica column J&W HP-5ms (30 m × 0.25 mm × 0.25 μm) was used. Three microlitres of each extract were injected into the CIS 4 injector (Gerstel, Mühlheim/Ruhr, Germany) equipped with a glass baffled liner in two replicate measurements. The instrument was operated in split mode (CIS initial temperature: 20°C (equilibration time 0.20 min, initial time 0.10 min), 12.0°C/s, 260°C (hold time 10.00 min); purge flow to split vent: 30 ml/min at 0.02 min, vent flow 20 ml/min, vent pressure 68.95 kPa until 1 min; total flow: 34 ml/min. The following temperature programme was used: 35°C (3 min), 10°C/min, 280°C (3 min). Linear retention indices<sup>[17]</sup> were calculated using a standard mixture of *n*-alkanes C<sub>6</sub>–C<sub>26</sub>.

Identification was carried out using a triple-axis quadrupole mass spectrometer (Agilent 5975C). The transfer line was kept at 280°C and mass spectra were acquired in a 'gain factor' Electromagnetic Voltage (EMV) mode in scan mode from 35 to 350 *m/z*. The mass spectrometer (MS) source was held at 230°C and the MS quadrupole temperature was set at 150°C. The measured mass spectra were identified by using the available mass spectral libraries (Wiley 9, NIST 2005, custom libraries).

Volatiles were quantified by preparing synthetic solutions containing known amounts of available odorants (labelled with a superscript 'a' in Table 1) and analysing them the same way as the extracts. Calibration plots were made, and the areas obtained in the analysis of the samples were interpolated in the corresponding calibration graphs to estimate the concentration of volatile organic compounds present in the extract. Concentrations of non-available compounds (labelled with superscript 'b' and 'c' in Table 1) were estimated by utilizing models developed using compounds with similar base and/or molecular ions and/or close retention time (Table 1).

### Dynamic Headspace Gas Chromatography–Mass Spectrometry

A dynamic headspace (DHS) analysis was run on the same machine using the Gerstel's multipurpose sampler MPS II XL and the DHS module. Four whole berries were placed into a 20 ml headspace vial. The DHS conditions were as follows. The fruits were conditioned for 5 min at 37°C. The DHS liners filled with Tenax resin (both from Gerstel) were used. The purge volume was 300 ml with a purge flow of 50 ml/min and 37°C trap temperature. The drying volume was 150 ml and was delivered at 10 ml/min and same trap temperature. The transfer heater temperature was set to 150°C.

The initial temperature of the twister desorption unit (TDU) was set to 40°C with a delay time of 0.5 min and zero initial time. A single ramp was used with a rate of 300°C/min up to at 280°C, where it was held for 4 min.

The CIS 4/TDU injector (Gerstel) was operated in a solvent vent mode [CIS initial temperature: –100°C (0.1 min), 12.0°C/s, 300°C (5 min); purge flow to split vent: 25 ml/min at 0.01 min, vent flow 40 ml/min, vent pressure 40 kPa until 0.01 min].

The same column and the same oven program as with the SPE extracts were used for the separation of the trapped volatiles.

### Gas Chromatography–Olfactometry

A panel of six judges, three women and three men (age 30–45 years) from the laboratory staff at the TU Graz, carried out the sniffings of the SPE extract. All panellists had extensive experience with GC-O and showed no signs of specific anosmia. The sniffing sessions did not exceed 30 min. All panellists used a seven-point anchored scale with seven levels of intensity: (zero); 1 = weak, hardly recognizable odour; 2 = clear but not much intense odour; 3 = extremely strong odour; for intensity evaluation of the eluting odour.<sup>[13,18]</sup> Intermediate values (e.g. 1.5) did not bear description. A HP 5890 Series II gas chromatograph (Hewlett Packard, Palo Alto, CA, USA) equipped with a polar fused silica column ZB Wax Plus (30 m × 0.32 mm × 0.25 μm film thickness) was used. One microlitre of the extract was injected in splitless mode into a split/splitless injector held at 220°C. The compounds were separated using the following oven program: 35°C (1 min), 10°C/min, 260°C (5 min). Eluting compounds were split 1:1 with

**Table 1.** Quantitative results from solid phase extraction (SPE) and semi-quantitative results from the dynamic headspace (DHS) of *Fragaria moschata* berries and compared to the quantitative data of *Fragaria vesca* berries

RI, DB-5	RI, DB-Wax	Compound	<i>F. moschata</i> (SPE, mg/kg)	<i>F. moschata</i> (DHS)	<i>F. vesca</i> (SPE, mg/kg)
605		Methyl acetate <sup>a</sup>	—	++	—
639		Ethyl acetate <sup>a</sup>	2.77	+++	1.20
649		Methyl propionate <sup>a</sup>	—	+	—
687		2-Pentanone <sup>a</sup>	0.20	—	<b>3.25</b>
695		3-Pentanone <sup>b</sup>	—	—	<b>0.04</b>
714		Ethyl propionate <sup>a</sup>	<b>0.03</b>	++	—
720		Propyl acetate <sup>a</sup>	—	+	—
724	953	Methyl butyrate <sup>a</sup>	<b>0.1</b>	++	—
762		Ethyl isobutyrate <sup>a</sup>	—	+	—
765	1103	Methyl ( <i>E</i> )-2-butenolate <sup>b</sup>	—	+	—
776		Isobutyl acetate <sup>a</sup>	—	+	—
779	979	Methyl 2-methylbutyrate <sup>a</sup>	<b>1.11–3.98</b>	++	0.16
799		Butyric acid <sup>a</sup>	<b>0.1–0.69</b>	+	—
801	1070	Hexanal <sup>a</sup>	0.46	—	1.21
804	1010	Ethyl butyrate <sup>a</sup>	0.44	++	0.63
814		Butyl acetate <sup>a</sup>	0.12	—	0.66
845	1673	2-Methylbutyric acid <sup>a</sup>	<b>0.73–2.65</b>	—	—
848	1164	Ethyl ( <i>E</i> )-2-butenolate <sup>a</sup>	—	++	—
853		Ethyl 2-methylbutyrate <sup>a</sup>	<b>0.01</b>	++	—
856		Ethyl isovalerate <sup>a</sup>	—	+	—
878		Isoamyl acetate <sup>a</sup>	—	++	—
856	1246	<i>trans</i> -2-Hexenal <sup>a</sup>	3.72–7.68	—	6.18
861	1497	Methyl 3-hydroxybutyrate <sup>b</sup>	<b>0.08</b>	—	—
880	1388	Methyl 3-hydroxy-3-methylbutyrate <sup>b</sup>	<b>0.01</b>	—	—
890		2-Heptanone <sup>a</sup>	—	—	<b>1.04</b>
925		Methyl hexanoate <sup>a</sup>	—	+	—
930	1582	Methyl 3-hydroxy-2-methylbutyrate <sup>b</sup>	<b>0.03</b>	—	—
938		$\alpha$ -Pinene <sup>a</sup>	0.13	—	0.16
938		<i>cis</i> - $\beta$ -Ocimene <sup>a</sup>	<b>0.2</b>	—	—
940		Ethyl 3-hydroxybutyrate <sup>a</sup>	<b>0.05</b>	—	—
955		Camphene <sup>a</sup>	<b>0.01</b>	—	—
968	1545	Benzaldehyde <sup>a</sup>	<b>0.09</b>	+	—
983		Hexanoic acid <sup>a</sup>	0–0.02	—	0.1
997		Myrcene <sup>a</sup>	—	+	—
1000	1508	Methyl 2-hydroxy-3-methylpentanoate <sup>b</sup>	<b>0.56–1.35</b>	+	—
1004		Ethyl hexanoate <sup>a</sup>	0.01	++	0.09
1013	1325	<i>cis</i> -3-Hexenyl acetate <sup>a</sup>	0.14	+	0.12
1018		Methyl 3-hydroxy-2-methylpentanoate <sup>a</sup>	<b>0.02</b>	—	—
1019	1280	Hexyl acetate <sup>a</sup>	0.05–0.14	+	0.25
1022	1343	<i>trans</i> -2-Hexenyl acetate <sup>a</sup>	0.66	—	0.21
1032		<i>p</i> -Cymene <sup>a</sup>	<b>0.03</b>	+	—
1036	1205	Limonene <sup>a</sup>	<b>0.03</b>	+	—
1039		1,8-Cineole <sup>a</sup>	<b>0.06</b>	—	—
1046	1550	Methyl 3-acetoxybutyrate <sup>b</sup>	<b>0.03</b>	—	—
1051	1650	Phenyl acetaldehyde <sup>a</sup>	0.04	—	0.04
1061	2046	Furaneol <sup>a</sup>	0.81	—	0.31
1065	1610	Mesifurane <sup>a</sup>	39.72	++	8.51
1074		Acetophenone <sup>a</sup>	—	+	—
1080		3-Hydroxy-2,6-dimethyl-4 <i>H</i> -pyran-4-one <sup>b</sup>	<b>0.01</b>	—	—
1088		<i>o</i> -Tolualdehyde <sup>a</sup>	<b>0.02</b>	—	—
1092		2-Nonanone <sup>a</sup>	—	—	<b>0.8</b>
1100	1835	$\delta$ -Hexalactone <sup>a</sup>	<b>0.05</b>	—	—
1106		Nonanal <sup>a</sup>	0.05	—	0.21
1115		Ethyl 3-acetoxybutyrate <sup>b</sup>	—	+	—
1133		$\alpha$ -Campholenic aldehyde <sup>b</sup>	<b>0.01</b>	—	—
1156		Octanoic acid <sup>a</sup>	—	—	<b>0.04</b>

Table 1. (Continued)

RI, DB-5	RI, DB-Wax	Compound	<i>F. moschata</i> (SPE, mg/kg)	<i>F. moschata</i> (DHS)	<i>F. vesca</i> (SPE, mg/kg)
1170		Benzyl acetate <sup>a</sup>	—	+	<b>0.12</b>
1204		Oestrageole <sup>a</sup>	<b>0.03</b>	++	—
1208		Decanal <sup>a</sup>	<b>0.04</b>	—	—
1221	1634	Unknown ( $\gamma$ -butanolactone analogue)	<b>0.08</b>	—	—
1244	1700	Unknown (furanol analogue)	<b>0.01</b>	—	—
1254		Nonanoic acid <sup>a</sup>	<b>0.16</b>	—	—
1257	2405	Chavicol <sup>b</sup>	<b>0.35–0.87</b>	+	—
1277		Phenethyl acetate <sup>a</sup>	—	+	—
1270		Unknown	<b>0.18</b>	—	—
1286		Sulfurol <sup>a</sup>	<b>0.08</b>	—	—
1286		Unknown ester	<b>0.02</b>	—	—
1291		Cinnamaldehyde <sup>a</sup>	<b>0.03</b>	—	—
1295		Thymol <sup>a</sup>	<b>0.02</b>	—	—
1308		Anethol <sup>a</sup>	—	+	—
1308		Safrole <sup>a</sup>	—	—	<b>0.07</b>
1317		Isoprenyl acetate <sup>b</sup>	—	+	—
1321		Unknown	<b>0.04</b>	—	—
1328		Unknown	<b>0.05</b>	—	—
1337		Myrtenyl acetate <sup>a</sup>	—	—	<b>0.4</b>
1362		Methyl anthranilate <sup>a</sup>	—	—	<b>1.95</b>
1366	2192	Eugenol <sup>a</sup>	<b>1.39</b>	+	0.11
1382		Unknown	<b>0.08</b>	—	—
1387	1629	Elemene (unknown string) <sup>c</sup>	<b>0.02</b>	++	—
1408	2020	Methyl eugenol <sup>a</sup>	<b>3.84</b>	++	—
1413		Vanillin <sup>a</sup>	<b>0.96</b>	—	0.05
1449		Valencene <sup>a</sup>	<b>0.60</b>	—	—
1450	2010	3-[2-(Acetoxy)ethyl]-dihydro-2(3H)- furanone <sup>b</sup>	<b>0.01</b>	—	—
1453		Unknown	<b>0.01</b>	—	—
1456		Cinnamyl acetate <sup>a</sup>	—	—	<b>0.04</b>
1464		Unknown	<b>0.02</b>	—	—
1477		$\gamma$ -Muurole (unknown string) <sup>c</sup>	<b>0.09</b>	++	—
1479		$\gamma$ -Decalactone <sup>a</sup>	0.04	—	0.01
1503	2202	<i>trans</i> -Methylisoeugenol <sup>a</sup>	<b>0.40</b>	+	—
1508		Veratryl alcohol <sup>a</sup>	<b>0.03</b>	—	—
1510		$\delta$ -Decalactone <sup>a</sup>	<b>0.05</b>	—	—
1512		1-Methylbutyl phenyl acetate	0.10	—	0.09
1578		Unknown	<b>0.03</b>	—	—
1610		Unknown	<b>0.02</b>	—	—
1620		Unknown	<b>0.01</b>	—	—
1628		Unknown (3-hydroxy- $\beta$ -damascone-like)	<b>0.01</b>	—	—
1632		Unknown	<b>0.01</b>	—	—
1640		<i>p</i> -Coumaryl alcohol <sup>c</sup>	<b>2.05</b>	—	0.11
1647		Benzophenone <sup>a</sup>	0.01	—	<b>0.12</b>
1661		Vanillyl ethyl ether <sup>a</sup>	<b>0.05</b>	—	—
1672		3-Hydroxy-7,8-dihydro- $\beta$ -ionol <sup>b</sup>	<b>0.04</b>	—	—
1681		Unknown (probably chavicol-like)	<b>0.06</b>	—	—
1684		Unknown (probably $\beta$ -ionone derivative)	<b>0.22</b>	—	—
1691		3-[(Z)-5-[(E)-but-2-enylidene]-4,4-dimethyl- tetrahydrofuran-2-yl]propanal <sup>b</sup>	<b>0.73–4.82</b>	—	—
1695		3-(3,4-Dimethoxyphenyl)-1-propanol <sup>b</sup>	<b>0.01</b>	—	—
1699		$\alpha$ -Bisabolol <sup>c</sup>	<b>0.01</b>	—	—
1721		Indole-3-acetaldehyde <sup>b</sup>	<b>0.06</b>	—	—
1751	2120	Coniferyl alcohol <sup>a</sup>	4.1–10.05	—	4.87
1759		Myristic acid <sup>b</sup>	<b>0.27</b>	—	—
1775		Unknown	<b>0.18–1.06</b>	—	—
1783		Unknown (5-allyl guajacol - like)	<b>0.2</b>	—	—

**Table 1.** (Continued)

RI, DB-5	RI, DB-Wax	Compound	<i>F. moschata</i> (SPE, mg/kg)	<i>F. moschata</i> (DHS)	<i>F. vesca</i> (SPE, mg/kg)
1790		Unknown	<b>0.3</b>	—	—
1806		Blumenol A <sup>b</sup>	<b>1.16–3.24</b>	—	—
1840		Benzyl phenylacetate	—	—	<b>0.01</b>
1883		3,4-Dimethoxycinnamyl acetate <sup>b</sup>	<b>2.86</b>	—	—
1923		Unknown	<b>0.09</b>	—	—
1960		Palmitic acid <sup>b</sup>	<b>0.25</b>	—	—
1969		Unknown (probably tricyclic)	<b>0.03</b>	—	—
1994		Hexadecanol <sup>c</sup>	<b>0.07</b>	—	—
2128		Linoleic acid <sup>b</sup>	<b>0.02</b>	—	—
2151		Stearic acid <sup>b</sup>	<b>0.15</b>	—	—
2182		Stearyl alcohol <sup>b</sup>	<b>0.05</b>	—	—

Quantitative data in bold type are at least 10 times higher than in the other strawberry type. The quantitative data for *F. moschata* are mean values of two harvests. In all samples, two replicate extractions were made and all the data were averaged. In the case of deviation greater than 50% a range of concentrations is given. The quantitative data from *F. vesca* are mean values of two replicate extractions from one harvest only.

<sup>a</sup>Identification based on coincidence of mass spectra and retention index of pure compounds.

<sup>b</sup>Tentative identification based on coincidence of mass spectra and retention index from retention index database.

<sup>c</sup>Tentative identification based on coincidence of mass spectra only.

+++ = higher than 5%, ++ = between 5 and 0.5%, + = lower than 0.5% of the TIC peak area.

a fixed splitter at the end of the column between the FID detector (260°C) and the olfactory detector port ODO-3 (Gerstel). Hydrogen as carrier gas was held at a constant pressure of 106 kPa.

Similarly, 1 µl of extract was injected into a HP 5890 Series II GC with a mass spectrometer detector (Hewlett Packard) to help identify the odorants perceived during GC-O. The injector was operated in splitless mode at 220°C and the volatiles were separated on a similar fused silica column (J&W DB-Wax with dimensions 30 m × 0.25 mm × 0.5 µm) and operated by the following temperature programme: 35°C (1 min), 5.6°C/min, 240°C (5 min). Carrier gas (helium) was held at a constant pressure of 81 kPa. Retention indices were calculated after injection of a series of *n*-alkanes (C<sub>6</sub>–C<sub>26</sub>).

To represent the response of the complete panel to a certain odour the 'adjusted frequencies' were used (the Dravnieks' 'percent applicability'<sup>[13,18]</sup>). This concept represents both average intensity and frequency of citation of the odour. It is calculated as:

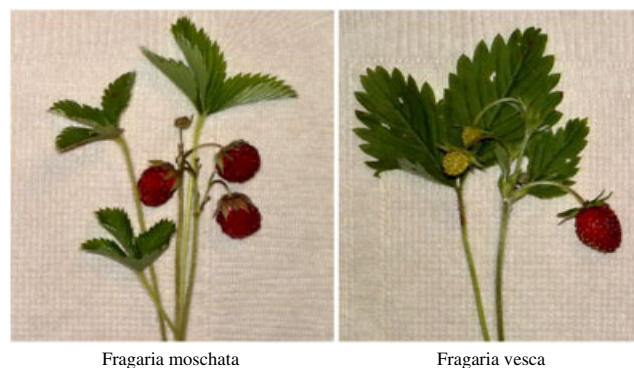
$$\text{adjusted frequency} = \sqrt{F \times I}$$

where *F* is the detection frequency of an aromatic attribute expressed as percentage, and *I* is the average intensity expressed as percentage of the panel maximum intensity. The identification of the odorants was carried out by a comparison of the recorded odours and their linear retention indices with those of pure reference compounds or available libraries.<sup>[19]</sup>

## Results

### Sensory Evaluation of Whole Fruits

Most of the berries were around 0.5–1 cm in size, hanging on 5–10 cm long stems. In contrast to woodland strawberries, fruits of *F. moschata* have roundish shape and mostly dark red to brown–pinkish colour with lighter (almost white) pink parts. The leafy crown of *F. moschata* is oriented towards the tip, in contrast to *F. vesca* (see Figure 1). Freshly picked berries had



**Figure 1.** Comparison of musk strawberry (*Fragaria moschata*) and woodland strawberry (*Fragaria vesca*) fruits

pronounced sweet-caramel and tropical orthonasal notes. The flavour in the mouth was balanced, sour–sweet, little astringent with green, caramel, seedy and clove-like retronasal notes. When fruits are put together, they create a very intense almost mango-like, tropical smell. No clear differences in flavour profile were found between the two harvests.

### Solid Phase Extract of Mashed Musk Strawberries

Almost 100 peaks were separated in the musk strawberry extract (Table 1). The most abundant volatiles of the SPE extract were mesifuran and coniferyl alcohol [(*E*)-4-(3-hydroxyprop-1-en-1-yl)-2-methoxyphenol], with the latter being reported for the first time in a strawberry extract.<sup>[20]</sup> We observed also a very high content of such strongly smelling chemicals as methyl eugenol (1,2-dimethoxy-4-prop-2-enylbenzene) and chavicol (4-prop-2-enylphenol) also reported for the first time in strawberries. Chavicol was, however, identified just tentatively (coincidence of

mass spectra and retention index from literature), as no reference standard is commercially available. It was not possible to identify 27 clearly resolved compounds (deconvolution purity by the AMDIS software higher than 70%), many of them seeming to share similar structures to ionones and furanones.

The remaining compounds are strikingly dominated by methyl esters, most notably those of various hydroxymethylbutanoic and -pentanoic acids (Table 1).

### Dynamic Headspace of Whole Musk Strawberries

Dynamic headspace is a very powerful technique to trap volatiles from the air surrounding the tested material. Due to the difficulty of relating DHS peak areas to real concentrations, we therefore present just semi-quantitative data in this work (Table 1).

This technique allowed the identification of additional esters besides those found with the SPE method (Table 1). Furthermore, the ratio of furaneol to mesifuran in the DHS profile was similar to that observed in the SPE extract. The possible conversion of furaneol to mesifuran during the process of extraction<sup>[21]</sup> therefore most likely did not occur.

### Gas Chromatography–Olfactometry of Musk Strawberry Solid Phase Extracts

Twenty-nine odours with adjusted frequencies higher than 0.25 (1 being the maximum) have been detected in the SPE extract

(Table 2). The highest adjusted frequencies (limit arbitrarily set to >0.5) were found for mesifuran (0.82), eugenol (2-methoxy-4-prop-2-enylphenol; 0.79) and methyl butyrate (0.75). Other prominent zones were those of furaneol (0.65) and 3-mercaptohexyl acetate (0.52). It is interesting to see that many of the odour-active volatile organic compounds from Table 2 were not detected by GC-MS (the most obvious case is 3-mercaptohexyl acetate (i.e. 3-sulfanylhhexyl acetate), described as 'tropical' in the GC-O).

We performed reconstitution of the musk strawberry flavours based on the quantification results using available chemicals (Table 1). The resulting flavouring was similar to that of fresh fruit, but it lacked the above-mentioned 'exotic' character of fresh berries. This character was successfully mimicked by adding a certain portion of 3-mercaptohexyl acetate and 1-octene-3-one to the flavour preparation.

### Musk Strawberry Versus Woodland Strawberry

During the 2011 harvest it was possible to gather some fruits of woodland strawberry too. The comparison of the SPE extracts can be found in Table 1. We were able to confirm previous findings<sup>[10]</sup> that musk strawberries contain more volatiles compared with woodland strawberries. In both cases, however, the most abundant volatile compound was mesifuran, and the second most abundant volatile compound was coniferyl alcohol.

**Table 2.** The results of GC-olfactometric measurements of musk strawberry extract, expressed as adjusted frequencies of panel responses (AF) sorted by decreasing AF values

RI, DB-Wax	Identification	Descriptor	AF
1610	Mesifuran <sup>a</sup>	Caramelic, strawberry, burnt	0.82
2186	Eugenol <sup>a</sup>	Clove, sweet, floral, rose, fatty, smoky, fish	0.79
<1100	Methyl butyrate <sup>a</sup>	Fruity, chewing-gum, sweet, ester	0.75
2046	Furaneol <sup>a</sup>	Chocolate, caramelic, fruity, burnt, Maltol	0.67
1735	3-Mercaptohexyl acetate <sup>b</sup>	Exotic, mango, green, fatty	0.52
1673	2-Methylbutyric acid <sup>a</sup>	Cheese, sweat, vanilla, sweet	0.49
1508	Methyl 2-hydroxy-3-methylpentanoate <sup>a</sup>	Fruity, ester, caramelic	0.47
1317	1-Octen-3-one <sup>b</sup>	Mushroom	0.46
2492	Unknown	Chocolate, cocoa, sweet, burnt	0.45
2010	3-[2-(Acetoxy)ethyl]-dihydro-2(3H)-furanone <sup>d</sup>	Caramelic, burnt	0.43
1475	1-Octen-3-ol <sup>a</sup>	Floral, plastic	0.41
1835	$\delta$ -Hexalactone <sup>a</sup>	Broth, sweet, fatty	0.41
2405	Chavicol <sup>d</sup>	Flowery, perfume, warm	0.39
<1100	Methyl 2-methylbutanoate <sup>a</sup>	Fruity, sweet, acetic	0.38
1719	$\gamma$ -Hexalactone <sup>a</sup>	Fruity, burnt, sweaty, green	0.38
1849	$\beta$ -Damascenone <sup>b</sup>	Fruity, dried plum	0.37
2020	Methyl eugenol <sup>a</sup>	Strawberry, exotic, green	0.37
2277	Methyl anthranilate <sup>c</sup>	Plastic, medicinal, smoky	0.35
<1100	Ethyl butanoate <sup>a</sup>	Sweet, fruity	0.32
1246	<i>trans</i> -2-Hexenal <sup>a</sup>	Green, apple, aldehydic	0.32
1556	Unknown	Woody, spicy, chemical	0.31
1650	Phenylacetaldehyde <sup>a</sup>	Phenolic, beer, warm	0.31
1634	Unknown ( $\gamma$ -butyrolactone analogue)	Vanilla, sweet	0.29
1919	Ethyl dihydrocinnamate <sup>c</sup>	Fruity, sweet, perfume-like, medicinal	0.29
1872	Guaiacol <sup>b</sup>	Phenolic, medicinal, rancid, green, fatty	0.27

<sup>a</sup>Identification based on coincidence of mass spectra, retention index of pure compounds and their odour.

<sup>b</sup>Identification based on coincidence of odour and retention index of pure compound (tentative).

<sup>c</sup>Identification based on coincidence of odour and retention index from retention index databases (tentative).

<sup>d</sup>Identification based on coincidence of mass spectra and retention index from retention index databases (tentative).

Nevertheless, only musk strawberries contain chavicol, sulfurol [2-(4-methyl-1,3-thiazol-5-yl)ethanol], methyl eugenol, methyl isoeugenol and some other 'heavy' compounds. In contrast, only woodland strawberries contained a series of 2-alkenones (from C<sub>5</sub> to C<sub>9</sub>), methyl anthranilate, myrtenyl acetate, safrole (5-allyl-1,3-benzodioxole) and benzophenone. It is also interesting to see that the two strawberry species showed a completely different acid pattern (Table 1).

## Discussion

Similar to the work by Ulrich *et al.*<sup>[10]</sup> we found that mesifuran is the most abundant volatile organic compound in the musk strawberry and, similarly, our GC-O results also found eugenol among the most important volatile organic compounds.

However, the ranking of the most important components does not match very well. One possible cause for the mismatch could be linked to the simple frequency method used by Ulrich *et al.*,<sup>[10]</sup> which fails at discriminating the most intense odorants. In addition, the absolute geographical altitude might play its role in the observed differences between the results obtained in both studies; the difference in elevation was about 600 m. We furthermore suspect that the extraction with Freon was not sufficient to extract the powerful aroma compounds such as 3-mercaptohexyl acetate and/or 1-octene-3-one and therefore these compounds did not show up in their GC-O profile of the musk strawberry 'Cotta'.

The unique flavour of strawberries is usually attributed to the balance of fruity character from methyl and ethyl esters, caramel notes from Maillard compounds and 'green' notes from C<sub>6</sub> compounds. The wild species are usually distinguishable with the presence of methyl anthranilate and methyl cinnamate.<sup>[10,22–24]</sup>

The C<sub>6</sub> compounds are dominant in unripe fruits and during ripening their content decreases while the content of esters, furanones, lactones and acids increases.<sup>[25]</sup> At full ripeness, mesifuran and furaneol dominate the profile of volatile compounds in strawberries. From the published literature it is not possible to conclusively say which furanone is present in higher concentrations in garden strawberry, and which one in woodland strawberry.<sup>[10,22,23,25,26]</sup> Apparently, there are so many cultivars available that it is quite problematic to be decisive on this issue. Nevertheless, it seems that furaneol is more abundant in garden strawberries and mesifuran in wild strawberries. This finding was also confirmed in our study for both strawberry types.

The musk strawberry bears two different names in German: *Moschuserdbeere*, which would be the equivalent to musk strawberry; and *Zimterdbeere*, which suggests that this strawberry is characterized by a cinnamon smell. This is most likely attributable to the high content and olfactory impact of eugenol, methyl eugenol and methyl isoeugenol.

The term 'musk' is used in many biological species, indicating strong smell.<sup>[27]</sup> However, no true macrocyclic ketones (typical examples of musk odours) were found in this or any other study in strawberry species. We think that the exotic-mango character could be a better candidate for the '*éminence gris*' behind the name of this fragrant strawberry. The structure of mango flavour is usually attributed to the combination of green/piney notes (myrcene, ocimene and/or diphenylether), caramel-like notes (maltol, furaneol), milky notes ( $\delta$ -lactones) and exotic notes (sulfur compounds). We could find all of these compounds in the musk strawberry extract: caramel-like notes of the furanones, milky notes of  $\delta$ -lactones and sulfurol and green, exotic

notes of 3-mercaptohexyl acetate. The clove/cinnamon notes, however, most likely make the difference compared to mango.

Additionally, the number of methyl and ethyl esters found in this study (23) was astonishing. Together they constituted 7.6% of the SPE extract.

Unfortunately, a high number of compounds remained unidentified. Even if none of them was found to be odour active, it would be still interesting to identify at least some of them in the future.

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