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New Biodegradable Mulching Films for Strawberry (*Fragaria* × *Ananassa* Duch.): Effects on the Volatile Profiles of the Fruit

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Abstract: The effects of mulching films on the profiles of volatile organic compounds (VOCs) from two strawberry cultivars, namely Elide and Sabrina, were evaluated by headspace solid-phase micro-extraction coupled to gas chromatography mass spectrometry (HS-SPME/GC-MS). Strawberries were grown on two biodegradable mulching films, named N5 and N18, in comparison to black polyethylene (PE). PCA models, separately performed on the VOCs dataset of the two cultivars, showed that the observations for each of the three mulching films lie in corresponding regions of the PCA plot, suggesting that the mulching films represented the main source of data variation, and hence, the variability of the VOCs profile induced by the mulching films overcame the cv-related one. For both the cultivars, a higher number of VOCs with a positive impact on the "sweet" taste sensation and consumers' preference were positively correlated with strawberries produced on the biodegradable films compared to PE. However, there was an interaction between the type of cultivar and the mulches, as Elide responded better to N18 and N5 mulching films, while Sabrina responded better only to N18. Altogether, these results could contribute to assessing the effects of mulching type on putative volatile markers of the desirable sensory perception and consumers' acceptability of strawberries.

Keywords: *Fragaria* × *ananassa* Duch.; plastic mulch disposal; volatile organic compounds (VOCs); headspace solid phase microextraction (HS-SPME/GC-MS); multivariate analysis

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1. Introduction

Strawberry fruit (*Fragaria* × *ananassa*) belongs to the Rosaceae family and is the most popular berry consumed as either fresh fruit or processed products, such as syrups, jam, flavourings, and ice creams, thanks to their unique organoleptic characteristics and nutraceutical value. The high content of phytochemical molecules, mostly ascorbic acid, anthocyanins, and flavonoids, confers on the strawberry a protective activity against reactive oxygen species and free radicals [1,2]. In the last decades, breeding programs have been focused on the development of new strawberry cultivars characterized by improved agronomic, organoleptic, and nutraceutical traits [3,4]. In fact, consumer quality acceptance is strongly related to specific perceived sensory traits, including fruit colour, shape, acidity, and sweetness, combined with flavour and aroma, which are defined by the pattern of volatile organic compounds (VOCs). Finally, in the overall evaluation of quality, the nutritional value, intended as the content of bioactive compounds with beneficial effects on human health, is considered [3].

Compared to other fruit crops, the nutritional and sensorial properties of strawberry can be ascribed to the interaction among genetic, agronomic, and environmental features. Specifically, as the quality of strawberries cannot be improved after harvest, consumer acceptance and nutritional aspects of fruit can be modulated by modifying the preharvest conditions, including soil type, irrigation, fertilization, mulching, ripening stage, and other agricultural practices [5,6]. Strawberries quickly deteriorate during the harvest campaign and in the post-harvest phase, so they are commonly grown on raised beds by covering the soil around plants with plastic mulching films [6–8]. The introduction of the mulching system in strawberry cultivation has brought multiple agronomic benefits with an improvement of fruit quality until harvest by avoiding direct contact of the fruit with the soil [7]. Notably, higher phenolic content has been detected in fruit from strawberry plants grown on plastic films than in those produced on bare soil. Moreover, white mulch increased the amount of the total phenolic compounds and ellagic acid and the overall antioxidant activity in strawberry fruit more than brown mulch, while the total anthocyanin level was the highest in berries obtained using brown mulch. Strawberries cultivated on red plastic mulch contained significantly higher amounts of flavour compounds than those grown on black mulch due to different light exposure caused by the mulch colour [3,5,9]. The impact of the colour of plastic mulches on strawberry quality is related to the quantity and quality of light reaching the plant, which in turn affects the plant growth and development and subsequently the production of plant secondary metabolites, including the aroma and phenolic compounds [3,5,9]. Although strawberries ripened over red plastic mulch gave the fruit a series of improved parameters, such as higher fruit size, concentration of phytonutrients, flavour, and aroma components, the most commonly used plastic mulch colour is black [3,5,9].

Plastic films, mainly made by resistant and durable low-density polyethylene (PE), are non-compostable and non-biodegradable, causing critical soil contaminations of phthalate and phthalic acid esters and also increasing agricultural plastic wastes (pipes, fittings, and agricultural packaging) [6,7]. Currently, there is a great effort towards the replacement of PE with biodegradable mulches that offer similar performance compared to the conventional films but disappear from the soil within 5–6 months, being completely converted in organic substances [6–8]. In the last years, several agronomic trials have demonstrated the efficiency of thermoplastic starch-based mulches, such as those derived from Mater-Bi, a proprietary trade name by Novamont, as a good alternative to PE films for strawberry cultivation [8]. Most of these trials have been conducted to evaluate the agronomical performance of biodegradable films on the plant yield and fruit quality, weed control, and biodegradability in soil, while only few studies have addressed the influences on strawberry qualitative and nutraceutical features [8].

Very recently, the impact of an innovative Mater-Bi film, grade EF08P0, indicated as N5, was compared to a commercial Mater-Bi mulch, grade EF04P0 (N18), and to a conventional non-biodegradable PE film under farm conditions over long-lasting cultivation on high-raised beds (typical of the Southern Italy) and was assessed on the properties of two strawberry cultivars (cvs), namely Elide and Sabrina [8]. In particular, for strawberry cultivation over a period of 230 days, Morra et al. (2022) evaluated at four harvesting dates (30 March, 4 May, 17 May, 1 June 2021) the soluble solid content, ascorbic acid, titratable acidity, polyphenols, anthocyanins, flavonoids, and antioxidant activity in relation to the cultivar and the three mulching films (PE, N5, and N18). The content of total soluble solid, polyphenols, and anthocyanins were modified by the mulching type, while antioxidant activity and ascorbic acid were substantially unaffected. The variation of relevant parameters was indicative of the fact that the mulching condition significantly affects the maturation of strawberries [8]. Furthermore, this study evaluated the composition of nutritionally relevant compounds in strawberries, while the organoleptic characteristics were not defined.

Perceived sweetness, which is correlated to the content of sugars, is the key factor for consumers' liking of strawberries, and it is one of the main traits considered for breeding programs [10]. Nevertheless, higher sugar contents alone may negatively affect other critical traits, including longer fruit development periods and yield losses [11]. Sensory-chemical studies have recognized that specific VOCs, referred to as sweetness-enhancing volatiles, can increase sweetness perception [10]. VOCs typically occur at much lower amount than sugars (up to 10^{-6} times), while even a slight increase of the sweetness-enhancing volatiles would likely result in improved sensations of sweetness and overall acceptance at a lower carbon cost for the industry-demanded agronomic features. Thus, non-sugar sources of sweetness would be a very good alternative to increasing liking, as this practice is not detrimental to the horticultural traits and can allow putative markers to engineer the strawberry flavour from breeding populations [10].

In light of the importance given to the sensory perception, the effects of both cvs and mulching films on the profile of VOCs were evaluated by headspace solid-phase micro-extraction coupled to gas chromatography mass spectrometry (HS-SPME/GC-MS). Two strawberry cvs., Elide and Sabrina, grown as previously reported in Morra et al. [8], were sampled at the most suitable harvest time (1 June 2022) to explore the number and content of the components previously indicated as putative sweetness-enhancing volatiles as influenced by different combinations of cultivar and mulching film.

2. Materials and Methods

2.1. Experimental Site and Innovative Mulch

Experimental cultivation was carried out in a private farm, located in Falciano del Massico (Caserta) (41°140′ N, 13°944′ E), under a multi-tunnels structure with a semi-circular cross-section (2.5 m high, 5.3 m wide, 38 m long). Tunnels were covered by clear ethylene-vinyl acetate plastic film with a thickness of 18 mm. Soil had a loam texture (USDA) composed of: 10% clay, 53% silt, 37% sand, pH 7.8, 2.2% organic matter, total N 1.3 g kg^{-1}, C/N 9.8, electrical conductivity 286 dS m^{-1}, and available p 99 mg kg^{-1}, as reported in Morra et al. (2022) [8]. Two biodegradable mulching films were compared to the standard black, low-density PE mulching film, 50 μm thick: (a) the innovative N5 (Mater-Bi, Novamont, Novara, Italy), grade EF08P0, black-coloured, 18 μm thick, 1.6 m width and (b) the commercial standard N18 (grade EF04P0 Mater-Bi), black-coloured, 18 μm thick, 1.6 m width. Compared to N18, the N5 film presented a durability optimized for a longer crop cycle [8]. Two strawberry cvs., namely Elide (CIV) and Sabrina (Planasa), were cropped on each type of the three mulching films. The main mechanical properties for a Mater-Bi film, 15 μm thick, grade EF04P are: tensile strength 35 MPa, parallel elongation at break 380%, Young's modulus 200 MPA; biodegradability, according to UNI EN standard 13432 and 14995, must be ≥90% conversion into CO_2 within 2 years under ambient soil conditions; water vapour pressure is 400 g x 30 μm/m^2 day.

As described in Morra et al. (2022), main plots hosted the different mulching films that covered raised beds along the entire length of a tunnel (38.5 m); each main plot was divided in two sub-plots to test the two cvs.. Each raised bed, with an isosceles trapezoidal cross-section, was 0.8 m wide at the bottom, 0.6 m at the top, 0.35 m high at both sides, and 0.4 m apart from the other. The whole area dedicated to the trial was 1200 m^2, and within each experimental plot, an area was delineated containing 10 plants in which ripe berries were harvested and sampled to assess the VOCs profiles. Fruits analysed in this study were sampled at the last harvest on 1 June 2021.

2.2. Reagents and Chemicals

Chemicals, analytical standards, and reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ultra-pure water (resistivity of 18 MΩ × cm at 25 °C) was from a Millipore Milli-Q purification system (Millipore Corp., Bedford, MA, USA), while helium, used as GC carrier gas, was at a purity of 99.999% (Rivoira, Milan, Italy). The HS-SPME fibres and the glass vials were obtained from Supelco (Bellofonte, PA, USA); the capillary GC-MS column HP-Innowax (30 m × 0.25 mm × 0.5 μm) was from Agilent J&W (Agilent Technologies Inc., Santa Clara, CA, USA).

2.3. Analysis of Volatile Organic Compounds (VOCs)

2.3.1. Sample Preparation and HS-SPME Procedure

Profiling of VOCs was obtained by HS-SPME/GC-MS according to Zorrilla-Fontanesi et al. (2012), utilizing DVB/CAR/PDMS (50/30 mm) fibre and 50 °C and 20 min as extraction temperature and extraction time, respectively [12]. Regarding the sample preparation, 1 g of strawberry sample was placed into a 20 mL screw-on cap HS vial containing 0.3 g of NaCl. Before sealing the vial, 1.5 µL of a stock solution of 20 ppm of 2-octanol was added as the internal standard (IS). Then, vials were sealed with a Teflon septum and an aluminium cap (Chromacol, Hertfordshire, UK) and stirred. The equilibration time and temperature were 10 min and 40 °C, respectively. The extraction and injection steps were automatically executed using an autosampler MPS 2 (Gerstel, Mülheim, Germany). HS-SPME extraction was performed by exposing the fibre to the headspace into the vial for 20 min at 40 °C to allow VOCs to be adsorbed onto the fibre surface.

2.3.2. Gas Chromatography–Quadrupole Mass Spectrometry Analysis (GC-MS)

The analysis of VOCs was carried out on a gas chromatograph model GC 7890A coupled to a mass spectrometer 5975 C (Agilent Technologies, Palo Alto, CA, USA). The HS-SPME fibre was hold for 10 min into the injector port of the GC system equipped with a capillary column HP-Innowax, using a temperature gradient starting at 50 °C for 3 min, then increased to 160 °C at 5 °C min^{-1}, kept at 160 °C for 1 min, ramped to 250 °C at 10 °C min^{-1}, and held at 250 °C for 2 min. VOCs were analysed at an ionization energy of 70 eV and detected by mass selective detector, operating in the 30–300 m/z mass range with a scanning speed of 2.7 scans/s. VOCs were identified comparing experimental mass spectra with those of the standard NIST05/Wiley07 libraries as well as through matching of the retention indices (RI) (as Kovats indices) with literature data and pure standards when available. Each sample was analysed in duplicate with a randomized sequence, including analysis of blanks. For each volatile compound, the peak area was measured from the total ion chromatogram (TIC) and semi-quantified by relative comparison with the peak area of the IS (relative peak area, RPA%).

2.4. Statistical Data Analysis

Exploratory data analysis was performed by principal component analysis (PCA) [13] and heatmap. For PCA, data were autoscaled, whereas the heatmap was built centring the data to the median of the PE group and applying autoscaling using the Euclidean distance and the Ward's method for clustering. The data set obtained for Elide and Sabrina cvs. were independently investigated by linear mixed-effect modelling (LME) to take into account the effects of the technical replicates. Specifically, the concentration of VOCs was modelled considering the mulching film as a fixed effect and the technical replicate as a random effect. Pair comparisons were performed to discover the VOCs that showed a different content in N5 and in N18 modes with respect to PE as well as between each other. Bonferroni correction was applied to counteract the multiple testing problem, and significance level $\alpha = 0.05$ was assumed. Data analysis was performed using in-house-developed R-functions implemented by R 4.0.4 platform (R Foundation for Statistical Computing, Vienna, Austria).

3. Results and Discussion

3.1. Comparative Determination of VOCs Profile from "Sabrina" and "Elide" Strawberry Samples

HS-SPME/GC-MS analysis of strawberry samples cultivated with three different mulch types (PE, N5, and N18) allowed to detect overall 69 and 65 VOCs in Elide and Sabrina cvs., respectively. In particular, the two strawberry cvs. contained VOCs of the same chemical classes, but only a few of these compounds, including alcohols (5), furans (3), lactones (3), and others (1), were common to the two cvs. Elide, in fact, presented 35 esters, 6 aldehydes, 8 acids, and 8 terpenes, while Sabrina showed 32 esters, 5 aldehydes, 10 acids, and 6 terpenes. At this regard, Table 1 lists the VOCs abbreviation code, the experimental and literature-reported Kovats index, and the identification methods for the two cvs.

Table 1. Volatile metabolites detected in Sabrina and Elide strawberry cultivars and their identification codes.

Metabolite	Code	RIt/RIsp	ID	Metabolite	Code	RIt/RIsp	ID
Esters							
Ethyl acetate	E1	921/921	RI/MS/S	Ethyl 3-(methylthio)propionate	E29	1569/1569	
Methyl propionate	E2	953/953	RI/MS/S	Ethyl 2-hydroxyhexanoate	E30	1596/1592	RI/MS/S
Methyl butyrate	E3	989/989	RI/MS/S	Hexyl hexanoate	E31	1609/1608	RI/MS/S
Methyl 2-methylbutyrate	E4	1016/1016	RI/MS/S	Methyl 3-hydroxyhexanoate	E32	1653/1641	RI/MS/S
Methyl 3-methylbutyrate	E5	1021/1021	RI/MS/S	Ethyl 3-hydroxyhexanoate	E33	1702/1696	RI/MS/S
Ethyl butyrate	E6	1037/1037	RI/MS/S	Benzyl acetate	E34	1729/1731	RI/MS/S
Ethyl 2-methylbutyrate	E7	1053/1053	RI/MS/S	Ethyl cinnamate	E35	2145/2145	
Ethyl 3-methylbutyrate	E8	1068/1068	RI/MS/S	**Aldehydes**			
Butyl acetate	E9	1074/1075	RI/MS/S	Hexanal	Ald1	1084/1084	RI/MS/S
Isoamyl acetate	E10	1121/1122	RI/MS/S	2-Hexenal	Ald2	1242/1242	RI/MS/S
Ethyl pentanoate	E11	1142/1142	RI/MS/S	Nonanal	Ald3	1404/1404	RI/MS/S
trans-Ethyl-2-butenoate	E12	1179/1169	RI/MS/S	Benzaldehyde	Ald4	1533/1533	RI/MS/S
Pentyl acetate	E13	1192/1192	RI/MS/S	Dodecanal	Ald5	1716/1716	RI/MS/S
Methyl hexanoate	E14	1204/1204	RI/MS	Tetradecanal	Ald6	1924/1924	MS
Ethyl hexanoate	E15	1249/1249	RI/MS/S	**Alcohols**			
Isoamyl butyrate	E16	1277/1267	RI/MS/S	1-Hexanol	Al1	1365/1365	RI/MS/S
Hexyl acetate	E17	1284/1285	RI/MS/S	*trans*-3-Hexen-1-ol	Al2	1375/1376	RI/MS/S
Methyl 2-hexenoate	E18	1288/1284	RI/MS	*cis*-3-Hexen-1-ol	Al3	1393/1394	RI/MS/S
cis-3-Hexen-1-ol acetate	E19	1326/1326	RI/MS/S	*trans*-2-Hexen-1-ol	Al4	1416/1416	RI/MS/S
trans-2-Hexen-1-ol acetate	E20	1344/1344	RI/MS/S	1-Octanol	Al5	1561/1561	RI/MS/S
Ethyl 2-hexenoate	E21	1354/1355	RI/MS	**Acids**			
Methyl octanoate	E22	1396/1398	RI/MS/S	Propanoic acid	Ac1	1545/1545	RI/MS/S
Hexyl isobutyrate	E23	1400/1392	RI/MS/S	Butyric acid	Ac2	1631/1631	RI/MS/S
Hexyl butyrate	E24	1420/1415		2-Methylbutanoic acid	Ac3	1676/1676	RI/MS/S
Ethyl octanoate	E25	1439–1439		Hexanoic acid	Ac4	1848/1848	RI/MS/S
trans-2-Hexenyl butyrate	E26	1478/1476	RI/MS/S	Heptanoic acid	Ac5	1952/1952	RI/MS/S
Ethyl 3-hydroxybutyrate	E27	1525/1525		Octanoic acid	Ac6	2073/2073	RI/MS/S
Methyl 3-(methylthio)propionate	E28	1529/1525	RI/MS/S	Nonanoic acid	Ac7	21762176	RI/MS/S
				Decanoic acid	Ac8	2278/2278	RI/MS/S
Terpenes				**Furanones**			
β-Pinene	T1	1042/1042		Mesifurane	F1	1592/1600	RI/MS/S
cis-Linalool oxide	T2	1451/1445		Furaneol	F2	2042/2042	RI/MS/S
trans-Linalool oxide	T3	1478/1483		*trans*-γ-Jasmolactone	F3	2188/2181	RI/MS
Linalool	T4	1553/1553	RI/MS/S	**Lactones**			
β-Farnesene	T5	1667/1667	RI/MS	γ-Octalactone	L1	1919/1919	RI/MS/S
α-Terpineol	T6	1702/1690	RI/MS/S	γ-Decalactone	L2	2158/2158	RI/MS/S
β-Damascenone	T7	1834/1835	RI/MS/S	γ-Dodecalactone	L3	2387/2384	RI/MS/S
Nerolidol	T8	2055/2055	RI/MS/S	**Others**			
				Phenol	O1	2005/2007	

For each sample, data are mean values of three replicates. RIt, relative retention indices on polar column reported in literature by www.pherobase.com; www.flavornet.org; www.ChemSpider.com; webbook.nist.gov (accessed on 1 July 2022); RIsp, relative retention indices calculated against n-alkanes (C8-C40) on HP-Innowax column; ID, identification method as indicated by the following: RI, Kovats retention index on a HP-Innowax column; MS, NIST and Wiley libraries spectra; S, co-injection with authentic standard compounds on the HP-Innowax column.

Tables S1 and S2 report the VOCs analysis of Elide and Sabrina, respectively, showing that the number and the content of aroma compounds varied between the two cvs. According to previous studies, esters were the most represented chemical class in both the cvs., accounting for about 71.2%, 59.8%, and 58.1% for PE, N5, and N18, respectively, in Elide and 50.4%, 55.0%, and 57.0% for PE, N5, and N18, respectively, in Sabrina (Tables S1 and S2). More in detail, methyl and ethyl esters appeared the most important contributors to the strawberry flavour, imparting fruity and floral aromas [14,15].

In Elide strawberries, the predominant esters were trans-2-hexen-1-ol acetate (E20), ethyl hexanoate (E15), and ethyl butyrate (E6) for PE and N5 mulching (30.2%, 10.7%, and 8.9% for PE, respectively, and 18.3%, 12.2%, and 9.2% for N5, respectively), while ethyl cinnamate (E35) followed by E15, E20, E6, and methyl hexanoate (E14) were the most abundant esters detected in fruits grown on N18 (14.0%, 12.3%, 9.2%, 5.6%, and 5.5%, respectively) (Table S1). Specifically, E6 and E15 were identified among the principal odorants in strawberries [14,15].

In Sabrina fruit grown on N5 mulch, the ester mainly present was trans-2-hexen-1-ol acetate (E20), followed by ethyl hexanoate (E15) and ethyl butyrate (E6) (25.0%, 7.5%, and 5.3%, respectively), while under PE conditions, the most abundant esters were ethyl hexanoate (E15), E20, and ethyl butyrate (E6) (18.3%, 10.0%, and 8.9%, respectively) and in N18 samples were *cis*-3-hexen-1-ol acetate (E19), methyl hexanoate (E14), and ethyl butyrate (E6) (16.1%, 14.2%, and 8.8%, respectively) (Table S2).

In Elide, the second-ranking class of volatiles was terpenes (9.7%, 9.7%, and 13.6% for PE, N5, and N18, respectively), with linalool (T4) as the most abundant component for all mulching conditions (7.0%, 5.3%, and 6.4% for PE, N5, and N18, respectively) (Table S1). On the other hand, the second major components of Sabrina cv. were acids, with a percentage of 34.6%, 21.3%, and 23.3% for PE, N5, and N18, respectively, with nonanoic acid (Ac8) representing the predominant component of the group for all treatments (27.6%, 18.5%, and 19.0% in PE, N5, and N18, respectively) (Table S2). Most of the volatiles listed in Table S2 have been already observed in Sabrina strawberries [15,16] even though the present study catalogued a larger number of VOCs compared to the previous ones, presumably owing to the different pedo-climatic environment, agronomic practices, and analytical method.

3.2. Statistical Analysis of the VOCs Profile in the Sabrina and Elide Strawberry Samples

The separate contributions of strawberry cultivar and mulching films to the profile of VOCs was inferred on a statistical basis. The data set consisted of 69 and 65 VOCs for Elide and Sabrina, respectively, and 18 observations (3 samples for each of the 3 mulching films and 2 technical replicates for each sample). The two biplots obtained by modelling by PCA the HS-SPME/GC-MS semi-quantitative data (% RPA), separately collected for the two cvs, are reported in Figure 1. The biplot was obtained by plotting the correlation loadings and the scaled scores of the PCA model on the same plot. The two components accounted for 96.1% and 98.4% of the variation in the dataset of Elide and Sabrina, respectively, since PC1 and PC2 explained the 66.7% and 29.4% of the total variance of the plot in panel A and 80.0% and 18.4% in panel B, respectively. For the two cvs, the observations for each of the three mulching films lie in corresponding regions of the PCA plot. In fact, PE fruit was characterized by a negative PC1 and a positive PC2, while samples obtained using N5 film presented both negative PC1 and PC2. On the other hand, strawberries obtained using N18 mulch presented a positive PC1 and a negative PC2 (Figure 1). These findings revealed that the mulching films (PE, N5, N18) represent the main source of data variation. Moreover, it can be observed that different VOCs characterise different mulching films and that the effect of each mulching film on the profile of VOCs is different for each cv. Indeed, in the case of Elide, L3, T3, E30, and E21 were higher in N5 than N18, and PE, E20, E19, E34, and E17 were higher in PE than N5 and N18, while Ald1, T7, O1, and E25 were higher in N18 than in N5 and PE, for example. On the other hand, in the case of Sabrina, Al5 was higher in N5 than N18, and PE, E15, Ac1, L3, and Ald3 were higher in PE than N5 and N18, while E3, E1, E22, and E28 were higher in N18 than in N5 and PE, for example. Consequently, it can be affirmed that the VOCs that characterise each mulching film depend on the cultivar.

The semiquantitative variability of individual VOCs induced by the mulching films was visualized through the heatmaps obtained for the two data sets. In Figure 2A, the heatmap for Elide is reported, while Figure 2B shows the heatmap for Sabrina. In both varieties, the VOCs showed very different trends: some VOCs increased both in N5 and N18 with respect to PE, while others decreased in both N5 and N18 with respect to PE, and some VOCs were higher in N18 and lower in N5 or vice versa, while others showed a level similar to PE. Since the two heatmaps are different, it is possible to conclude that the effect of the mulching film on the VOCs composition depends on the cultivar, in line with the outcomes of PCA analysis.

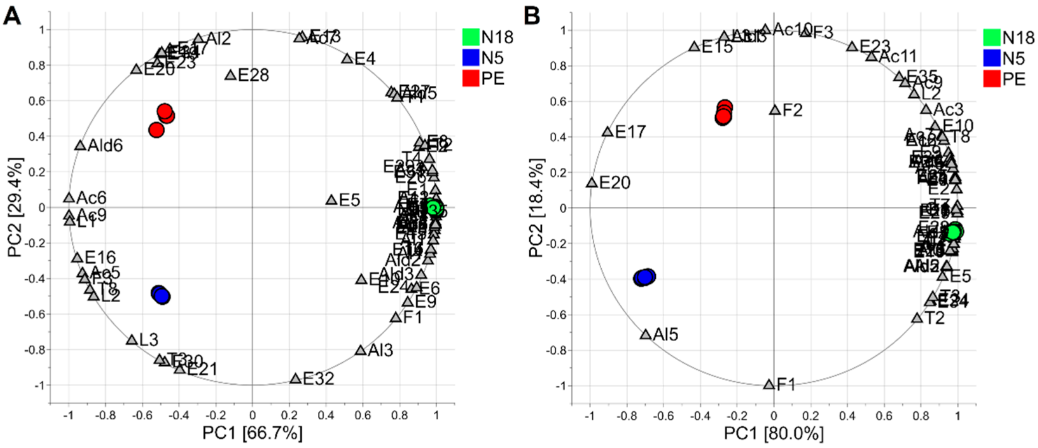

Figure 1. Biplots of the PCA models for the two cultivars: Elide (R2 = 96.1%, panel (**A**)) and Sabrina (R2 = 98.4%, panel (**B**)); VOCs are reported as grey triangles.

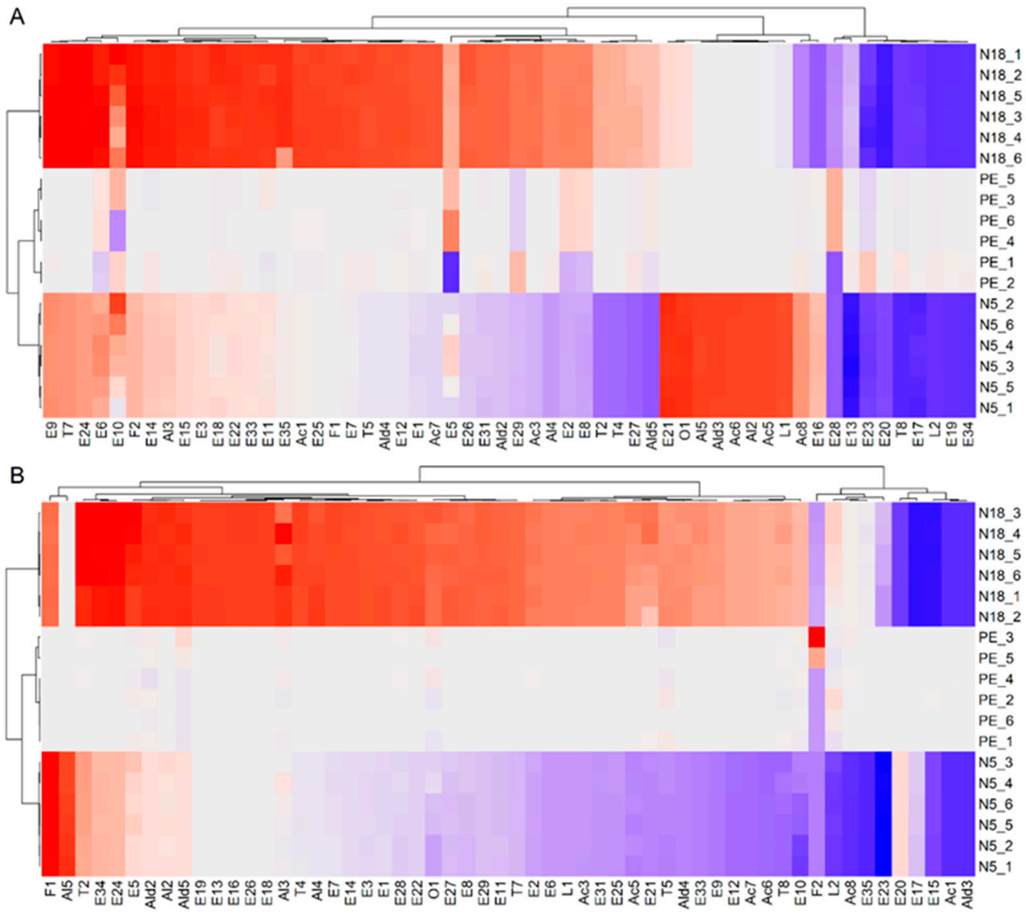

Figure 2. Heatmaps: Elide (panel (**A**)) and Sabrina (panel (**B**)). The colour scale ranges from blue, which indicates values lower than the median of the PE group, to red for values greater than the median of the PE group.

3.2.1. Linear Mixed-Effect Modelling (LME) on Elide Data Set

The LME analysis conducted on the VOCs profiles of Elide discovered 40 VOCs differently expressed between PE and N5 mulching, 52 VOCs for the comparison of PE and N18, and 58 matching N5 and N18. In particular, out of 40 VOCs differently expressed between PE and N5 in Elide cv, 26 (E3, E9, E15, E16, E21, E24, E30, E32, E33, E35, Ald2, Ald4, Al1, Al3, Ac4, Ac5, Ac8, T3, T6, T8, F1–F3, L2, L3, and O1) presented amounts statistically higher in the fruit grown using N5 film, while 14 (E13, E17, E19, E20, E26, E27, E34, Ald1, Ald5, Ald6, Ac2, T1, T2, and T4) were more abundant in the strawberries produced on the PE mulch. The amount of the remaining 29 volatiles was comparable in both the treatments (PE and N5) (Figure 1; Table S1).

Among the 26 volatiles characterizing Elide N5 fruit, some compounds, including methyl butyrate (E3), butyl acetate (E9), ethyl hexanoate (E15), hexyl butyrate (E24), benzaldehyde (Ald4), 2-methylbutanoic acid (Ac4), α-terpineol (T6), mesifuran (F1), γ-decalactone (L2), and γ-dodecalactone (L3), exhibited a sweetness- and/or flavour-enhancing ability regardless of the sugar content, while isoamyl butyrate (E16) and 2-hexenal (Ald2) showed negative coefficients related to liking [10,17,18]. Moreover, some components, including hexanoic acid (Ac5), mesifuran (F1), furaneol (F2), γ-decalactone (L2), γ-dodecalactone (L3), and several esters, such as methyl butyrate (E3), identified as key odorants to strawberry flavour, increased during the fruit ripening [18–21].

In fruit, the enzymatic biosynthesis of volatile esters is due to the esterification of alcohols and acyl-CoA, derived from both fatty acid and amino acid metabolism, and occurs during the late steps of ripening [21]. In strawberries at an advanced degree of maturation, alcohol acyltransferase (AAT), the enzyme involved in the last step of ester formation, can display a 16-fold increase from the half-red to the full-red ripeness stage, and a statistical positive correlation occurs among the AAT gene expression, the AAT activity, and the content of some volatile esters [19]. Moreover, the availability of precursors is probably a limiting step in the biosynthesis of volatile esters, causing specific flavour profiles at different ripeness stages [19–21]. The formation of C6 aldehydes generally increases during ripening as a result of both the increment from 25 up to 200% in the activity of the enzymes lipoxygenase (LOX) and hydroperoxide lyase (HPL), which is involved in the biosynthetic pathway, and to the increase up to 200% of the substrate amount (linolenic acid) [20]. The contents of mesifuran (F1) and furaneol (F2) rise considerably during the strawberries' ripening, as the enzymes responsible for their synthesis, O-methyltransferase for F1 and quinine oxidoreductase (FaQR) for F2, show the maximum activity at the full-red stage, allowing potential targets to engineer the strawberry flavour from breeding populations [19].

Among the 14 VOCs (E13, E17, E19, E20, E26, E27, E34, Ald1, Ald5, Ald6, Ac2, T1, T2, and T4) that are regularly more abundant in the Elide strawberries produced on PE with respect to N5, pentyl acetate (E13), hexyl acetate (E17), *cis*-3-hexen-1-ol-acetate (E19), *cis*-linalool oxide (T2), and linalool (T4) are positively correlated to the taste sensation "sweet", while *trans*-2-hexen-1-ol-acetate (E20) exhibits strong negative correlations with both sweetness and consumers' liking [10,17]. Finally, hexanal (Ald1) and T4 are associated with the fruit maturation, as the enzymes involved in their biosynthetic pathway exhibited the highest activity at the full-red stage [19–21]. The findings reported above suggest that the samples obtained using the N5 film can meet the consumers' expectations of sweetness more that the PE samples, as N5 fruit is characterized by a larger number of the so-called sweetness-and/or aroma-enhancing volatiles [10,17]. In fact, among all the sensory attributes, sweetness is considered the principal driver for consumers, who particularly prefer sweet and aromatic ("intense fruity") strawberries [10,17]. Moreover, based on the higher number of ripening-associated VOCs found in the N5 samples, it can be hypothesized that the N5 film favours an early ripening of strawberries.

Within the Elide cv, 38 VOCs (E1, E3, E7, E9, E11, E12, E14, E15, E18, E21, E22, E24–E26, E31–E33, Ald1–Ald4, Al1, Al3–Al5, Ac1, Ac2, Ac4, Ac8, T1, T2, T4–T7, F1, F2, and O1) were up-regulated in the fruits cultivated using N18 in comparison to PE, while 14 compounds (E16, E17, E19, E20, E34, Ald6, Ac5, Ac6, Ac9, T8, F3, and L1–L3) showed down-regulated levels in berries grown using PE film. The amount of the remaining 17 volatiles was comparable between the treatments (PE and N18) (Figure 1; Table S1). Among the 38 volatiles up-regulated in Elide N18 fruits, eight esters, including methyl butyrate (E3), butyl acetate (E9), ethyl pentanoate (E11), *trans*-ethyl 2-butyrate (E12), methyl hexanoate (E14), ethyl hexanoate (E15), hexyl butyrate (E24), and ethyl octanoate (E25); six terpenes, most of all *cis*-linalool oxide (T2) and linalool (T4); two aldehydes, nonanal (Ald3) and benzaldehyde (Ald4); two lactones, γ-decalactone (L2) and γ-dodecalactone (L3); and mesinfuran (F1) and 2-methylbutanoic acid (Ac4) are generally believed as positively correlated with "acceptance" regardless of the sugar content, while two compounds, ethyl 2-methylbutyrate (E7) and 2-hexenal (Ald2), have been reported to have a negative correlation related to liking [10,17,18]. Many of the above-described volatiles, including ethyl pentanoate (E11), methyl hexanoate (E14), ethyl hexanoate (E15), *trans*-2-hexenyl butyrate (E26), hexanal (Ald1), 2-hexenal (Ald2), hexanoic acid (Ac4), mesifuran (F1), and furaneol (F2), also indicated as key odorants in strawberry flavour, increase during the fruit ripening, as the enzymes involved in their biosynthetic pathway exhibit the highest activity at the full-red stage [18–21].

Among the 14 (E16, E17, E19, E20, E34, Ald6, Ac5, Ac6, Ac9, T8, F3, and L1–L3) VOCs statistically more abundant in the PE Elide strawberries, compared to N18, hexyl acetate (E17), *cis*-3-hexen-1-ol acetate (E19), decanoic acid (Ac9), L2, and L3 have been directly associated with the "sweet" taste sensation, while isoamyl butyrate (E16) and *trans*-2-hexen-1-ol-acetate (E20) are negatively associated with both sweetness and consumers' liking [10,17]. Overall, the results obtained by the comparison of PE and N18 in Elide cv. suggest that fruit grown using the N18 film can be preferred by consumers, as it is described by a higher number of VOCs, showing an enhancing effect on sweetness and/or associated with the fruit ripening. In fact, notwithstanding that both γ-decalactone and γ-dodecalactone have been detected only in PE strawberries (Table S1), no direct correlation between aroma sensation and the lactone content have been observed, suggesting that the other VOCs can significantly contribute to the sensory perception [22].

Out of the 58 differently expressed VOCs between N5 and N18 in Elide cv, 43 compounds (E1–E4, E7–E9, E11–E15, E18, E22, E24–E29, E31, E33, Ald1–Ald5, Al1, Al4, Al5, Ac1, Ac2, Ac4, Ac8, T1, T2, T4–T7, F1, F2, and O1) showed a higher level in berries grown using N18 mulching film (Table S1). On the contrary, 15 (E16, E20, E21, E30, E32, Ald6, Ac5, Ac6, Ac9, T3, T8, F3, and L1–L3) were more abundant in fruit produced using the N5 film, while the amount of the remaining 11 volatile constituents was comparable between the treatments (N5 and N18) (Figure 1; Table S1).

Among the 43 volatiles predominant in Elide N18 fruit, methyl butyrate (E3), methyl 2-methylbutyrate (E4), ethyl 3-methylbutyrate (E8), butyl acetate (E9), ethyl pentanoate (E11), pentyl acetate (E13), methyl hexanoate (E14), ethyl hexanoate (E15), hexyl butyrate (E24), ethyl octanoate (E25), hexyl hexanoate (E31), nonanal (Ald3), benzaldehyde (Ald4), 2-methylbutanoic acid (Ac4), *cis*-linalool oxide (T2), linalool (T4), mesifuran (F1), and furaneol (F2) have been indicated as positively correlated with the attributes "sweet" and/or "aromatic" [10,17,18]. Some of these volatiles, including E3, E11, E14, E15, Ald1, Ald3, Ald4, Al5, Ac4, and F1 and F2, are crucial to the characteristic fruity notes of mature strawberries [10,23]. On the contrary, ethyl 2-methylbutyrate (E7) and 2-hexenal (Ald2) have shown negative correlations with "acceptance" and "aromatic" retronasal sensation, respectively [17].

Among the 15 VOCs (E16, E20, E21, E30, E32, Ald6, Ac5, Ac6, Ac9, T3, T8, F3, and L1–L3) more abundant in N5 fruit, only γ-decalactone (L2) and γ-dodecalactone (L3) have been indicated directly correlated with both sweetness and liking independently of sugars, while isoamyl butyrate (E16) and *trans*-2-hexen-1-ol-acetate (E20) are inversely associated to consumers' acceptance [10,17]. According to these results, the N18 strawberries could be preferred by the consumers compared to the N5 counterparts, as it induces the development of a larger number of volatiles with a positive impact on the consumers' preference. Moreover, the higher number of VOCs associated with ripe fruit in N18 than in N5 suggests that N18 film anticipates the ripening time of the fruit.

3.2.2. Linear Mixed-Effect Modelling (LME) on Sabrina Data Set

The LME analysis conducted on the VOCs profiles of Sabrina discovered 50 VOCs differently expressed comparing PE and N5 mulching, 56 between PE and N18, and 59 matching N5 and N18. Among 50 VOCs differently expressed between PE and N5 mulching, only 7 volatiles (E20, E24, E34, Al5, T2, T3, and F1) showed a statistically significant up-regulation in the strawberries produced on N5 mulch, while 43 VOCs (E1–E3, E6, E9, E11–E15, E18, E19, E21–E23, E25, E27, E29, E31, E33, E35, Ald1, Ald3, Ald4, Al4, Ac1–Ac4, Ac6–Ac11, T4, T5, T7, T8, F3, and L1–L3) were down-regulated compared to the PE film. The amount of the remaining 15 compounds showed a non-statistical difference between the two treatments (PE vs. N5) (Figure 2; Table S2).

A substantially different picture emerged from the PE vs. N18 comparison since out of 56 VOCs differently expressed between the two films, 47 components (E1–E3, E5–E14, E16, E18, E19, E22, E24–E29, E31, E33, E34, Ald1, Ald2, Ald4, Ald5, Al1–Al4, Ac3, Ac4, Ac7, Ac8, T2–T5, T7, T8, F1, L1, and O1) were up-regulated in the fruit grown on N18 mulch, while only 9 VOCs (E15, E17, E20, Ald3, Ac1, Ac10, Ac11, F3, and L3) were down-regulated (Figure 2; Table S2). The content of the remaining nine VOCs was not statistically different between the two treatments (PE vs. N18) (Figure 1; Table S2). In Sabrina cv, 54 (E1–E3, E5–E14, E16, E18, E19, E22–E29, E31, E33–E35, Ald1, Ald2, Ald4, Ald5, Al1–Al4, Ac2–Ac4, Ac6–Ac9, Ac11, T2–T5, T7, T8, F3, L1, L2, and O1) out of 59 VOCs differently expressed between N5 and N18 were up-regulated in berries grown using N18 mulching film. On the contrary, five (E15, E17, E20, Al5, and F1) were more abundant in fruit produced using N5 film, while the amount of the remaining six VOCs was comparable between the treatments (N5 and N18) (Figure 2; Table S2).

In relation to the comparison of PE vs. N5 for Sabrina cv, among the seven volatiles (E20, E24, E34, Al5, T2, T3, and F1) up-regulated in N5 strawberries, hexyl butyrate (E24), *cis*-linalool oxide (T2), and mesifuran (F1) are sweetness-enhancing volatiles, while *trans*-2-hexen-1-ol-acetate (E20) has shown a strong negative association to liking [10,17,18]. Moreover, octanol (Al5) has been previously reported in fruit at late stages of maturation, and mesifurane (F1) is known to increase during the fruit ripening, imparting a caramel or cotton-candy-like flavour in red strawberries [14,24]. Among the 43 volatiles (E1–E3, E6, E9, E11–E15, E18, E19, E21–E23, E25, E27, E29, E31, E33, E35, Ald1, Ald3, Ald4, Al4, Ac1–Ac4, Ac6–Ac11, T4, T5, T7, T8, F3, and L1–L3) that dominate the VOCs profile of Sabrina PE fruit, methyl butyrate (E3), ethyl butyrate (E6), butyl acetate (E9), ethyl pentanoate (E11), pentyl acetate (E13), methyl hexanoate (E14), ethyl hexanoate (E15), *cis*-3-hexen-1-ol-acetate (E19), ethyl octanoate (E25), hexyl hexanoate (E31), nonanal (Ald3), benzaldehyde (Ald4), 2-methylbutanoic acid (Ac4), decanoic acid (Ac9), linalool (T4), γ-decalactone (L2), and γ-dodecalactone (L3) positively affect liking and/or sweetness [10,17,18]. Several of these VOCs, including E3, E6, E11, E14, E15, Ald1, T4, L2, and L3, are increased in ripe fruit, imparting the characteristic fruity notes of mature strawberries [10,23]. Consistent with these findings, the strawberries cv. Sabrina grown on PE could be preferred by the consumers compared to the N5 fruit because of an increased number of volatiles, with a positive impact on the consumers' liking. Moreover, since in the N5 samples, a larger number of volatiles associated with ripe strawberries were detected, it can be hypothesized that the N5 film promotes an early ripening of the fruit with respect to PE.

Concerning the comparison of PE vs. N18 for Sabrina cv, among the 47 VOCs (E1–E3, E5–E14, E16, E18, E19, E22, E24–E29, E31, E33, E34, Ald1, Ald2, Ald4, Ald5, Al1–Al4, Ac3, Ac4, Ac7, Ac8, T2–T5, T7, T8, F1, L1, and O1) that were up-regulated in the N18 fruit, methyl butyrate (E3), ethyl butyrate (E6), ethyl 3-methylbutyrate (E8), butyl acetate (E9), ethyl pentanoate (E11), pentyl acetate (E13), methyl hexanoate (E14), *cis*-3-hexen1-ol-acetate (E19), hexyl butyrate (E24), ethyl octanoate (E25), hexyl hexanoate (E31), benzaldehyde (Ald4), 2-methyl butanoic acid (Ac4), *cis*-linalool oxide (T2), linalool (T4), and mesinfuran (F1) enhance strawberry sweetness and consumer preference [10,17,18]. Several of the above-mentioned VOCs, including E3, E6, E11, E15, and E17, are commonly designated as key odorants in strawberries [14,23,24]. Among the volatiles positively associated with N18 cv. Sabrina fruit, ethyl 2-methylbutyrate (E7) and 2-hexenal (Ald2) showed a negative correlation to consumers' preference [10,17]. On the other hand, nine volatiles (E15, E17, E20, Ald3, Ac1, Ac10, Ac11, F3, and L3) presented a higher content in Sabrina berries grown on PE compared to N18 film. Among them, ethyl hexanoate (E15), hexyl acetate (E17), nonanal (Ald3), and γ-dodecalactone (L3) exhibit a sweetness- and/or flavour-enhancing capability regardless of the sugar content, while *trans*-2-hexen-1-ol acetate (E20) seems to display the strongest negative correlations with both liking and sweetness [10]. In line with these results, the N18 strawberries cv. Sabrina could be preferentially chosen by the consumers compared to the PE fruit, as a higher number of volatiles with a positive impact on the consumers' linking were observed. Moreover, similarly to N5, the higher number of volatiles associated with ripe strawberries observed suggests that the N18 film promotes an earlier ripening of the fruit compared to PE.

Among the 54 compounds (E1–E3, E5–E14, E16, E18, E19, E22–E29, E31, E33–E35, Ald1, Ald2, Ald4, Ald5, Al1–Al4, Ac2–Ac4, Ac6–Ac9, Ac11, T2–T5, T7, T8, F3, L1, L2, and O1) showing higher contents in N18 compared to N5 Sabrina strawberries, methyl butyrate (E3), ethyl butyrate (E6), ethyl 3-methylbutyrate (E8), butyl acetate (E9), ethyl pentanoate (E11), pentyl acetate (E13), methyl hexanoate (E14), *cis*-3-hexen1-ol-acetate (E19), hexyl butyrate (E24), ethyl octanoate (E25), hexyl hexanoate (E31), benzaldehyde (Ald4), 2-methyl butyric acid (Ac4), decanoic acid (Ac9), *cis*-linalool oxide (T2), linalool (T4), and γ-decalactone (L2) have positive correlations with "acceptance" [10,17,18]. Moreover, some of the above 54 VOCs, including E3, E6, E11, E14, Ald1, E15, and E17, have been described as key odorants and are commonly observed in ripe strawberries [14,23,24]. On the other hand, ethyl 2-methylbutyrate (E7) and 2-hexenal (Ald2) are negatively correlated with liking or sweetness [10,17,18].

Among the five VOCs (E15, E17, E20, Al5, and F1) most abundant in Sabrina N5 fruit, ethyl hexanoate (E15), hexyl acetate (E17), and mesifuran (F1) have been reported to positively influence the taste sensation "sweet", while *trans*-2-hexen-1-ol acetate (E20) seems to have a strong detrimental effect on consumers' linking [10,17,18]. Based on the above reported findings, N18 strawberries cv. Sabrina could be more preferred by the consumers compared to the N5 fruit because of the higher presence of volatiles, with a positive influence on the consumers' preference and generally related to ripe fruit.

4. Conclusions

In this study, the qualitative and semi-quantitative changes of VOCs in strawberries cv. Elide and cv. Sabrina, grown on three mulching films, namely PE, N5, and N18, were evaluated on a statistical basis. Overall, for both the cvs., the data analysis highlighted a positive effect of the biodegradable mulching films in improving the acceptability of strawberries to the consumer. However, there emerged a combined effect of cv. and mulching film, as the performance of biodegradable films (N18 and N5) compared to PE was more evident for Elide than for Sabrina cv. In fact, in Elide cv., some VOCs with a positive impact on the "sweet" taste sensation and consumers' preference were positively associated with strawberries produced on N18 and N5 mulching films. In turn, Sabrina responded positively only to N18, which induced the production of a higher number of sweetness-enhancing volatiles compared to PE and N5. Moreover, the up-regulation of VOCs directly related to consumers' preferences improved the perceived quality of the fruit cultivated on the biodegradable mulches in spite of a slightly lower °Brix value. This result is in agreement with the one recently reported for Elide, which had higher °Brix values in fruits produced on PE than N5 and N18 [8]. However, although no univocal optimal VOCs profile can be connected with a high level of liking-associated compounds, and taking into account that the contribution of distinct VOCs to a characteristic aroma depends on both the intrinsic aroma threshold value (ATV) and its content, a correlation between sensory parameters and the patterns of VOCs could help to define possible relationships within the multiple aspects of complex biosystems, such as fruit. As a result, the characterization of the VOCs related to the sensory perception of Elide and Sabrina strawberries require further trials aiming to combine the information supplied by the chemical characterization of VOCs with the odour perception by gas-chromatography-olfactometry (GC-O), which is a valuable tool to elucidate the aroma active components, and/or to analyse the linkage of aroma or aroma/taste in all samples.

In conclusion, the explorative results here described might be of interest for producers to identify putative volatile markers useful for the objective assessment of the effects of mulching type on metabolic activity of fruits and to enhance the strawberry sensory perception and satisfy consumers' preference.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12102514/s1, Table S1: VOCs analysis of Elide cultivar; ID is the identifier of the VOC and annotation specifies its chemical name; data are reported as median (5th percentile–95th percentile); *p* is the *p*-value of the pair comparison calculated by LME; * is used to indicate significant differences applying Bonferroni correction with $\alpha = 0.05$.; Table S2: VOCs analysis of Sabrina cultivar; ID is the identifier of the VOC and annotation specifies its chemical name; data are reported as median (5th percentile–95th percentile); *p* is the *p*-value of the pair comparison calculated by LME; * is used to indicate significant differences applying Bonferroni correction with $\alpha = 0.05$.

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