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Nontargeted Metabolite Profiles and Sensory Properties of Strawberry Cultivars Grown both Organically and Conventionally

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ABSTRACT: Strawberry (*Fragaria* × *ananassa* Duch.) contains many secondary metabolites potentially beneficial for human health, and several of these compounds contribute to strawberry sensory properties, as well. In this study, three strawberry cultivars grown both conventionally and organically were subjected to nontargeted metabolite profiling analysis with LC-qTOF-ESI-MS and to descriptive sensory evaluation by a trained panel. Combined metabolome and sensory data (PLS model) revealed that 79% variation in the metabolome explained 88% variation in the sensory profiles. Flavonoids and condensed and hydrolyzable tannins determined the orosensory properties, and fatty acids contributed to the odor attributes of strawberry. Overall, the results indicated that the chemical composition and sensory quality of strawberries grown in different cultivation systems vary mostly according to cultivar. Organic farming practices may enhance the accumulation of some plant metabolites in specific strawberry genotypes. Careful cultivar selection is a key factor for the improvement of nutritional quality and marketing value of organic strawberries.

KEYWORDS: *Fragaria* × *ananassa Duch., nontargeted metabolite profiling, sensory analysis, organic farming, cultivars*

INTRODUCTION

Strawberry (*Fragaria* × *ananassa* Duch.) is globally one of the most important cultivated berries in terms of economy, yield, and cultivation surface area.[1−3] Strawberries are largely consumed fresh, but they are also applicable for freezing, institutional kitchens, and many processed products, such as jams, juices, and jellies.[4] Strawberries are a rich source of, for example, vitamin C, folate, and dietary fiber.[3,5] In addition, strawberries contain many secondary metabolites that are potentially beneficial to human health. These metabolites include several types of phenolic compounds (flavonoids, phenolic acids, hydrolyzable tannins, procyanidins), terpenes, and saponins.[3,6−9]

Genetic background is a significant factor affecting the contents of secondary metabolites in strawberry tissues.[1,10,11] In plants, secondary metabolites often act against biotic and abiotic threats in plants' growth environments;[12−14] consequently, both cultivation practices and climatic and weather conditions affect the secondary metabolism of crop plants.[1,15,16] Because of the possible health benefits of strawberry's secondary metabolites (e.g., anticarcinogenicity, anti-inflammatory activity, ability to reduce the risk of cardiovascular disease, lipid and carbohydrate metabolism-modifying effects),[3] it would be desirable for breeders and farmers to be able to predict more precisely the cultivar × environment interactions affecting the metabolite contents and profiles of strawberry cultivars.

Traditionally, good disease resistance, storage longevity, high yield, and berry size and shape have been among the main goals of strawberry breeding.[10,17] Today, it is possible to utilize comprehensive molecular biology tools, combined with omics-based analytical approaches, to find cultivars with elevated secondary metabolite levels and to further enhance the supply of these health-benefiting compounds from the every-day diet.[4,18] However, from the consumer point of view pleasant sensory characteristics are the most important attributes of strawberries.[1,19] Although good for human health, many secondary plant metabolites contribute to berry taste properties often found to be unpleasant, such as astringency and bitterness.[20] In addition, domestication and breeding of strawberry have diminished some desirable sensory quality-enhancing chemical traits.[17] Therefore, it can be challenging to produce high-quality strawberry cultivars and strawberry products with elevated secondary metabolite concentrations and yet accepted by consumers.

Strawberry flavor is determined by sweet and sour notes; soluble solids content (sugars) and acids and their ratio are the main determinants of these properties.[21,22] Phenolic compounds, in turn, are known to have bitter and astringent properties.[19,23] Strawberry odor is often described to have, for instance, fruity and green notes.[24,25] Volatile compounds, including esters, furanones, aldehydes, terpenes, sulfur compounds, and alcohols, contribute to the odor of strawberry and might participate in strawberry flavor formation.[22,24−26]

Organic farming has been a considerable trend over the past decades, as consumers appreciate naturally produced food products. Organic plants are considered to be better-tasting, purer, and healthier in comparison to conventionally produced crop plants, due to less usage of pesticides and fertilizers. However, the superiority of organic strawberries has been

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difficult to establish scientifically.[15,16,24,27] Moreover, comparative studies on the sensory quality of conventional and organic strawberries are rather scarce, and studies investigating strawberry chemical composition usually concentrate on restricted compound groups such as polyphenols or volatile compounds.

In this study, three strawberry cultivars grown both conventionally and organically were subjected to nontargeted metabolite profiling analysis with liquid chromatography connected with negative electrospray ionization in a quadrupole−time-of-flight−mass spectrometer (LC-qTOF-ESI-MS) and to descriptive sensory evaluation by a trained panel in a sensory laboratory. Metabolite and sensory data were tested statistically to investigate the dependence of strawberry metabolome on cultivar and farming practice and to examine the effects of cultivar, farming practice, and chemical composition on the sensory quality of strawberry.

MATERIALS AND METHODS

Strawberries. Samples from three strawberry (*Fragaria* × *ananassa* Duch.) cultivars, 'Bounty' (B), 'Jonsok' (J), and 'Polka' (P), were collected from both conventional (CON) and organic (OR) fields in northern Savonia, eastern Finland, in June−July 2013. The farming locations were Leppävirta (62.30° N, 27.475° E; J-CON and P-CON), Kaavi (62.586° N, 28.295° E; B-CON), and Lapinlahti (63.216° N, 27.232° E, B-OR, J-OR, and P-OR). Organic cultivation was implemented according to EU Council Regulation (EC 834/2007). The collected berries were in the same, fully mature stage and grown approximately in the same compass direction. Of each cultivar (conventional and organic), ∼7 kg was harvested; ∼2 and ∼5 kg were used for metabolome analysis and sensory evaluation, respectively. After harvesting, pooled whole berry samples were immediately placed on ice and frozen as soon as possible. Berries were stored at −20 °C until further processing.

Nontargeted Metabolite Profiling. *Sample Preparation.* For the metabolite extraction, 10 frozen berries (81.46−149.09 g) for each cultivar × farming practice combination were gently thawed in a microwave oven (defrost setting, 1.5 min; AM Kodinkoneet Inc., Helsinki, Finland) and homogenized with a stick blender. Chemical compounds were extracted from pureed strawberries with 80% methanol; for 10 g of strawberry puree, 30 mL of methanol was used. Suspensions were quickly vortexed and extracted in a horizontal shaker (Unimax 2010, Heidolph Instruments, Schwabach, Germany) at 400 rpm for 15 min at room temperature. Extracts were filtered through a gauze (Mölnlycke Health Care, Gothenburg, Sweden) and centrifuged (Centrifuge 5810R, Eppendorf, Hamburg, Germany) at 3220*g* for 5 min. The clarified supernatants were stored at −75 °C until analysis. Prior to the metabolite analysis, samples were again centrifuged and filtered (Acrodisc 0.22 μm PTFE filter, PALL, Port Washington, NY, USA).

The total phenolic contents of strawberry extracts were measured with the Folin−Ciocalteu method,[28] with some modifications. Briefly, 200 μL of strawberry extract dilution (1:10 or 1:20) was combined with 1000 μL of 10% (v/v) Folin−Ciocalteu reagent (VWR International, Darmstadt, Germany) and 800 μL of 7.5% (w/v) Na_2CO_3 (Sigma-Aldrich, St. Louis, MO, USA) solution; the reagent mixture was incubated at room temperature for 60 min and vortexed every 20 min. The mixture absorbance was measured at 765 nm (Ultrospec 2000, Pharmacia Biotech, Uppsala, Sweden), and the total phenolic content was calculated as gallic acid (GA) (Sigma-Aldrich) equivalent according to a standard curve (0.01, 0.03, 0.05, 0.07, and 0.1 mg GA/mL).

LC-MS Conditions. The samples were analyzed by LC-qTOF-ESI-MS (Agilent Technologies, Waldbronn, Karlsruhe, Germany) that consisted of a 1290 LC system, a Jetstream electrospray ionization (ESI) source, and a 6540 UHD accurate-mass qTOF spectrometer. The samples were analyzed with reversed phase (RP) chromatographic separation, and data were acquired in negative electrospray ionization (ESI−). The sample tray was kept at 4 °C during the analysis.

Two microliters of the sample solution was injected onto a column (Zorbax Eclipse XDB-C18, 2.1 × 100 mm, 1.8 μm) (Agilent Technologies, Palo Alto, CA, USA) kept at 50 °C. Mobile phases, delivered at 0.4 mL/min, consisted of water (eluent A) and methanol (eluent B) (Sigma-Aldrich), both containing 0.1% (v/v) of formic acid (Sigma-Aldrich). The following gradient profile was employed: 0−10 min, 2 → 100% B; 10−14.5 min, 100% B; 14.5−14.51 min, 100 → 2% B; 14.51−16.5 min, 2% B.

A Jetstream ESI source was operated in negative ionization mode. The conditions were as follows: drying gas temperature, 325 °C, and flow, 10 L/min; sheath gas temperature, 350 °C, and flow, 11 L/min; nebulizer pressure, 45 psi; capillary voltage, 3500 V; nozzle voltage, 1000 V; fragmentor voltage, 100 V; and skimmer, 45 V. For data acquisition, 2 GHz extended dynamic range mode was used, and the instrument was set to acquire over m/z 20−1600. For automatic data-dependent MS/MS analyses, precursor isolation width was 1.3 Da, and from every precursor scan cycle, the four most abundant ions were selected for fragmentation. Collision energies were 10, 20, and 40 V in subsequent runs. Continuous mass axis calibration was performed by monitoring two reference ions from an infusion solution throughout the runs. The reference ions were m/z 112.985587 and 966.000725.

Data Processing. Initial data processing was performed using MassHunter Qualitative Analysis B.05.00 (Agilent Technologies, Palo Alto, CA, USA). Ions were extracted to compounds exhibiting isotopic peaks, dimers, and common adducts. Mass Profiler Professional software (version 2.2, Agilent Technologies, Palo Alto, CA, USA) was used for compound alignment. Noise and low-abundance metabolites were removed from the data matrix according to their abundance and appearance frequency in the samples. The final data set contained 3669 molecular features. For the comparison of the sample groups, the data matrix was exported to Excel and filtered according to their maximum peak area value, indicating high relative abundance in one or more sample types focusing on the 131 most intense compound signals. After initial reduction, preliminary partial least-squares regression (PLS) analysis (Unscrambler 9.8, Camo Process AS, Oslo, Norway), using molecular features as *X*-variables, that is, predictors, and sensory properties as *Y*-variables, that is, responses, was performed to perceive the most relevant compounds in terms of sensory characteristics; this resulted in 60 final entities, which were then subjected to spectral inspection and identification.

Principal component analysis (PCA) (Simca 13.0, Umeå, Sweden) was utilized to monitor the relationship between samples and sample replicates in their metabolite profiles. Hierarchical clustering analysis with heat map representation (TM4Microarray Software Suite, available at www.tm4.org/mev.html; algorithm according to Eisen et al.[29]) was applied to analyze and display the similarity of sample replicates and compound accumulation patterns and to visualize the differences in normalized metabolite signal abundances between sample types.

The identification of metabolites was performed on the basis of the fragmentation patterns in the data-dependent MS/MS acquisition. The MS/MS data and the molecular masses were compared against The METLIN Metabolite Database (http://metlin.scripps.edu/), SciFinder (https://scifinder.cas.org), Dictionary of Natural Products (http://dnp.chemnetbase.com/), or earlier published work describing fragmentation patterns.

Sensory Analyses. *Sample Preparation.* For sensory analyses, 200 g of frozen strawberries/cultivar × farming practice combination was gently thawed in a microwave oven (defrost setting, 120 W, 2.5 min; Rosenlew, Stockholm, Sweden). Thawed berries were homogenized with a stick blender, and 30 g of strawberry puree was measured into 50 mL beakers. The beakers were covered with small watch glasses, and the samples were kept at room temperature to stabilize the headspace and to reach the serving temperature (∼20 °C).

Sensory Panel. The descriptive profiling was carried out with 15 voluntary assessors familiar with sensory profiling. Assessors were known to be able to rank taste solutions and recognize flavor differences and odor samples.

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Table 1. Attributes, Definitions, and References Used in Sensory Evaluations

attribute	description	reference	intensity
orosensory			
total intensity of flavor	perceived first expression of flavor		
sourness	taste of acids	0.1% citric acid	8
bitterness	bitter taste	0.07% caffeine	8
astringency 1	soft, mouth-drying mouthfeel	0.1% $AlNH_4(SO_4)_2$	8
astringency 2	rough, sharp mouthfeel	0.1% $Al_2(SO_4)_3$	8
sweetness	taste of sugars and other sweeteners	2% sucrose	8
off-flavor	flavors that are not innate for strawberries		
odor			
total intensity of odor	perceived first expression of odor		
fruity	an ester-like odor	5 mL of 10 ppm ethyl butyrate	7
green	freshly cut grass, leaves, or vegetables	1 μL of 200 ppm *cis*-3-hexenol	9
strawberry-like		1 μL of wild strawberry aroma concentrate (Roberts, Turku, Finland)	8
off-odor	odors that are not innate for strawberries		

Panel Training. The panelists were trained according to ISO 8586 standard. Descriptions for strawberry samples were adapted from Hakala et al.[24] and Sandell et al.[30] In four 1 h sessions, assessors were trained to recognize and evaluate the intensity of seven orosensory attributes and five odor attributes in reference samples (Table 1) and in strawberry puree samples.

Intensity Evaluations. The sensory evaluations were performed in December 2013 in a sensory laboratory (ISO 8589). Three parallel evaluation sessions were organized, and each panelist assessed the same six-sample set in each session. The sample set consisted of three cultivars cultivated both conventionally and organically (B-CON, B-OR, J-CON, J-OR, P-CON, and P-OR). Sample cups were coded with three-digit numbers, and the sample order was randomized. The intensities of sensory attributes were rated with a line scale (from 0 = "none" to 10 = "very strong") in nonrandom order, using reference samples (Table 1) in every session. In addition, the possible off-flavors and off-odors were described freely in writing. Data were collected with Compusense *five* software (Compusense Inc., Guelph, Canada).

Statistical Analyses. Differences among the sensory attributes of strawberry samples were analyzed by ANOVA together with Tukey's *t* test and the Tamhane test ($p < 0.05$) (SPSS 16.0, SPSS Inc., H, Chicago, IL, USA). Relationships between sensory and chemical characteristics were tested with the PLS method (Unascrambler 9.8, Camo Process AS, Oslo, Norway). Cross-validation was used to estimate the number of principal components for a statistically reliable model. The reproducibility, agreement, and sensitivity of assessors were also tested (PanelCheck, Nofima, Tromsø, Norway).

RESULTS AND DISCUSSION

Sensory-Active Metabolites in Organic and Conventional Strawberries. From the 3669 molecular features observed in the strawberry metabolite profiling analysis, 60 compounds were selected for further analysis on the basis of preliminary partial least-squares regression (PLS) analysis: the peak areas of 131 molecular features having highest abundance of response in the LC-MS analysis were used as predictors, and the intensities of 7 orosensory attributes and 5 odor attributes (Table 1) evaluated from frozen, pureed strawberry samples were used as responses. The 60 major compounds found to be related to the sensory profile were tentatively identified on the basis of their accurate mass and product ion spectrum. This compound group included mainly flavonoids, hydrolyzable and condensed tannins, organic and phenolic acids, and fatty acids, as well as some terpenoids, furans, and a lactone derivative (Table 2).[6,8,21,31−37] The identified compounds are depicted in the total ion chromatogram of conventional 'Jonsok' sample (Figure 1).

The relatively good stability of 'Bounty" and 'Polka' chemical composition in different horticultural systems was demonstrated by the PCA separating individual sample replicates on the basis of the 60 tentatively identified metabolites: for these two cultivars, conventional and organic samples were closely accumulated (Figure 2). In the hierarchical clustering analysis, conventional 'Polka' (P-CON) and organic 'Polka' (P-OR) further indicated quite similar metabolite profiles regardless of cultivation system (Figure 3). The sample replicates of conventional 'Jonsok' (J-CON) and organic 'Jonsok' (J-OR) were clearly clustered according to farming practice, therefore demonstrating the most conspicuous intracultivar differences between organic and conventional samples (Figures 2 and 3). Especially many organic and cinnamic acids were markedly more abundant in J-CON in comparison to J-OR or to any other sample (Figure 3). When organic 'Bounty' (B-OR) and conventional 'Bounty' (B-CON) were compared, for example, isorhamnetin aglycone, tryptophan, salidroside, an epiafzelechin hexose, a sesquiterpenoid, and a dicaffeoylquinic acid were on average significantly more abundant in B-OR (Figure 3).

In general, the compositional differences between cultivars were much more pronounced than the differences caused by cultivation methods. For example, differing flavonoid glycosylation patterns between cultivars were visualized in the heat map (Figure 3). Both J-CON and J-OR totally lacked a kaempferol pentose. The abundances of some small carboxylic and/or aromatic compounds and salidroside were regulated in a cultivar-dependent manner. A sapogenin compound was found to be more abundant in all organic samples in comparison to conventional analogues, but no other consistent, significant differences were found according to farming practice within the selected compound group.

Earlier studies have shown that flavonoids (e.g., anthocyanins and quercetin glycosides), hydroxycinnamic acids, flavan-3-ols, hydrolyzable and condensed tannins, and triterpene-derived metabolites are important constituents in many strawberry tissues.[7−9,11,14,38,39] Several members of these compound groups were identified as orosensory-active features in this study. In nature, phenolic compounds and terpenoids are often associated with bitter taste perceived by a large family of receptors,[17,40] and the astringent mouthfeel of berries and other polyphenol-rich foods is a consequence of phenolic compounds binding to salivary proteins.[41] Bitterness and astringency, although important quality traits of many beverages (e.g.,

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Table 2. Metabolites Tentatively Identified from Strawberry Fruit by LC-qTOF-MS and MS/MS Analysis[a]

peak	t_R (min)	MM	m/z ESI(−)	tentative identification	ref
3	1.09	174.0165	173.0799, 155.0327, 111.0059	dehydroascorbic acid	6, 31
12	1.12	130.02638	129.0199, 116.9357, 85.0290	2-methyl-2-butenedioic acid variant	Dictionary of Natural Products, Metlin
14	1.12	192.02711	191.0186, 173.0080, 111.0086	citric acid	Metlin
62	1.28	104.0475	103.0400, 59.0141	malonic acid	Metlin
78	1.32	134.05775	133.0129, 115.0032, 71.0137	malic acid	Metlin
216	1.68	112.0161	111.0071, 67.0190	furoic acid	Metlin
240	1.73	206.04272	205.0286, 111.0088, 67.0179	furan derivative	Metlin
370	2.08	369.10675	368.1009, 161.0486, 131.0372, 101.0249	methyl cinnamate	Metlin, SciFinder
384	2.12	302.06467	301.0563, 272.4473, 227.0569	quercetin aglycone	SciFinder
466	2.31	316.08017	315.0723, 152.0110, 109.0291	isorhamnetin aglycone	Metlin
498	2.36	296.1112	295.1046, 249.1008, 87.0454, 44.9982	butyric acid conjugate	Metlin
523	2.40	866.20764	865.1974, 575.1197, 287.0560	proanthocyanidin trimer	8
536	2.43	204.08987	203.0797, 186.0565, 142.0644, 116.0497, 74.0248	tryptophan	Metlin
611	2.59	300.08524	299.1139, 137.0618, 89.0232, 59.0137	salidroside	Metlin
618	2.60	332.11142	331.1027, 169.0098, 125.0250	glucogallin	8, 32
655	2.69	144.0242	143.0349, 84.1061, 55.0182	caprylic acid	33, Metlin
661	2.71	346.12735	345.1220, 299.1138, 179.0564, 161.0452, 89.0243, 59.0142	salidroside derivative	Metlin
675	2.72	176.06854	175.0223, 115.0028, 87.0069, 71.1334	L-ascorbic acid	Metlin
844	3.06	418.11182	417.1046, 285.0600, 241.0698, 163.0388, 152.0110	kaempferol pentose	8, 32, SciFinder
887	3.15	326.10114	325.0933, 307.0819, 163.0399, 145.0296	coumaric acid hexose	8
973	3.30	292.0224	291.0156, 247.0288, 163.0413, 147.0457, 117.0344	coumaric acid derivative	Metlin
1083	3.51	466.11182	465.1032, 447.0832, 285.0388, 241.0457, 151.0024	kaempferol hexose	8, 32
1090	3.53	432.10712	431.0996, 269.0462, 253.0528, 225.0552, 147.0082	pelargonidin hexose	6, Metlin
1111	3.56	596.1752	595.1669, 269.0438, 224.6179, 105.5180, 79.2842	unknown	Metlin
1139	3.64	450.1177	449.1094, 431.1024, 287.0583, 269.0448	ferulic acid hexose derivative	21
1185	3.71	578.1647	577.1592, 425.0822, 407.0565, 289.0669, 269.0441, 147.0060	flavan-3-ol derivative	34, Metlin
1211	3.75	480.12738	479.1204, 271.0628, 253.0506, 227.0704, 164.0107, 83.0130	naringenin derivative	32, SciFinder
1244	3.82	356.1118	355.1046, 309.0967, 147.0453, 103.0537	cinnamic acid derivative	Metlin
1253	3.84	332.09055	(751.1387, 561.9384) 331.0821, 289.0719, 271.0554, 125.0237	propelargonidin cleavage product	8
1254	3.84	376.0803	(751.1387, 561.9384) 375.0720, 289.0705, 271.0592, 125.0241	propelargonidin cleavage product	8
1368	4.02	520.1591	519.1185, 271.0613, 253.0503, 227.0734, 164.0103	naringenin derivative	32, SciFinder
1388	4.07	358.12668	357.1193, 195.0649, 151.0765	phenylpropanoid hexose derivative	35, Metlin
1473	4.23	434.12186	433.2065, 271.0583, 253.5903, 227.6108	naringenin hexose	8, SciFinder
1507	4.29	518.1069	517.0959, 473.1056, 269.0456, 225.0560, 163.0040, 147.0404	pelargonidin malonylhexose	6, Metlin
1660	4.54	448.1021	447.0932, 315.0104, 284.0331, 227.0324	kaempferol hexose	Metlin
1763	4.69	434.04922	433.0329, 299.9912, 271.9984, 216.0056, 145.0287	quercetin pentose	8, Metlin
1821	4.77	478.07626	477.0670, 301.0358, 151.0027	quercetin glucuronide	34, Metlin
1823	4.77	956.1505	955.1465, 477.0684, 301.0311, 227.0457	quercetin glucuronide dimer	
1839	4.80	310.1058	309.0983, 147.0426	cinnamic acid hexose	6
1857	4.83	358.12726	357.1203, 195.0662, 151.0761	phenylpropanoid hexose derivative	35, Metlin
1874	4.85	448.06552	447.0584, 300.9994, 257.0115, 229.0145, 200.0048	ellagic acid deoxyhexose	8
1933	4.96	302.00665	300.9998, 284.0042, 257.6011, 229.0150, 200.0099	ellagic acid aglycone	8
1997	5.06	476.1328	475.0.682	unknown	
2089	5.21	462.0812	461.0724, 285.0410, 229.0505, 175.0228, 113.0246	kaempferol glucuronide	8, Metlin
2090	5.21	436.12785	435.1299, 273.0772, 167.0359	epiafzelechin hexose	8, Metlin
2133	5.27	462.08133	461.0738, 285.0408, 175.0246, 113.0247	kaempferol glucuronide	8, Metlin
2272	2.48	436.10193	435.1285, 273.0761, 167.0343	epiafzelechin hexose	8, Metlin
2326	5.57	490.1129	489.1046, 285.0406, 229.0494	kaempferol acetylhexose	8, Metlin
2407	5.74	140.12	139.1123	unknown	
2529	6.09	350.1946	349.1867, 179.0558, 169.1227, 89.0242, 87.0057, 59.0130, 57.0347	lactone hexose	Metlin
2675	6.51	464.26373	463.2546, 417.2496, 255.1918	sesquiterpenoid	8
2744	6.74	464.26276	463.2550, 417.2463, 255.1844, 161.0466	sesquiterpenoid	8
3040	8.64	562.29956	561.2902, 515.2831, 191.0532, 161.0454	dicaffeoylquinic acid	Metlin

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Table 2. continued

peak	t_R (min)	MM	m/z ESI(−)	tentative identification	ref
3046	8.71	562.29987	561.2930, 515.2878, 353.6063, 179.4866, 85.0302, 44.9973	dicaffeoylquinic acid	Metlin
3056	8.80	488.35153	487.3432, 469.3330, 407.3308, 135.0753	sapogenin	8, Metlin
3322	10.09	541.33966	540.3286, 480.3092, 255.2323	palmitic acid derivative	6, 36
3358	10.19	567.35455	566.3363, 506.3248, 281.2486, 245.0757	oleic acid derivative	6, 36
3470	10.51	280.24063	279.2334, 153.6057, 75.2451	linoleic acid	6, 36
3533	10.73	282.2561	281.2489, 126.5730	oleic acid	6, 36
3655	11.60	622.446	621.4382, 486.8462, 271.7586, 153.4179	triterpenoid	37, Metlin

[a]Data were acquired in negative electrospray ionization (ESI−). t_R, retention time.

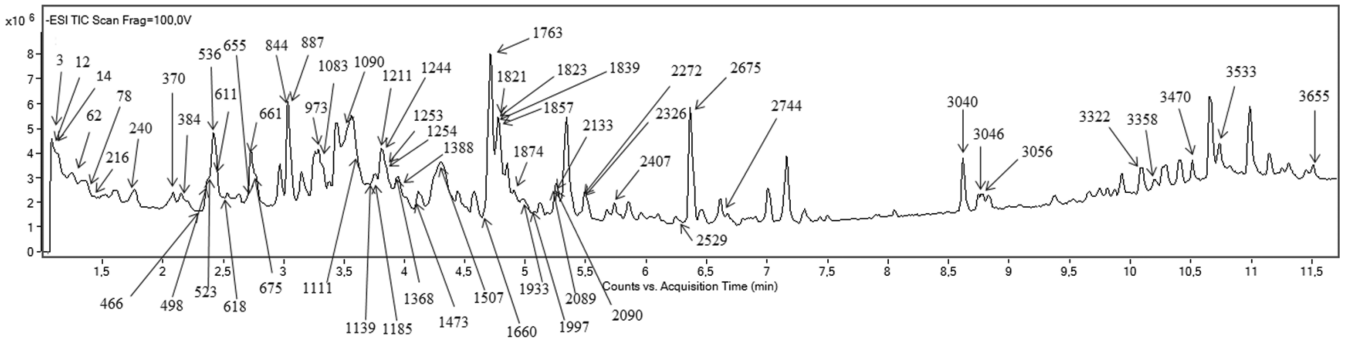

Figure 1. Total ion chromatogram of strawberry obtained in reversed phase liquid chromatography connected with negative electrospray ionization in qTOF-MS analysis. Sixty sensory-active strawberry metabolites are depicted. The peaks are marked by numbers, referring to the tentative identifications in Table 2.

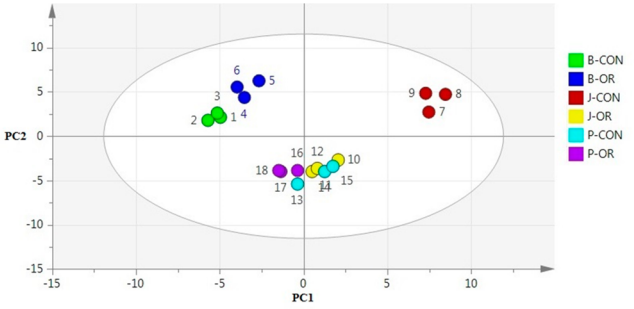

Figure 2. Principal component analysis (PCA) of the metabolite contents of three conventionally and organically grown strawberry cultivars. The PCA plot shows differences between strawberry sample replicates according to their metabolite profiles based on metabolite-specific signal abundances. Each replicate is a pool of 10 strawberry fruits. Numbered circles indicate sample replicates. 1−3, B-CON, conventional 'Bounty'; 4−6, B-OR, organic 'Bounty'; 7−9, J-CON, conventional 'Jonsok'; 10−12, J-OR, organic 'Jonsok'; 13−15, P-CON, conventional 'Polka'; 16−18, P-OR, organic 'Polka'.

wine, tea),[41] are frequently experienced as unpleasant properties of berries.[20]

For instance, citric, malic, and malonic acids were indicated to contribute to strawberry sensory quality in our analyses. Organic acids are an important compound group in strawberry and, indeed, participate and act in synergy with sugars in strawberry flavor formation and perception.[2]

Esters, alcohols, aldehydes, ketones, lactones, furanones, and terpenoids are dominant factors in the strawberry aroma profile.[22,26,42] In addition to furans, terpenoids, and a lactone derivative, many types of fatty acids were among the selected compounds considered in the present study. Several volatile aroma- and/or flavor-active plant secondary metabolites are, in fact, derived from primary metabolites:[17,22,32] for example, lactones are typical products of essential fatty acid degradation[17] and are known to participate in, for instance, the formation of strawberry fruity aroma.[25]

Environment × genotype interactions are important determinants of strawberry phenolic content and metabolite profile. Growth season temperature,[43] fertilization level,[15] harvesting time,[42] and growth location[1] may affect the strawberry metabolome in inter- and intracultivar manners, and some compounds show more plasticity according to environmental conditions.[1] Wounding, microbial attack, and UV radiation, for instance, may induce kaempferol derivative accumulation.[12] As 'Jonsok' samples contained all kaempferol derivatives analyzed in this study except the pentose derivative, it can be proposed that genotype-dependent patterns regulate the glycosylation of flavonols in response to a stress factor. Saponins are synthesized during normal plant development, but biotic threats can also trigger their accumulation.[13]

Total Phenolic Contents of Strawberry Samples. When compared to any other sample, J-CON showed significantly higher total phenolic (TP) values (199 ± 11 mg/100 g fw GAE). J-OR had the second highest TP content (176 ± 11 mg/100 g fw). B-CON had the lowest TP concentration (155 ± 6 mg/100 g fw). The TP contents of J-OR and B-CON differed significantly from each other. The TP contents of P-OR, P-CON, and B-OR were 165 ± 5, 170 ± 4, and 171 ± 2 mg/100 g fw, respectively.

The TP contents of our samples were in line with previous studies.[1,39] No consistent intracultivar alteration of TP content has been observed according to organic versus conventional production.[14,27] Cultivar-dependent, significant differences in the TP values as well as in absolute and relative concentrations of strawberry metabolites have been observed frequently in earlier studies.[1,10,25,27,39]

Strawberry Sensory Properties and Their Relationships with the Identified Metabolites. Some significant

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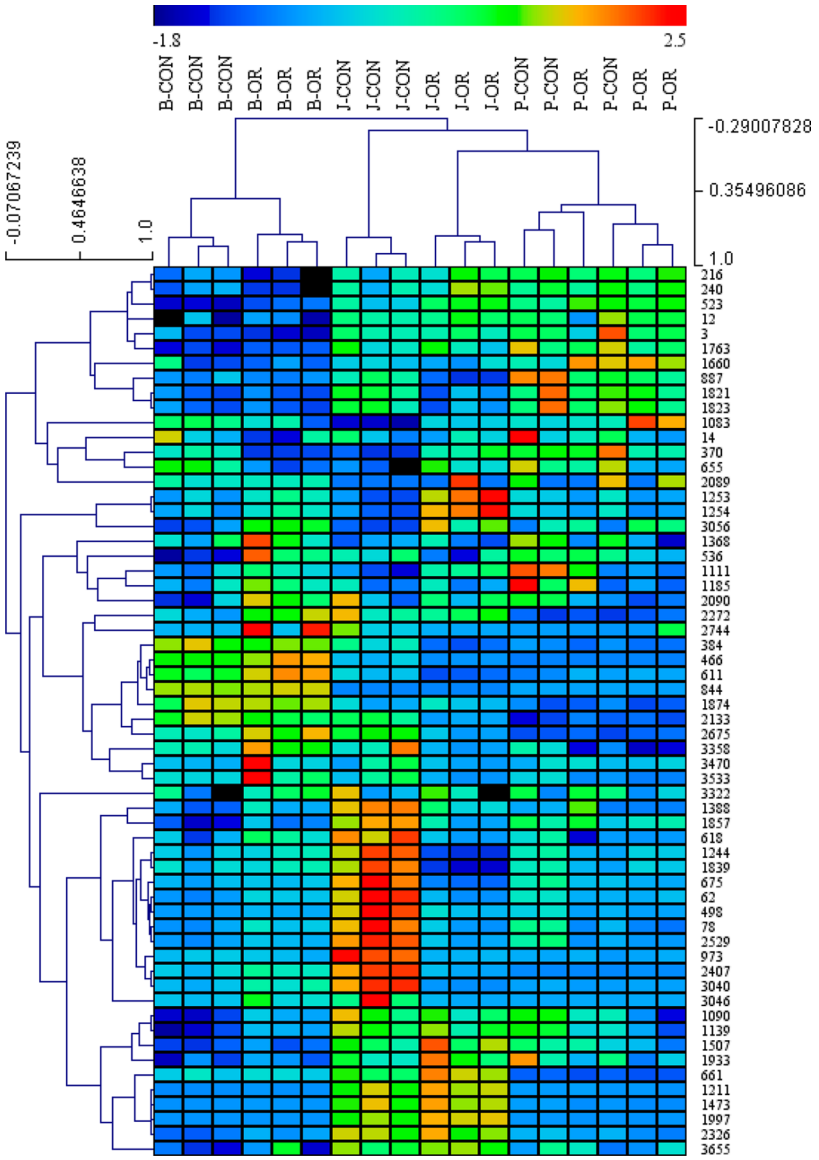

Figure 3. Heat map representation of the hierarchical clustering analysis based on Pearson correlation of the strawberry sample replicates. The analysis is based on the normalized signal abundances of the tentatively identified strawberry metabolites across all analyzed samples. The metabolites are marked by numbers, referring to the tentative identifications in Table 2. The color-coding scale indicates the relative abundance within each metabolite: blue, low abundance; red, high abundance; green/yellow, average abundance. Each replicate is a pool of 10 strawberry fruits. B, 'Bounty'; J, 'Jonsok'; P, 'Polka'; CON, conventional; OR, organic.

differences were detected in all orosensory attribute intensities evaluated from the frozen, pureed strawberry samples (Figure 4A). Nevertheless, there was no consistent correlation between cultivar or farming practice and orosensory property intensities. Sourness and sweetness were the dominating taste properties. J-OR showed both the lowest sweetness intensity and the highest sourness intensity, whereas B-OR was the sweetest and the least sour. B-OR had significantly lower type 1 astringency intensity in comparison to all other samples. Off-flavor was detected in every sample type in at least one evaluation session by at least one assessor.

Green and strawberry odors demonstrated statistically significant differences between samples (Figure 4B). With respect to strawberry odor intensity, J-CON and J-OR represented the extremes and were significantly different from each other; J-CON had considerably higher strawberry odor intensity than J-OR. B-OR had significantly higher green odor intensity than J-OR and P-OR. Off-odors were observed in all sample types with no significant intensity differences.

When the identified chemical compounds were used to explain the sensory attributes of strawberry samples in the PLS model (Figure 5), with three factors, 79% variation in the metabolome data explained 88% variation in the sensory profiles. Bitterness and astringencies were related to the sample-specific combinations of several flavonoid and flavan-3-ol derivatives, a salidroside conjugate, and a triterpenoid. Astringencies and bitterness, in turn, were important contributors in the total intensity of flavor. Two propelargonidin-derived products, citric and caprylic acids and methyl cinnamate, were related to both sourness and rough, sharp type 2 astringency. The aglycones of quercetin and isorhamnetin seemed to be somewhat meaningful in sweet taste intensity, whereas ellagic acid aglycone contributed to bitterness. Green and strawberry odors, as well as sweet taste, were connected

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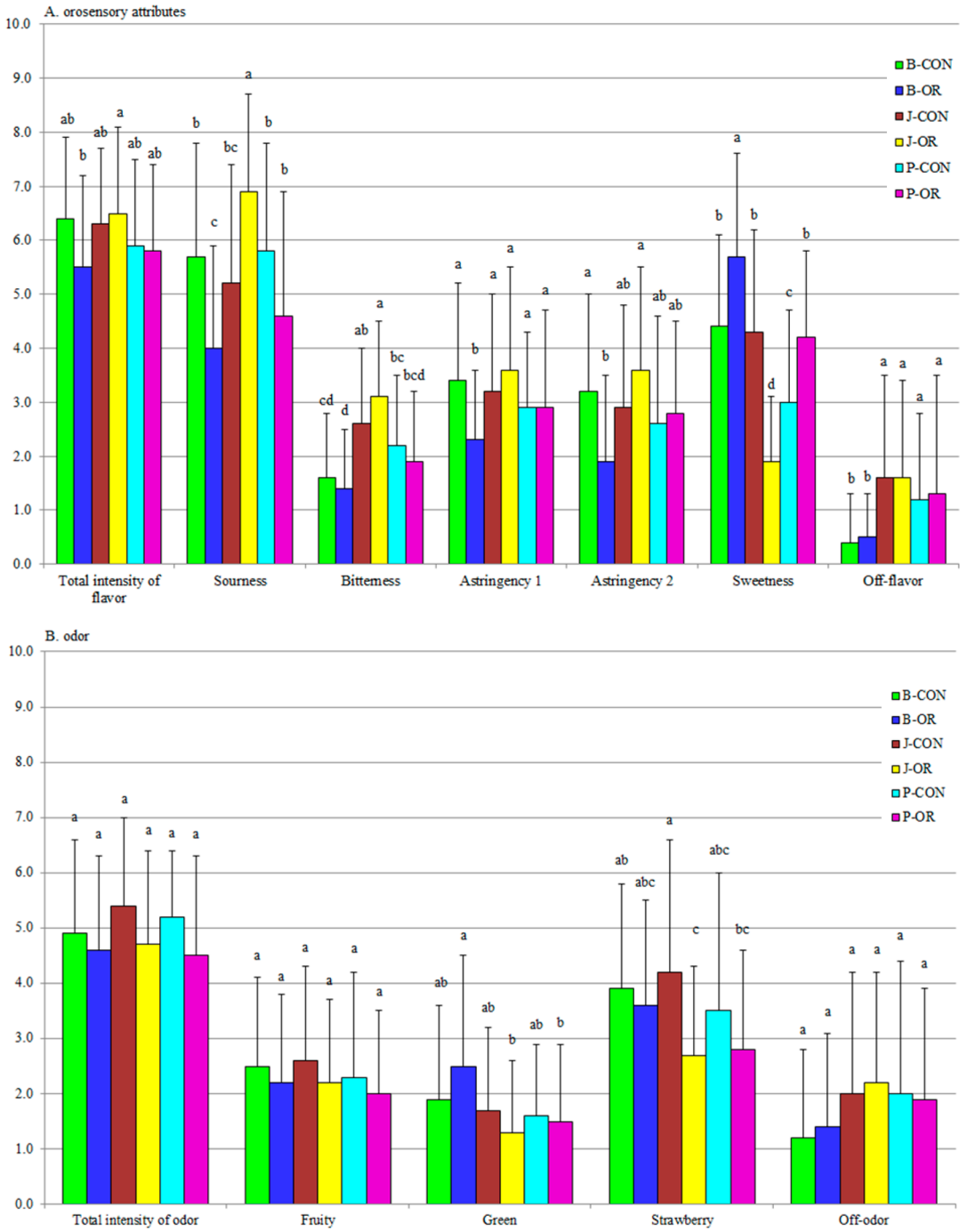

Figure 4. Mean intensities (±SD) of (A) orosensory attributes and (B) odor attributes of three strawberry cultivars (B, 'Bounty'; J, 'Jonsok'; P, 'Polka') cultivated conventionally (CON) and organically (OR). Samples with the same letters for each attribute do not differ significantly.

with fatty acids and fatty acid derivatives. Oleic and linoleic acids were related to green odor formation; an oleic acid derivative was a more determinant factor in strawberry odor. A sesquiterpenoid compound contributed to strawberry odor and salidroside to green odor.

Flavonol glycosides are strongly astringent and contribute especially to velvety, mouth-drying astringency.[20,44−46] Glycosylation patterns of different flavonols are, however, crucial for astringency detection thresholds, depending on the aglycone structure.[44,45] Proanthocyanidins, a compound group including propelargonidins, induce puckering and rough astringency at low concentrations.[45,46] Anthocyanins and proanthocyanidins may also form polymeric pigment compounds having astringent properties in a food matrix.[47] Many compounds having astringent properties may have bitter properties as well, depending on their structure, polymerization level, and concentration.[45,46] Because flavan-3-ols (e.g., (epi)catechin and (epi)afzelechin) tend to polymerize,[8] they possibly contribute to bitterness, along with astringency. Moreover, when chemical mixtures are assessed for their sensory quality, astringency and bitterness are easily linked.[45,46] In a previous study, flavonol aglycones have been observed to correlate with the bitterness of wine,[48] but in our data, flavonol aglycones seemed to be related to the sweetness of B-OR. As for

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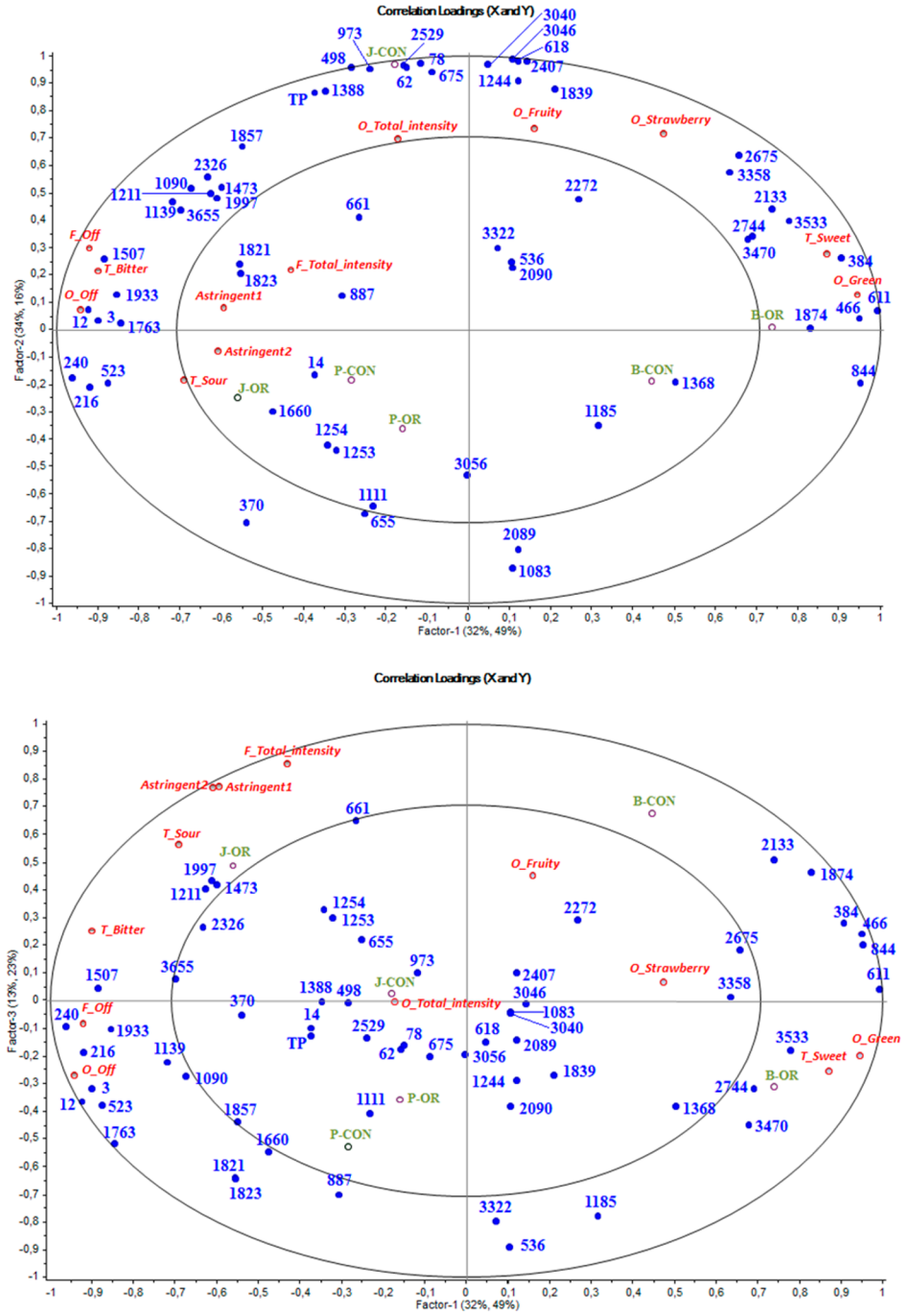

Figure 5. Partial least-squares regression (PLS) plots of the interactions between sensory and tentative metabolite profiles in three strawberry cultivars (B, 'Bounty'; J, 'Jonsok'; P, 'Polka') cultivated conventionally (CON) and organically (OR). Tentatively identified metabolites are used as *X*-variables (predictors) and sensory properties as *Y*-variables (responses). The metabolites are marked by numbers, referring to the tentative identifications in Table 2. Factors 1 and 2 are in the upper plot, and factors 1 and 3 are in the lower plot. Sensory attributes are in italics. O, odor; T, taste; F, flavor; TP, total phenolics.

terpenoids in general, they are well-known bitter, toxicity-implying defense compounds.[17]

Organic acids have been previously found to contribute to both sourness and astringency, two attributes having positive correlation with total acid content and titratable acidity and negative correlation with sugar content.[49] In sensory evaluations, astringency and sourness can be correlated with each other in a berry fruit sample,[49] and this makes distinguishing the effects of individual chemical compounds on fruit sensory properties more difficult.

Oleic and linoleic acids—being long-chained, *cis*-polymerized fatty acids—are distinctive precursors of volatiles involved in plant flavor formation.[17] The catabolism of essential fatty acids is also apt to cause off-flavors.[17] Salidroside is a glucoside of phenylethyl alcohol, and the cleavage of its moieties is likely to profit, for example, rosy odor.[50,51] Yet, the orthonasally perceived aroma of strawberry—derived from the balanced combination of hundreds of plant metabolites, their conjugates. and derivatives—is a complex, polygenic trait fluctuating from

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one cultivar to the other and between plants cultivated under different environmental conditions.[22,50]

Sucrose content is a determinant factor in strawberry sweetness and also correlates positively with the total volatile content of strawberry; in addition, some volatile compounds have been observed to enhance sweet taste perception from strawberry samples.[22] The contents and ratios of strawberry sugars (i.e., fructose, glucose, and sucrose) vary according to cultivar, and this may affect the sweet taste perception because sugars differ in their relative sweetness.[2,52] Sweetness may decrease both astringency and bitterness: increased sucrose levels have been found to reduce these properties in polyphenol-rich solutions, and this can be explained both physiologically and cognitively.[53] In addition, sample color can modify flavor perception.[54]

The sugar contents or more accurate volatile profiles of frozen strawberries were not determined in this study, and overall, all samples had moderate sensory attribute intensities. Yet it can be concluded that either high sucrose content, the low concentrations of some flavonoid glycosides, distinct fatty acid metabolism, advantageous volatile profile, or all of these factors may explain the relatively high green odor and sweet taste intensities and relatively low astringency and bitterness intensities evaluated from B-OR (Figures 4 and 5). In this study, we have considered a small but prominent fraction of the strawberry metabolome. Adequate peak area, indicating metabolite abundance in the strawberry sample, was one of the criteria used for compound qualification in our analyses. Among the thousands of chemical compounds detected in our samples, there are yet many minor constituents that undoubtedly contribute to the taste, flavor, and odor of strawberries. Therefore, it is our aim to take a broader perspective in clarifying the relationship between the metabolome and sensory quality of strawberries.

The present results indicate that the stability of strawberry chemical composition and sensory quality in different horticultural systems vary mainly according to cultivar. Organic farming practices may enhance the accumulation of some health-related plant metabolites in specific strawberry genotypes. Hence, careful cultivar selection is a key factor in combining sustainable farming practices and the enhanced nutritional quality of strawberry. Furthermore, appropriate cultivar selection may help to increase the marketing value of organically grown strawberry. This study has further verified that strawberry cultivars differ in their chemical composition and sensory characteristics. Many types of flavonoids, hydrolyzable tannins, and flavan-3-ol derivatives are among the most abundant compounds in strawberries, and these compounds contribute to strawberry sensory attributes, as well.

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